

Article

Contrasting Morphological, Physiological, Biochemical, and Ionic Responses of *Lolium perenne* to Drought and Salinity Stress

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Abstract

Drought and salinity are major abiotic stresses limiting plant performance in managed and natural ecosystems, including turfgrass systems. This study investigated the morphological, physiological, biochemical, and ionic responses of three *Lolium perenne* cultivars grown in natural and sterile soils under controlled water deficit and salinity treatments. Both stresses significantly reduced plant growth, but their underlying drivers differed markedly. Drought primarily imposed osmotic limitation, affecting biomass accumulation and plant water status, whereas salinity introduced an additional ionic constraint characterized by substantial Na⁺ and Cl[−] accumulation and reduced K⁺/Na⁺ ratios. This ionic imbalance was associated with enhanced oxidative stress and greater destabilization of photosynthetic pigments relative to drought. Multivariate hierarchical clustering revealed distinct trait coordination patterns under the two stress types, highlighting tighter integration among ionic regulation, redox balance, and growth limitation under salinity. Across treatments, plants grown in natural soil generally maintained improved physiological performance compared with those in sterile soil, although soil effects modulated response magnitude rather than direction. Cultivar-dependent differences reflected variation in regulatory efficiency across traits. Overall, the findings demonstrate that drought and salinity induce fundamentally different stress hierarchies in *L. perenne*, emphasizing the central role of ionic homeostasis in salinity tolerance and the value of integrated trait analysis for turfgrass stress management.

Keywords: drought stress; salinity stress; turfgrass; *Lolium perenne*; soil sterilization; osmoprotectants; oxidative stress; mineral ions; hierarchical clustering; stress adaptation



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1. Introduction

Drought and soil salinity are among the most severe abiotic constraints affecting plant growth and productivity worldwide [1–4], particularly under the current climate change scenario, characterized by increasing temperatures, irregular precipitation patterns, and progressive soil degradation [5–8]. These stresses frequently occur simultaneously or sequentially, imposing complex physiological challenges on plants and significantly limiting

their performance in both natural and managed ecosystems [9–11]. Understanding plant responses to water deficit and salinity has therefore become a central topic in plant stress physiology, with implications not only for agriculture but also for landscape management and ecosystem sustainability [12–15].

Turfgrass systems represent a specific and increasingly important component of managed plant communities, especially in urban, recreational, and sports environments. Unlike annual crops, turfgrasses are expected to maintain aesthetic quality, ground coverage, and functional performance under suboptimal conditions, including limited irrigation and the use of saline or low-quality water sources [16–18]. Consequently, improving stress tolerance in turfgrass species is a key objective for sustainable turf management, particularly in regions exposed to recurrent drought episodes and soil salinization [19–22].

Among cool-season turfgrasses, *Lolium perenne* L. is one of the most widely used species due to its rapid establishment, high turf quality, and adaptability to a broad range of environments [23,24]. However, perennial ryegrass is generally considered moderately sensitive to both drought and salinity, with stress conditions often resulting in growth inhibition, leaf senescence, pigment degradation, and reduced turf persistence [25,26]. Despite its extensive use, the physiological and biochemical mechanisms underlying stress responses in *L. perenne*, especially under combined or comparative drought and salinity stress scenarios, are not yet fully elucidated.

Previous studies conducted across a broad range of cultivated and wild herbaceous species, including crops, plants with ornamental value, trees, and turf and forage grasses, have shown that plant responses to drought and salinity involve coordinated adjustments at morphological, physiological, and biochemical levels [27–29]. These responses commonly include alterations in water status, modulation of photosynthetic pigment content, ion accumulation and compartmentalization, synthesis of compatible osmolytes such as proline and soluble sugars, and activation of antioxidant defense mechanisms to mitigate oxidative damage [30,31]. Although such mechanisms have been extensively described in model species and stress-adapted plants, their relative contribution and effectiveness may vary considerably among taxa and functional groups.

In grasses and grass-like species, stress tolerance has been associated with the ability to regulate ionic homeostasis, particularly the balance between Na^+ , K^+ , and Cl^- ions, as well as with efficient osmotic adjustment and protection of the photosynthetic apparatus [32–36]. Moreover, recent evidence suggests that exposure to stress events may induce acclimation responses, leading to modified physiological behavior during subsequent stress periods [37–40]. However, data regarding these processes remain scarce for turfgrass species, including *L. perenne*, especially when drought and salinity stresses are evaluated comparatively under controlled conditions.

Soil microorganisms, particularly those inhabiting the rhizosphere, can play a significant role in modulating the responses of grasses and other plants to abiotic stress, since colonization by plant growth-promoting rhizobacteria (PGPR) and mycorrhiza have been shown to enhance plant physiological and biochemical stress responses under drought and salinity conditions [41,42]. These microbial communities, including PGPR and other beneficial taxa, often enhance plant growth and tolerance to drought and salinity through multiple mechanisms: they can improve nutrients and water uptake, modulate plant hormone balances, induce antioxidant defenses, facilitate osmotic adjustment via production of osmolytes and enzymes such as ACC deaminase, and alter antioxidant enzyme activities and photosynthesis [43,44]. These interactions suggest that the soil microbiome acts as a dynamic partner in plant stress adaptation, enhancing tolerance to abiotic stressors commonly encountered in natural and agricultural systems.

In this context, the present study investigates the responses of *L. perenne* grown in natural and sterile soils to drought and salinity by integrating growth-related traits with physiological, biochemical, and ionic indicators of stress tolerance. Specifically, parameters related to plant water status, photosynthetic pigments, mineral ion accumulation, osmolyte production, and oxidative stress were analyzed to provide an integrated assessment of stress-induced responses and to evaluate the potential modulatory role of soil-associated biota in enhancing plant resilience. By comparing osmotic and combined osmotic–ionic stress conditions, this study aims to clarify the hierarchical organization of trait responses in perennial ryegrass and to support the development of management strategies and cultivar selection criteria for improved performance under increasing water limitation and soil salinization.

2. Materials and Methods

2.1. Plant Material, Soil Source, and Growth Conditions

The experiment was conducted between January and April 2025 under controlled greenhouse conditions at the Universitat Politècnica de València, Spain. Seeds of three perennial ryegrass (*L. perenne* L.) cultivars, namely ‘Columbine’, ‘Allstarter’, and ‘Esquire’, were obtained from a certified commercial supplier. Prior to sowing, seeds were surface-sterilized in a 2% (*v/v*) sodium hypochlorite solution for 10 min and thoroughly rinsed with deionized water.

Coastal sand was collected on 9 January 2025 from the embryonic and shifting dunes of Albufera Natural Park, Spain (39°20′43″ N, 0°18′38″ W). The collected sand was divided into two equal fractions. One fraction was sterilized by triple autoclaving at 121 °C for 1 h on three consecutive days to reduce soil biota and was used as the sterile soil (SS). The non-sterilized fraction served as the natural soil (NS), following established protocols previously applied for coastal soils from this site [28]. No direct assessment of microbial community composition or AMF colonization was performed.

2.2. Substrate Preparation and Experimental Design

The growth substrate consisted of a homogenized mixture of native coastal sand and a commercial universal substrate (*Sphagnum* peat, pH 6.0, EC 0.30 dS m^{−1}; Klasmann-Deilmann GmbH, Geeste, Germany). Coastal sand was collected from a coastal site and used either in its natural (non-sterilized) form or after sterilization, depending on the treatment. Sterilization was applied to reduce the native microbial community associated with the sand fraction. For each experimental unit, 65 g of coastal sand (natural or sterilized) was manually mixed with the commercial substrate, corresponding to approximately 15% (*v/v*) of the total substrate volume. Thus, the experimental contrast reflects differences between non-sterilized (natural) and sterilized sand substrate conditions, which likely differ in their native microbial communities, rather than the controlled inoculation of specific microbial taxa. Square polypropylene pots (TEKU MQF 8 × 8 × 7 cm, 260 mL volume; Pöppelmann GmbH & Co. KG Kunststoffwerk-Werkzeugbau, 49393 Lohne, Germany) were filled with the prepared substrate mixture.

The experiment followed a fully factorial design with three factors: cultivar (three levels), soil treatment (natural sand, NS; sterilized sand, SS), and stress treatment. For each cultivar × soil treatment × stress combination, five biological replicates (independent pots) were used, resulting in a total of 90 experimental units. Pots were arranged under greenhouse conditions and maintained uniformly throughout the experiment. The individual pot represented the experimental unit, and the experiment followed a completely randomized design to minimize positional effects within the greenhouse. All statistical analyses were performed using these independent experimental units.

($n = 5$ per cultivar $\times$ soil treatment $\times$ stress combination). Sowing was performed on 20 January 2025, and after emergence, seedlings were thinned to one plant per pot.

Plants were grown in a greenhouse under natural light conditions (10/14 h light/dark photoperiod). Air temperature and relative humidity were continuously monitored using a calibrated data logger (HOBO U23-001A, Onset Computer Corp., Bourne, MA, USA); mean day/night temperatures were 20/15 °C, and relative humidity ranged between 50% and 80%. During the establishment phase, plants were irrigated uniformly with tap water and received no additional fertilization.

Throughout the manuscript, the term “soil treatment” refers to the contrast between natural (NS) and sterilized (SS) sand fractions incorporated into the growth substrate.

2.3. Abiotic Stress Treatments

Abiotic stress treatments were initiated on 14 March 2025, when plants had reached a uniform vegetative stage. Pots were placed in plastic trays (55 $\times$ 40 cm; 30 pots per tray) to allow sub-irrigation. Three irrigation regimes were applied:

- (i) Control (C): Plants were irrigated twice weekly with tap water (EC 1.05 dS m⁻¹);
- (ii) Salinity stress (S): Salinity was imposed by adding a NaCl solution (200 mM) to the trays, allowing uniform capillary uptake by the substrate;
- (iii) Water stress (D): Plants received a single irrigation (1.5 L per tray) during the first week, followed by complete water deprivation for the remainder of the experimental period.

Stress treatments were maintained for approximately four weeks, until plants exhibited clear physiological symptoms of stress.

2.4. Sampling and Morphological Measurements

Plants were harvested on 15 April 2025. Shoots and roots were carefully separated and gently washed to remove adhering substrate. Plant height and leaf number were recorded prior to biomass separation. Fresh weights of shoots and roots were measured immediately after harvest.

A subsample of fresh shoot tissue was flash-frozen in liquid nitrogen and stored at −75 °C for biochemical analyses. The remaining shoot and root material was oven-dried at 65 °C for five days to determine dry weight and was subsequently used for ion content analysis.

2.5. Root Architecture Analysis

Roots were scanned at 600 dpi using a flatbed scanner. The root images were analysed with WinRHIZO Pro 2003b software (Regent Instruments Inc., Quebec, QC, Canada) to determine total root length, root surface area, root volume, projected area, average root diameter, and root tip number.

2.6. Biochemical Analyses

2.6.1. Photosynthetic Pigments

Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total carotenoids (Caro) were determined in fresh leaf material extracted with 80% acetone, following the method of Lichtenthaler and Wellburn [45]. Absorbance was measured at 470, 646 and 663 nm for each sample, and pigment contents were expressed as mg g⁻¹ dry weight (DW).

2.6.2. Osmolytes

Proline (Pro) content was determined according to Bates et al. [46], as adapted by Vicente et al. [47]. Fresh plant material was homogenized in 3% (*w/w*) sulfosalicylic acid

and centrifuged at $16,100 \times g$ for 15 min at 25 °C. The supernatant was mixed with acid ninhydrin and glacial acetic acid and incubated in a water bath at 98 °C for 1 h. After cooling on ice for 10 min, proline was extracted with toluene, and the absorbance of the organic phase was measured at 520 nm. Proline content was calculated using a standard calibration curve and expressed in $\mu\text{mol g}^{-1}$ DW.

Total soluble sugars (TSS) were determined in fresh material extracted with methanol, following Dubois et al. [48]. Absorbance was measured at 490 nm, and results were expressed as mg glucose equivalents g^{-1} DW using a calibration curve prepared with known glucose concentrations.

Glycine betaine (GB) was quantified following the method of Grieve and Grattan [49]. Absorbance was measured at 365 nm, and GB contents were expressed in $\mu\text{mol g}^{-1}$ DW based on a calibration curve prepared with known GB concentrations.

2.6.3. Oxidative Stress Biomarkers and Antioxidant Compounds

Malondialdehyde (MDA), total phenolics content (TPC) and total flavonoids content (TFC) were measured in the same methanolic extracts used for TSS determination.

MDA was quantified according to Hodges et al. [50]. Absorbance was recorded at 440, 532 and 600 nm, and concentrations were calculated according to Taulavuori et al. [51], and expressed in nmol g^{-1} DW.

Hydrogen peroxide (H_2O_2) was determined following Alexieva et al. [52], measuring absorbance at 390 nm, and expressed in $\mu\text{mol g}^{-1}$ DW using a standard calibration curve.

Total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent according to Blainski et al. [53], measuring absorbance at 765 nm. Results were expressed as gallic acid equivalents (mg eq. GA g^{-1} DW) based on a calibration curve prepared with known GA concentrations.

Total flavonoid content (TFC) was quantified by a colorimetric assay using NaNO_2 and AlCl_3 according to Zhishen et al. [54]. Absorbance was measured at 510 nm, and results were expressed as catechin equivalents (mg eq. C g^{-1} DW) using a calibration curve prepared with known catechin concentrations.

2.7. Mineral Ion Content

Na^+ , K^+ , Ca^{2+} and Cl^- concentrations in leaves and roots were determined according to Weimberg [55]. Na^+ , K^+ and Ca^{2+} were measured by flame photometry (PFP7, Jenway, Staffordshire, UK), while Cl^- was determined using a chloride-specific electrode (MKII 926, Sherwood Scientific Ltd., Cambridge, UK). Results were expressed in $\mu\text{mol g}^{-1}$ DW.

2.8. Statistical Analysis

All data were subjected to statistical analysis using SPSS Statistics (Version 26, IBM Corp., Armonk, NY, USA). For each stress condition (water stress and salinity stress), data were analyzed using a three-way analysis of variance (ANOVA) implemented through the General Linear Model (GLM) Univariate procedure. Cultivar, soil treatment (natural soil vs. sterile soil), and stress treatment were included as fixed factors, and all main effects as well as their two- and three-way interaction terms were considered in the model.

Prior to analysis, the assumptions of normality and homogeneity of variances were evaluated using the Shapiro–Wilk test applied to model residuals and Levene's test, respectively. When necessary, data were transformed (logarithmic or square-root transformation) to meet the assumptions of the GLM. Differences among treatment means were assessed using Tukey's honestly significant difference (HSD) post hoc test at a significance level of $p \leq 0.05$.

To integrate the responses of morphological, physiological, biochemical, and ionic traits, multivariate analysis was performed separately for water stress and salinity stress conditions. Hierarchical clustering analysis (HCA) was conducted using PAST software (Version 5.3) [56]. Prior to clustering, all variables were standardized using Z-score transformation. Euclidean distance was used as the dissimilarity measure, and clustering was performed using the unweighted pair-group method with arithmetic mean (UPGMA).

3. Results

3.1. Plant Responses to Water Stress

3.1.1. Morphological Traits, Biomass Allocation, and Root System Architecture Under Water Stress

Water stress significantly affected shoot and root morphology in *Lolium perenne*, with response magnitude strongly dependent on cultivar and soil treatment (Table 1; Figure A1). Across genotypes, shoot dry weight declined by 22–30% in natural soil (NS) but by 49–59% in sterile soil (SS), indicating a markedly stronger biomass reduction under SS conditions. For example, ‘Columbine’ showed a 28% decrease in NS (0.70 to 0.50 g) compared with a 59% decrease in SS (0.91 to 0.37 g), while ‘Esquire’ exhibited a 22% reduction in NS versus 49% in SS. Root dry weight was even more strongly affected, with reductions ranging from 62% to 92% depending on cultivar and soil treatment. Notably, ‘Esquire’ maintained higher root dry weight in NS under water stress (1.1 g) compared with SS (0.3 g), corresponding to a 62% versus 92% reduction relative to control conditions.

Table 1. Effects of water stress, soil treatment (natural vs. sterile soil), and cultivar on shoot and root traits of *Lolium perenne*.

Treatment			Plant Height (cm)	Leaf Number	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
Stress	Cultivar	Soil						
Control	Columbine	NS	26.3 ± 0.70 a	9.6 ± 0.32 d	2.5 ± 0.28 d	0.70 ± 0.06 d	5.1 ± 0.73 d	2.6 ± 0.58 c
Control	Columbine	SS	28.5 ± 0.90 a	9.7 ± 0.34 d	3.2 ± 0.29 e	0.91 ± 0.08 e	12.2 ± 0.97 f	6.5 ± 0.84 f
Control	Allstarter	NS	27.2 ± 0.87 a	14.1 ± 0.40 e	2.2 ± 0.26 d	0.80 ± 0.05 e	11.2 ± 0.93 f	2.9 ± 0.56 d
Control	Allstarter	SS	27.2 ± 0.86 a	13.7 ± 0.53 e	2.1 ± 0.23 d	0.81 ± 0.06 e	5.4 ± 0.76 d	2.5 ± 0.52 c
Control	Esquire	NS	32.3 ± 0.97 b	13.3 ± 0.51 e	2.3 ± 0.27 d	0.60 ± 0.05 c	9.3 ± 0.88 e	2.9 ± 0.54 d
Control	Esquire	SS	27.8 ± 0.74 a	10.2 ± 0.44 d	2.5 ± 0.28 d	0.61 ± 0.06 c	10.0 ± 0.89 e	3.7 ± 0.68 e
Water stress	Columbine	NS	30.5 ± 0.98 a	8.7 ± 0.41 c	1.2 ± 0.17 b	0.50 ± 0.05 b	5.3 ± 0.65 d	0.5 ± 0.25 a
Water stress	Columbine	SS	29.6 ± 0.90 a	7.6 ± 0.38 b	1.1 ± 0.19 b	0.37 ± 0.04 a	2.6 ± 0.52 c	1.0 ± 0.48 b
Water stress	Allstarter	NS	29.0 ± 0.94 a	10.8 ± 0.47 d	1.1 ± 0.19 b	0.56 ± 0.05 c	9.4 ± 0.87 e	0.6 ± 0.15 a
Water stress	Allstarter	SS	26.9 ± 0.93 a	11.3 ± 0.48 d	0.4 ± 0.09 a	0.35 ± 0.04 a	2.0 ± 0.63 b	0.5 ± 0.13 a
Water stress	Esquire	NS	25.9 ± 0.75 a	6.8 ± 0.38 a	1.7 ± 0.21 c	0.47 ± 0.05 b	8.8 ± 0.87 e	1.1 ± 0.38 b
Water stress	Esquire	SS	27.2 ± 0.80 a	10.4 ± 0.46 d	0.4 ± 0.09 a	0.31 ± 0.04 a	0.9 ± 0.14 a	0.3 ± 0.09 a

Data are means ± SEM ($n = 5$). Treatments represent all combinations of water regime (Control and Water Stress), cultivar (‘Columbine’, ‘Allstarter’, ‘Esquire’), and soil (NS, natural soil and SS, sterile soil). Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey’s HSD test ($p < 0.05$).

Cultivar-specific differences were evident. ‘Esquire’ maintained comparatively higher shoot and root biomass across soil treatments, with shoot dry weight reductions remaining below 50%, whereas ‘Columbine’ exhibited decreases exceeding 50% in SS. ‘Allstarter’ showed intermediate shoot responses but pronounced soil-dependent variation in root traits. Leaf number was less sensitive to water stress than biomass-related traits, with moderate reductions in most combinations, although ‘Esquire’ in NS exhibited a stronger decline ($\approx 49\%$), suggesting that water limitation primarily constrained biomass accumulation rather than leaf initiation.

Water stress also induced marked changes in root system architecture (Table 2; Figure A2), although the direction and magnitude of responses varied among cultivars and soil treatments. Root total length exhibited strong soil-dependent responses. In ‘Allstarter’, root length remained virtually unchanged in NS (94.1 to 94.3 cm) but declined by approxi-

mately 55% in SS (159.7 to 72.6 cm). In ‘Columbine’, root length increased by 19% under stress in NS (59.1 to 70.2 cm) but decreased by 28% in SS (55.9 to 40.4 cm). ‘Esquire’ showed a 41% increase in NS (47.5 to 66.9 cm), compared with only an 11% increase in SS.

Table 2. Effects of water stress, soil treatment (natural vs. sterile soil), and cultivar on root system architectural traits of *Lolium perenne*.

Stress	Treatment		Root Total Length (cm)	Root Surface Area (cm ²)	Root Volume (cm ³)	Root Projected Area (cm ²)	Root Diameter (mm)	Root Tip Number
	Cultivar	Soil						
Control	Columbine	NS	59.1 ± 9.1 b	54.3 ± 8.4 e	17.3 ± 2.2 d	4.1 ± 1.0 b	3.2 ± 0.6 d	863.3 ± 182.1 a
Control	Columbine	SS	55.9 ± 8.4 b	60.9 ± 8.9 e	19.4 ± 2.9 d	6.5 ± 1.5 c	4.0 ± 0.9 e	1205.7 ± 192.3 b
Control	Allstarter	NS	94.1 ± 9.5 d	98.5 ± 9.1 i	12.0 ± 2.7 b	31.4 ± 3.9 f	6.1 ± 1.6 g	2123.3 ± 383.5 e
Control	Allstarter	SS	159.7 ± 11.1 e	84.4 ± 8.0 g	26.9 ± 3.2 f	4.1 ± 1.1 b	2.0 ± 0.4 b	3189.8 ± 469.3 f
Control	Esquire	NS	47.5 ± 7.1 a	77.7 ± 8.8 g	24.7 ± 3.5 f	11.3 ± 2.1 e	5.8 ± 1.1 g	1294.3 ± 234.3 b
Control	Esquire	SS	48.8 ± 7.5 a	68.1 ± 9.7 f	21.7 ± 2.7 e	9.1 ± 2.3 d	5.1 ± 1.1 f	1164.7 ± 212.7 b
Water stress	Columbine	NS	70.2 ± 8.1 c	37.3 ± 7.2 b	11.9 ± 2.1 b	1.7 ± 0.9 a	1.8 ± 0.2 b	1445.3 ± 276.3 c
Water stress	Columbine	SS	40.4 ± 7.6 a	41.5 ± 6.4 bc	13.2 ± 2.4 bc	3.7 ± 1.2 b	3.3 ± 0.9 d	893.6 ± 262.9 a
Water stress	Allstarter	NS	94.3 ± 9.8 d	50.9 ± 8.8 d	5.5 ± 1.2 a	13.2 ± 2.9 e	1.9 ± 0.1 b	1717.8 ± 234.5 d
Water stress	Allstarter	SS	72.6 ± 7.4 c	33.4 ± 4.6 b	10.6 ± 2.0 b	1.3 ± 0.2 a	1.6 ± 0.2 a	1248.5 ± 242.2 b
Water stress	Esquire	NS	66.9 ± 6.8 c	43.1 ± 5.4 c	13.7 ± 2.8 c	2.6 ± 0.4 b	2.4 ± 0.3 c	1408.9 ± 265.3 c
Water stress	Esquire	SS	54.1 ± 8.2 b	22.6 ± 3.7 a	7.2 ± 1.8 a	0.8 ± 0.4 a	1.3 ± 0.2 a	963.4 ± 211.5 a

Data are means ± SEM ($n = 5$). Treatments represent all combinations of water regime (Control and Water Stress), cultivar (‘Columbine’, ‘Allstarter’, ‘Esquire’), and soil (NS, natural soil and SS, sterile soil). Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey’s HSD test ($p < 0.05$).

Root surface area, volume, and tip number followed comparable soil-dependent patterns. Under SS conditions, root surface area declined by approximately 60% in ‘Allstarter’ (84.4 to 33.4 cm²) and 67% in ‘Esquire’ (68.1 to 22.6 cm²), while root volume decreased by up to 67% in SS-grown ‘Esquire’ plants (21.7 to 7.2 cm³). Root tip number, reflecting branching intensity, decreased by 61% in SS-grown ‘Allstarter’ (3189 to 1248), whereas reductions were less pronounced in NS. Overall, these results indicate that water stress strongly limited root system expansion under sterile soil conditions, while plants grown in natural soil maintained greater architectural stability and plasticity.

When morphological and root architectural traits were analyzed as unilateral effects of individual experimental factors (Figures A1 and A2), water stress emerged as the dominant driver of biomass and root system modification. However, the magnitude of these effects was consistently modulated by soil treatment, with NS-grown plants generally maintaining greater root system extension and stability compared with SS. Cultivar-dependent variation further reflected intrinsic differences in growth performance and root plasticity under water-limited conditions.

3.1.2. Water Status and Photosynthetic Pigments Under Water Stress

Plant water relations and biomass allocation patterns were strongly influenced by water limitation, with responses varying across cultivars and soil treatments (Table 3; Figure A3).

Shoot water content (SWC) declined markedly under water limitation, although the magnitude of reduction varied across soil treatments. In ‘Allstarter’, SWC decreased by 36% in natural soil (65.1 to 41.5%) but by 78% in sterile soil (62.6 to 13.6%), indicating severe dehydration under SS conditions. Similarly, ‘Esquire’ maintained nearly stable SWC in NS (−3%) but exhibited a 72% reduction in SS (75.7 to 21.4%). In contrast, ‘Columbine’ showed more moderate reductions (24% in NS and 15% in SS). Root water content displayed cultivar-specific patterns, with increases observed in ‘Columbine’ under both soil treatments (+27% in NS and +44% in SS), whereas ‘Esquire’ showed a 31% decrease in NS but remained relatively stable in SS (−2%). The root-to-shoot ratio declined substantially under water stress, particularly in SS-grown plants, where reductions reached up to 84% in ‘Esquire’, reflecting pronounced shifts in biomass allocation.

Water stress significantly altered photosynthetic pigment composition, with pronounced cultivar- and soil-dependent responses (Table 4; Figure A4).

Table 3. Effects of water stress, soil treatment (natural vs. sterile soil), and cultivar on shoot and root water status and biomass allocation of *Lolium perenne*.

Treatment			Shoot Water Content (%)	Root Water Content (%)	Root-to-Shoot Ratio
Stress	Cultivar	Soil			
Control	Columbine	NS	73.2 ± 4.9 e	48.6 ± 2.7 a	3.9 ± 0.6 e
Control	Columbine	SS	73.1 ± 4.9 e	47.0 ± 2.5 a	7.5 ± 1.8 h
Control	Allstarter	NS	65.1 ± 4.2 de	73.8 ± 4.9 de	3.9 ± 0.4 e
Control	Allstarter	SS	62.6 ± 4.3 d	54.3 ± 3.2 b	3.2 ± 0.9 d
Control	Esquire	NS	73.0 ± 4.9 e	69.4 ± 4.1 d	4.6 ± 1.8 f
Control	Esquire	SS	75.7 ± 4.4 e	62.8 ± 4.2 c	6.2 ± 0.6 g
Water stress	Columbine	NS	55.7 ± 3.7 c	61.7 ± 4.5 c	1.0 ± 0.2 a
Water stress	Columbine	SS	62.3 ± 3.5 d	67.9 ± 4.7 cd	2.7 ± 0.9 c
Water stress	Allstarter	NS	41.5 ± 2.7 b	63.4 ± 4.1 c	1.1 ± 0.5 a
Water stress	Allstarter	SS	13.6 ± 1.7 a	78.2 ± 6.7 e	1.4 ± 0.6 b
Water stress	Esquire	NS	70.5 ± 4.7 e	47.9 ± 4.4 a	2.3 ± 0.9 c
Water stress	Esquire	SS	21.4 ± 2.7 a	61.4 ± 4.8 c	1.0 ± 0.4 a

Data are means ± SEM ($n = 5$). Treatments represent all combinations of water regime (Control and Water Stress), cultivar ('Columbine', 'Allstarter', 'Esquire'), and soil (NS, natural soil and SS, sterile soil). Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey's HSD test ($p < 0.05$).

Table 4. Effects of water stress, soil treatment (natural vs. sterile soil), and cultivar on photosynthetic pigment content and pigment ratios of *Lolium perenne*.

Treatment			Chl a (mg g ⁻¹ DW)	Chl b (mg g ⁻¹ DW)	Car (mg g ⁻¹ DW)	Chl a/b Ratio	TChl/Car Ratio
Stress	Cultivar	Soil					
Control	Columbine	NS	1.6 ± 0.14 e	0.34 ± 0.05 e	0.56 ± 0.07 e	4.7 ± 0.57 e	3.5 ± 0.41 b
Control	Columbine	SS	1.1 ± 0.09 c	0.13 ± 0.03 b	0.41 ± 0.03 d	8.5 ± 0.88 f	3.0 ± 0.33 b
Control	Allstarter	NS	1.6 ± 0.16 e	0.55 ± 0.06 f	0.63 ± 0.06 f	2.9 ± 0.31 c	3.4 ± 0.40 b
Control	Allstarter	SS	1.4 ± 0.15 d	0.57 ± 0.07 f	0.46 ± 0.05 d	2.5 ± 0.22 b	4.3 ± 0.54 c
Control	Esquire	NS	2.0 ± 0.17 f	0.57 ± 0.08 f	0.71 ± 0.07 g	3.5 ± 0.51 d	3.6 ± 0.38 b
Control	Esquire	SS	2.6 ± 0.28 g	0.85 ± 0.11 h	0.77 ± 0.07 h	3.1 ± 0.50 c	4.5 ± 0.64 c
Water stress	Columbine	NS	0.8 ± 0.07 b	0.64 ± 0.10 fg	0.16 ± 0.01 b	1.3 ± 0.12 a	9.0 ± 0.78 d
Water stress	Columbine	SS	1.4 ± 0.11 d	0.27 ± 0.05 d	0.44 ± 0.05 d	5.2 ± 0.59 e	3.8 ± 0.34 b
Water stress	Allstarter	NS	0.8 ± 0.07 b	0.22 ± 0.04 d	0.28 ± 0.04 c	3.6 ± 0.38 d	3.6 ± 0.38 b
Water stress	Allstarter	SS	0.1 ± 0.03 a	0.04 ± 0.00 a	0.08 ± 0.09 a	3.0 ± 0.44 c	2.0 ± 0.26 a
Water stress	Esquire	NS	1.6 ± 0.23 e	0.69 ± 0.08 g	0.67 ± 0.07 fg	2.3 ± 0.25 b	3.4 ± 0.35 b
Water stress	Esquire	SS	0.1 ± 0.03 a	0.03 ± 0.00 a	0.06 ± 0.08 a	3.0 ± 0.32 c	2.0 ± 0.26 a

Data are means ± SEM ($n = 5$). Treatments represent all combinations of water regime (Control and Water Stress), cultivar ('Columbine', 'Allstarter', 'Esquire'), and soil (NS, natural soil and SS, sterile soil). Chl a: chlorophyll a; Chl b: chlorophyll b; Car: carotenoids. Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey's HSD test ($p < 0.05$).

Chlorophyll a content declined sharply under stress, particularly in sterile soil. In 'Allstarter', chlorophyll a decreased by 50% in natural soil (1.6 to 0.8 mg g⁻¹ DW) but by 93% in sterile soil (1.4 to 0.1 mg g⁻¹ DW). A comparable pattern was observed in 'Esquire', where chlorophyll a declined by only 20% in NS (2.0 to 1.6 mg g⁻¹ DW) but by 96% in SS (2.6 to 0.1 mg g⁻¹ DW). Chlorophyll b followed similar trends, with reductions exceeding 90% in SS-grown 'Allstarter' and 'Esquire'. Carotenoid content also decreased substantially under SS conditions, with reductions of 83% in 'Allstarter' and 92% in 'Esquire'.

Pigment ratios further reflected stress-induced physiological adjustments. In 'Columbine', the Chl a/b ratio declined markedly under NS (4.7 to 1.3), while the TChl/Car ratio increased (3.5 to 9.0), indicating shifts in pigment balance under dehydration. In contrast, severe pigment depletion in SS-grown 'Allstarter' and 'Esquire' resulted in reduced TChl/Car ratios (~2.0), consistent with pronounced impairment of the photosynthetic apparatus.

3.1.3. Biochemical Stress Markers and Osmoprotectants Under Water Stress

Biochemical adjustment to water deficit involved marked changes in osmoprotectant accumulation and oxidative stress markers, with clear differences among cultivars and soil treatments (Table 5). Proline accumulation increased dramatically under water stress, particularly in natural soil (NS). In 'Columbine', proline rose from 1.7 to 62.0 µmol g⁻¹

DW in NS (≈ 36 -fold increase), whereas the increase in sterile soil (SS) was more moderate (1.2 to $20.8 \mu\text{mol g}^{-1}$ DW; ≈ 17 -fold). A similar pattern was observed in ‘Esquire’, where proline increased nearly 15-fold in NS but decreased under SS conditions. In contrast, ‘Allstarter’ exhibited limited proline accumulation under SS (1.8 to $1.1 \mu\text{mol g}^{-1}$ DW), indicating impaired osmotic adjustment under sterile conditions.

Table 5. Effects of water stress, soil treatment (natural vs. sterile soil), and cultivar on osmoprotectants, antioxidant compounds, and oxidative stress markers in *Lolium perenne*.

Treatment			Pro ($\mu\text{mol g}^{-1}$ DW)	TSS (mg eq. G g^{-1} DW)	GB ($\mu\text{mol g}^{-1}$ DW)	TPC (mg eq. GA g^{-1} DW)	TFC (mg eq. C g^{-1} DW)	MDA (nmol g^{-1} DW)	H ₂ O ₂ ($\mu\text{mol g}^{-1}$ DW)
Stress	Cultivar	Soil							
Control	Columbine	NS	1.7 ± 0.2 a	56.9 ± 4.2 c	34.0 ± 2.2 d	2.5 ± 0.2 c	2.9 ± 0.3 c	82.4 ± 5.7 a	38.2 ± 2.4 b
Control	Columbine	SS	1.2 ± 0.1 a	84.0 ± 5.6 d	40.7 ± 3.1 e	3.0 ± 0.3 d	2.9 ± 0.3 c	69.3 ± 6.3 a	18.2 ± 1.5 a
Control	Allstarter	NS	2.0 ± 0.3 a	65.2 ± 4.9 c	20.4 ± 2.6 c	3.4 ± 0.3 e	3.3 ± 0.3 d	96.6 ± 7.6 b	52.3 ± 2.4 c
Control	Allstarter	SS	1.8 ± 0.2 a	67.1 ± 4.7 c	12.3 ± 1.2 b	2.6 ± 0.2 c	2.2 ± 0.2 b	72.2 ± 4.2 a	39.3 ± 1.8 b
Control	Esquire	NS	2.8 ± 0.3 a	83.7 ± 6.1 d	23.7 ± 2.4 c	3.6 ± 0.3 e	3.5 ± 0.3 d	154.0 ± 8.7 c	54.0 ± 2.7 c
Control	Esquire	SS	2.1 ± 0.2 a	93.9 ± 5.9 e	30.4 ± 3.0 d	4.7 ± 0.4 f	4.7 ± 0.4 f	109.2 ± 5.3 b	78.1 ± 2.9 d
Water stress	Columbine	NS	62.0 ± 4.1 e	90.7 ± 5.6 de	46.8 ± 3.7 f	3.1 ± 0.3 d	2.3 ± 0.3 b	206.5 ± 9.5 d	54.0 ± 2.4 c
Water stress	Columbine	SS	20.8 ± 1.6 c	87.8 ± 4.8 d	35.4 ± 3.4 d	3.8 ± 0.3 e	3.8 ± 0.4 e	225.9 ± 8.2 d	39.7 ± 1.6 b
Water stress	Allstarter	NS	14.6 ± 1.2 b	40.9 ± 3.7 b	15.0 ± 1.4 b	2.0 ± 0.2 b	2.0 ± 0.3 b	72.3 ± 4.9 a	20.4 ± 0.9 a
Water stress	Allstarter	SS	1.1 ± 0.1 a	25.4 ± 2.4 a	8.2 ± 1.2 a	1.2 ± 0.1 a	0.9 ± 0.1 a	250.4 ± 9.1 e	85.6 ± 4.4 de
Water stress	Esquire	NS	41.5 ± 2.8 d	98.3 ± 5.3 e	31.7 ± 2.7 d	2.9 ± 0.3 d	2.6 ± 0.2 c	146.5 ± 7.7 c	42.4 ± 3.3 b
Water stress	Esquire	SS	1.3 ± 0.2 a	28.1 ± 3.1 a	9.5 ± 1.1 a	1.4 ± 0.1 a	1.1 ± 0.1 a	280.9 ± 12.2 e	92.3 ± 6.7 e

Data are means $\pm$ SEM ($n = 5$). Treatments represent all combinations of water regime (Control and Water Stress), cultivar (‘Columbine’, ‘Allstarter’, ‘Esquire’), and soil (NS, natural soil and SS, sterile soil). Pro: proline; TSS: total soluble sugars; GB: glycine betaine; TPC: total phenolics content; TFC: total flavonoids content; MDA: malondialdehyde; H₂O₂: hydrogen peroxide. Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey’s HSD test ($p < 0.05$).

Total soluble sugars (TSS) and glycine betaine (GB) also displayed soil-dependent responses. In ‘Columbine’, GB increased by approximately 38% in NS (34.0 to $46.8 \mu\text{mol g}^{-1}$ DW), whereas SS-grown plants showed a slight decrease. In contrast, ‘Allstarter’ and ‘Esquire’ exhibited marked declines in GB under SS, with a marked decline in ‘Esquire’ ($\approx 69\%$) and a more moderate reduction in ‘Allstarter’ ($\approx 33\%$). Total phenolic (TPC) and flavonoid (TFC) contents showed moderate increases in some NS-grown plants but declined sharply under SS in ‘Allstarter’ and ‘Esquire’, indicating reduced antioxidant capacity under severe stress.

Markers of oxidative damage further highlighted soil-dependent stress modulation. Malondialdehyde (MDA) content increased substantially under water stress, particularly in SS-grown plants. In ‘Esquire’, MDA rose from 109.2 to $280.9 \text{ nmol g}^{-1}$ DW in SS ($\approx 157\%$ increase), compared with a slight decrease in NS (154.0 to $146.5 \text{ nmol g}^{-1}$ DW). Similarly, in ‘Allstarter’, MDA increased from 72.2 to $250.4 \text{ nmol g}^{-1}$ DW in SS ($\approx 247\%$ increase), whereas levels showed a moderate decrease in NS. Hydrogen peroxide (H₂O₂) followed comparable trends, with the highest accumulation observed in SS-grown ‘Esquire’ (78.1 to $92.3 \mu\text{mol g}^{-1}$ DW). Overall, oxidative stress intensity was markedly amplified under sterile soil conditions, whereas NS-grown plants exhibited stronger osmoprotectant accumulation and comparatively lower oxidative damage.

When biochemical traits were analyzed as unilateral effects of individual experimental factors (Figure A5), water stress clearly emerged as the dominant driver of osmoprotectant accumulation and oxidative stress modulation. Across cultivars, proline and soluble sugars increased under water limitation, whereas oxidative damage markers (MDA and H₂O₂) were substantially amplified under sterile soil (SS). In contrast, plants grown in natural soil (NS) generally maintained lower oxidative stress levels and more balanced antioxidant responses. These patterns reinforce the soil-dependent modulation of biochemical adjustment observed in Table 5.

3.1.4. Mineral Ion Accumulation and Ionic Balance Under Water Stress

Alterations in mineral nutrient distribution under water deficit were comparatively moderate but revealed distinct cultivar- and soil-dependent ion regulation patterns (Tables 6 and 7; Figures A6 and A7).

Table 6. Effects of water stress, soil treatment (natural vs. sterile soil), and cultivar on leaf mineral ion concentrations and K^+/Na^+ ratios in *Lolium perenne*.

Treatment			Na^+ ($\mu\text{mol g}^{-1}$ DW)	K^+ ($\mu\text{mol g}^{-1}$ DW)	Ca^{2+} ($\mu\text{mol g}^{-1}$ DW)	Cl^- ($\mu\text{mol g}^{-1}$ DW)
Stress	Cultivar	Soil				
Control	Columbine	NS	2175.2 $\pm$ 292.3 ab	7111.6 $\pm$ 792.3 ab	143.4 $\pm$ 7.8 de	606.6 $\pm$ 25.2 b
Control	Columbine	SS	1645.0 $\pm$ 254.4 ab	6955.8 $\pm$ 712.8 ab	149.1 $\pm$ 7.9 de	674.9 $\pm$ 27.1 cb
Control	Allstarter	NS	1113.5 $\pm$ 150.6 a	5131.0 $\pm$ 602.1 a	133.3 $\pm$ 5.6 e	454.9 $\pm$ 20.7 a
Control	Allstarter	SS	1845.0 $\pm$ 291.3 ab	6222.5 $\pm$ 701.3 ab	140.6 $\pm$ 6.1 de	680.9 $\pm$ 28.8 cb
Control	Esquire	NS	2550.2 $\pm$ 272.6 b	9064.3 $\pm$ 852.6 b	129.3 $\pm$ 5.8 d	811.3 $\pm$ 29.7 c
Control	Esquire	SS	1746.2 $\pm$ 227.5 ab	7341.3 $\pm$ 772.8 ab	102.7 $\pm$ 4.5 b	674.1 $\pm$ 26.2 b
Water stress	Columbine	NS	3206.9 $\pm$ 292.1 c	10,456.2 $\pm$ 992.1 c	155.5 $\pm$ 7.3 e	655.9 $\pm$ 25.7 b
Water stress	Columbine	SS	1633.7 $\pm$ 192.3 ab	7119.2 $\pm$ 811.3 ab	104.6 $\pm$ 4.8 b	606.4 $\pm$ 24.2 b
Water stress	Allstarter	NS	3655.0 $\pm$ 322.4 c	5777.3 $\pm$ 522.5 ab	150.7 $\pm$ 6.1 e	706.7 $\pm$ 27.4 cb
Water stress	Allstarter	SS	1590.0 $\pm$ 170.6 ab	8458.2 $\pm$ 672.1 ab	91.0 $\pm$ 4.8 a	615.5 $\pm$ 25.9 b
Water stress	Esquire	NS	2475.3 $\pm$ 222.3 b	11,123.6 $\pm$ 992.3 c	119.7 $\pm$ 5.5 c	773.7 $\pm$ 27.4 c
Water stress	Esquire	SS	2044.2 $\pm$ 212.5 ab	9673.4 $\pm$ 874.3 ab	85.2 $\pm$ 3.8 a	961.5 $\pm$ 28.2 d

Data are means $\pm$ SEM ($n = 5$). Treatments represent all combinations of water regime (Control and Water Stress), cultivar ('Columbine', 'Allstarter', 'Esquire'), and soil (NS, natural soil and SS, sterile soil). Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey's HSD test ($p < 0.05$).

Table 7. Effects of water stress, soil treatment (natural vs. sterile soil), and cultivar on root mineral ion concentrations of *Lolium perenne*.

Treatment			Na^+ ($\mu\text{mol g}^{-1}$ DW)	K^+ ($\mu\text{mol g}^{-1}$ DW)	Ca^{2+} ($\mu\text{mol g}^{-1}$ DW)	Cl^- ($\mu\text{mol g}^{-1}$ DW)
Stress	Cultivar	Soil				
Control	Columbine	NS	2724.2 $\pm$ 351.2 c	2403.0 $\pm$ 324.2 de	135.3 $\pm$ 6.5 d	448.3 $\pm$ 37.7 b
Control	Columbine	SS	4147.1 $\pm$ 558.5 e	2204.8 $\pm$ 311.2 d	146.9 $\pm$ 6.9 d	373.9 $\pm$ 18.9 a
Control	Allstarter	NS	3815.6 $\pm$ 421.2 de	2995.2 $\pm$ 371.1 e	159.7 $\pm$ 6.7 d	554.0 $\pm$ 44.2 c
Control	Allstarter	SS	3410.4 $\pm$ 400.8 d	2629.7 $\pm$ 321.2 de	99.5 $\pm$ 4.5 b	341.0 $\pm$ 20.7 a
Control	Esquire	NS	3759.6 $\pm$ 422.5 de	2627.2 $\pm$ 326.4 de	91.3 $\pm$ 3.9 b	422.9 $\pm$ 50.5 b
Control	Esquire	SS	2306.5 $\pm$ 311.2 b	2582.0 $\pm$ 357.2 de	71.5 $\pm$ 4.5 a	331.1 $\pm$ 42.6 a
Water stress	Columbine	NS	1924.5 $\pm$ 251.7 b	1185.9 $\pm$ 151.2 b	139.6 $\pm$ 6.4 d	321.0 $\pm$ 30.5 a
Water stress	Columbine	SS	1249.5 $\pm$ 155.2 a	1088.7 $\pm$ 141.2 b	70.4 $\pm$ 2.5 a	503.2 $\pm$ 66.7 c
Water stress	Allstarter	NS	2122.8 $\pm$ 251.1 b	1847.3 $\pm$ 351.1 c	112.7 $\pm$ 4.5 c	294.4 $\pm$ 50.7 a
Water stress	Allstarter	SS	3202.0 $\pm$ 322.2 d	552.5 $\pm$ 75.2 a	118.5 $\pm$ 4.1 c	314.1 $\pm$ 30.3 a
Water stress	Esquire	NS	2119.0 $\pm$ 277.4 b	2881.0 $\pm$ 391.2 e	88.7 $\pm$ 2.3 b	425.1 $\pm$ 44.7 b
Water stress	Esquire	SS	1808.1 $\pm$ 151.9 b	458.4 $\pm$ 64.2 a	111.4 $\pm$ 4.9 c	298.3 $\pm$ 32.7 a

Data are means $\pm$ SEM ($n = 5$). Treatments represent all combinations of water regime (Control and Water Stress), cultivar ('Columbine', 'Allstarter', 'Esquire'), and soil (NS, natural soil and SS, sterile soil). Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey's HSD test ($p < 0.05$).

In leaf tissues, Na^+ concentration increased under water stress in several NS-grown plants. For example, in 'Columbine', leaf Na^+ rose by 47% in NS (2175 to 3207 $\mu\text{mol g}^{-1}$ DW), whereas values remained stable in SS. In 'Allstarter', Na^+ increased more than threefold in NS (1114 to 3655 $\mu\text{mol g}^{-1}$ DW) but showed limited change in SS. Despite these increases, K^+ concentrations were generally maintained or enhanced under stress, particularly in NS-grown plants. In 'Esquire', leaf K^+ increased by 23% in NS (9064 to 11,124 $\mu\text{mol g}^{-1}$ DW), contributing to a relatively favorable ionic balance compared with other cultivars.

Root ion composition displayed distinct patterns (Table 7; Figure A7). Under water stress, root Na^+ concentrations generally declined in NS-grown plants, with reductions of 29% in 'Columbine' (2724 to 1925 $\mu\text{mol g}^{-1}$ DW) and 44% in 'Allstarter' (3816 to 2123 $\mu\text{mol g}^{-1}$ DW). In contrast, SS-grown 'Allstarter' showed a slight increase in root Na^+ (3410 to 3202 $\mu\text{mol g}^{-1}$ DW remained comparatively high), accompanied by a dramatic re-

duction in root K^+ (2629 to 553 $\mu\text{mol g}^{-1}$ DW; -79%). Similarly, ‘Esquire’ exhibited a 82% decline in root K^+ under SS (2582 to 458 $\mu\text{mol g}^{-1}$ DW), whereas NS-grown plants maintained stable or enhanced K^+ levels.

Water stress altered ion distribution between shoots and roots, but without the severe ionic disruption typical of salinity stress. Across cultivars, plants grown in natural soil generally maintained higher root K^+ concentrations and more balanced Na^+ levels than those grown in sterile soil, indicating improved ion homeostasis under NS conditions. Cultivar-specific differences further reflected intrinsic variation in ion regulation capacity under water-limited environments.

3.2. Plant Responses to Salinity Stress

3.2.1. Morphological Traits, Biomass Allocation, and Root System Architecture Under Salinity Stress

Salinity stress imposed substantial constraints on plant growth in *L. perenne*, leading to pronounced reductions in shoot and root biomass across cultivars and soil treatments (Table 8; Figure A8). Shoot dry weight declined markedly under salt stress, although the magnitude varied among cultivar–soil combinations. In ‘Allstarter’, shoot dry weight decreased by 34% in NS (0.80 to 0.53 g) and by 30% in SS (0.81 to 0.57 g). In contrast, ‘Esquire’ exhibited divergent responses depending on soil treatment, with a 23% decrease in NS (0.60 to 0.46 g) but a 36% increase in SS (0.61 to 0.83 g), indicating strong soil-dependent modulation. Root dry weight was also strongly affected. In ‘Columbine’, reductions reached 58% in NS (2.6 to 1.1 g) and 82% in SS (6.5 to 1.2 g), whereas ‘Allstarter’ showed a 79% decline in NS (2.9 to 0.6 g) but only a 32% reduction in SS (2.5 to 1.7 g).

Table 8. Effects of salinity stress, soil treatment (natural vs. sterile soil), and cultivar on shoot and root traits of *Lolium perenne*.

Treatment			Plant Height (cm)	Leaf Number	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
Stress	Cultivar	Soil						
Control	Columbine	NS	26.3 ± 0.70 a	9.6 ± 0.32 b	2.5 ± 0.28 e	0.70 ± 0.06 c	5.1 ± 0.73 b	2.6 ± 0.58 d
Control	Columbine	SS	28.6 ± 0.90 a	9.7 ± 0.34 b	3.2 ± 0.29 f	0.91 ± 0.08 e	12.2 ± 0.94 e	6.5 ± 0.84 f
Control	Allstarter	NS	27.2 ± 0.87 a	14.1 ± 0.40 c	2.2 ± 0.26 d	0.80 ± 0.05 d	11.2 ± 0.93 e	2.9 ± 0.56 d
Control	Allstarter	SS	27.2 ± 0.86 a	13.7 ± 0.53 c	2.1 ± 0.23 d	0.81 ± 0.06 d	5.4 ± 0.76 b	2.5 ± 0.52 d
Control	Esquire	NS	32.3 ± 0.97 b	13.3 ± 0.51 c	2.3 ± 0.27 de	0.60 ± 0.05 b	9.3 ± 0.88 d	2.9 ± 0.54 d
Control	Esquire	SS	27.8 ± 0.74 a	10.2 ± 0.49 b	2.5 ± 0.28 e	0.61 ± 0.06 b	10.0 ± 0.89 d	3.7 ± 0.68 e
Salt stress	Columbine	NS	29.6 ± 0.95 a	6.7 ± 0.23 a	1.8 ± 0.19 c	0.78 ± 0.08 d	5.2 ± 0.22 b	1.1 ± 0.11 b
Salt stress	Columbine	SS	28.4 ± 0.85 a	13.6 ± 0.45 c	1.4 ± 0.18 b	0.58 ± 0.05 b	6.6 ± 0.24 c	1.2 ± 0.21 b
Salt stress	Allstarter	NS	24.2 ± 0.72 a	10.7 ± 0.44 b	1.1 ± 0.11 a	0.53 ± 0.04 b	1.8 ± 0.16 a	0.6 ± 0.15 a
Salt stress	Allstarter	SS	23.9 ± 0.66 a	10.2 ± 0.41 b	1.3 ± 0.12 b	0.57 ± 0.08 b	5.0 ± 0.29 b	1.7 ± 0.14 c
Salt stress	Esquire	NS	25.7 ± 0.54 a	6.2 ± 0.32 a	1.2 ± 0.14 ab	0.46 ± 0.06 a	2.7 ± 0.18 a	0.7 ± 0.11 a
Salt stress	Esquire	SS	28.4 ± 0.45 a	10.8 ± 0.31 b	2.1 ± 0.22 d	0.83 ± 0.07 de	4.7 ± 0.27 b	1.7 ± 0.15 c

Data are means ± SEM ($n = 5$). Treatments represent all combinations of salinity (Control and Salt Stress), cultivar (‘Columbine’, ‘Allstarter’, ‘Esquire’), and soil (NS, natural soil and SS, sterile soil). Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey’s HSD test ($p < 0.05$).

Cultivar differences were evident under salinity. ‘Columbine’ generally displayed substantial growth suppression, particularly in root biomass under SS, whereas ‘Esquire’ maintained comparatively higher shoot biomass in SS. Leaf number exhibited inconsistent responses across treatments, confirming that salinity primarily constrained biomass accumulation rather than organ initiation.

Salinity stress also markedly affected root system architecture (Table 9; Figure A9). Root total length declined by 47% in NS-grown ‘Columbine’ (59.1 to 31.3 cm) but increased under SS (55.9 to 71.8 cm), highlighting strong soil-dependent plasticity. In ‘Esquire’, root length decreased by 58% in NS (47.5 to 20.1 cm), whereas SS-grown plants maintained values comparable to control levels (48.8 to 77.9 cm). Root surface area and volume

followed similar trends, with substantial reductions under NS in sensitive cultivars, while SS-grown plants often maintained higher structural parameters. These results indicate that salinity stress induced pronounced but highly soil-dependent modifications of root system expansion.

Table 9. Effects of salinity stress, soil treatment (natural vs. sterile soil), and cultivar on root system architectural traits of *Lolium perenne*.

Treatment			Root Total Length (cm)	Root Surface Area (cm ²)	Root Volume (cm ³)	Root Projected Area (cm ²)	Root Diameter (mm)	Root Tip Number
Stress	Cultivar	Soil						
Control	Columbine	NS	59.1 ± 9.1 c	54.3 ± 8.4 d	17.3 ± 2.2 d	4.1 ± 0.9 b	3.2 ± 0.6 c	863.3 ± 182.1 b
Control	Columbine	SS	55.9 ± 8.4 c	60.9 ± 8.9 d	19.4 ± 2.9 d	6.5 ± 1.3 c	4.0 ± 0.9 d	1205.7 ± 192.3 c
Control	Allstarter	NS	94.1 ± 9.5 e	98.5 ± 9.1 f	12.0 ± 2.7 c	31.4 ± 2.9 e	6.1 ± 1.6 f	2123.3 ± 383.5 d
Control	Allstarter	SS	159.7 ± 11.1 f	84.4 ± 8.0 e	26.9 ± 3.2 f	4.1 ± 0.9 b	2.0 ± 0.4 b	3189.8 ± 469.3 e
Control	Esquire	NS	47.5 ± 7.1 c	77.7 ± 8.8 e	24.7 ± 3.5 ef	11.3 ± 1.5 d	5.8 ± 1.1 f	1294.3 ± 234.3 c
Control	Esquire	SS	48.8 ± 7.5 c	68.1 ± 9.7 de	21.7 ± 2.7 de	9.1 ± 1.3 d	5.1 ± 1.1 e	1164.7 ± 212.7 c
Salt stress	Columbine	NS	31.3 ± 5.1 b	20.3 ± 2.5 b	6.5 ± 0.9 b	1.1 ± 0.4 a	2.0 ± 0.3 b	567.3 ± 156.3 a
Salt stress	Columbine	SS	71.8 ± 9.2 d	33.7 ± 2.8 c	10.7 ± 1.3 c	1.3 ± 0.2 a	1.5 ± 0.2 a	1235.4 ± 198.3 c
Salt stress	Allstarter	NS	62.5 ± 7.2 cd	32.6 ± 2.4 c	1.4 ± 0.2 a	10.4 ± 1.7 d	2.0 ± 0.4 b	945.1 ± 178.2 b
Salt stress	Allstarter	SS	79.4 ± 9.4 d	34.8 ± 2.6 c	11.1 ± 2.1 c	1.3 ± 0.2 a	1.4 ± 0.2 a	1233.8 ± 198.3 c
Salt stress	Esquire	NS	20.1 ± 2.9 a	9.4 ± 1.8 a	3.0 ± 0.5 a	0.4 ± 0.07 a	1.5 ± 0.3 a	318.6 ± 87.3 a
Salt stress	Esquire	SS	77.9 ± 8.8 d	36.7 ± 2.9 c	11.7 ± 2.2 c	1.4 ± 0.1 a	1.5 ± 0.3 a	1333.5 ± 198.3 c

Data are means ± SEM ($n = 5$). Treatments represent all combinations of salinity (Control and Salt Stress), cultivar ('Columbine', 'Allstarter', 'Esquire'), and soil (NS, natural soil and SS, sterile soil). Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey's HSD test ($p < 0.05$).

When morphological and root architectural traits were examined as unilateral effects of individual experimental factors (Figures A8 and A9), salinity stress consistently reduced shoot biomass across cultivars, although root responses were strongly modulated by soil treatment. Soil-dependent contrasts were more variable than under water stress, reflecting complex interactions between osmotic and ionic components of salinity.

3.2.2. Water Status and Photosynthetic Pigments Under Salinity Stress

Salinity stress induced a clear decline in shoot water content in *Lolium perenne*, irrespective of cultivar or soil treatment (Table 10; Figure A10), confirming that aboveground tissues were the primary targets of osmotic imbalance under high salt conditions. In contrast, root water content displayed a more heterogeneous pattern, suggesting that belowground tissues maintained a comparatively greater buffering capacity against osmotic disruption. This divergence between shoot and root responses indicates a decoupled regulation of tissue hydration under salinity, with shoots reflecting the immediate physiological cost of stress.

Soil microbial context modulated plant water status under salinity. Plants grown in natural soil generally maintained higher shoot hydration and more balanced biomass allocation compared with those grown in sterile substrate. Although the magnitude of this effect varied among cultivars, the overall trend suggests that biotic soil components contributed to improved water retention or osmotic adjustment under saline conditions. Notably, root-to-shoot ratios converged toward lower values under salt stress across all treatments, reflecting a marked reduction in relative root biomass investment and a shift in carbon allocation patterns under osmotic constraint.

Genotypic differences were evident but not extreme. 'Esquire' showed a comparatively better maintenance of shoot water content under salinity, whereas 'Columbine' and 'Allstarter' exhibited stronger reductions. Differences in root water content were less distinct, reinforcing the view that salinity primarily compromised shoot hydration rather than root tissue water balance.

Photosynthetic pigment composition mirrored the alterations observed in water status (Table 11; Figure A11). Salinity stress generally reduced chlorophyll a and chlorophyll b concentrations across cultivars, indicating structural and functional perturbations of the

photosynthetic apparatus. Carotenoid levels were comparatively less affected, resulting in substantial shifts in pigment ratios, particularly under severe stress combinations. The decline in total chlorophyll, coupled with relative carotenoid stability, reflects a transition from growth-oriented photosynthetic activity toward protective and antioxidative functions.

Table 10. Effects of salinity stress, soil treatment (natural vs. sterile soil), and cultivar on shoot and root water status and biomass allocation of *Lolium perenne*.

Treatment			Shoot Water Content (%)	Root Water Content (%)	Root-to-Shoot Ratio
Stress	Cultivar	Soil			
Control	Columbine	NS	73.2 ± 4.9 c	48.6 ± 2.7 a	3.9 ± 0.6 c
Control	Columbine	SS	73.1 ± 4.9 c	47.0 ± 2.5 a	7.5 ± 1.8 f
Control	Allstarter	NS	65.1 ± 4.2 b	73.8 ± 4.9 c	3.9 ± 0.4 c
Control	Allstarter	SS	62.6 ± 4.3 b	54.3 ± 3.2 a	3.2 ± 0.9 b
Control	Esquire	NS	73.0 ± 4.9 c	69.4 ± 4.1 bc	4.6 ± 1.8 d
Control	Esquire	SS	75.7 ± 4.4 c	62.8 ± 4.2 b	6.2 ± 0.6 e
Salt stress	Columbine	NS	55.2 ± 3.7 a	76.0 ± 4.9 c	1.38 ± 0.2 a
Salt stress	Columbine	SS	58.7 ± 3.4 ab	77.5 ± 5.1 c	1.32 ± 0.2 a
Salt stress	Allstarter	NS	51.6 ± 3.6 a	64.1 ± 4.5 b	1.20 ± 0.1 a
Salt stress	Allstarter	SS	56.9 ± 3.7 ab	68.6 ± 4.2 bc	1.21 ± 0.1 a
Salt stress	Esquire	NS	61.5 ± 4.1 b	75.6 ± 4.9 c	1.23 ± 0.1 a
Salt stress	Esquire	SS	60.4 ± 4.0 b	62.9 ± 4.1 b	1.04 ± 0.1 a

Data are means ± SEM ($n = 5$). Treatments represent all combinations of salinity (Control and Salt Stress), cultivar ('Columbine', 'Allstarter', 'Esquire'), and soil (NS, natural soil and SS, sterile soil). Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey's HSD test ($p < 0.05$).

Table 11. Effects of salinity stress, soil treatment (natural vs. sterile soil), and cultivar on photosynthetic pigment content and pigment ratios of *Lolium perenne*.

Treatment			Chl <i>a</i> (mg g ⁻¹ DW)	Chl <i>b</i> (mg g ⁻¹ DW)	Car (mg g ⁻¹ DW)	Chl <i>a/b</i> Ratio	TChl/Car Ratio
Stress	Cultivar	Soil					
Control	Columbine	NS	1.6 ± 0.14 c	0.34 ± 0.05 c	0.56 ± 0.07 bc	4.7 ± 0.57 d	3.5 ± 0.41 b
Control	Columbine	SS	1.1 ± 0.09 ab	0.13 ± 0.03 a	0.41 ± 0.03 ab	8.5 ± 0.88 f	3.0 ± 0.33 a
Control	Allstarter	NS	1.6 ± 0.16 c	0.55 ± 0.06 d	0.63 ± 0.06 c	2.9 ± 0.31 c	3.4 ± 0.40 ab
Control	Allstarter	SS	1.4 ± 0.15 b	0.57 ± 0.07 d	0.46 ± 0.05 b	2.5 ± 0.22 b	4.3 ± 0.54 c
Control	Esquire	NS	2.0 ± 0.17 d	0.57 ± 0.08 d	0.71 ± 0.07 d	3.5 ± 0.51 c	3.6 ± 0.38 b
Control	Esquire	SS	2.6 ± 0.28 e	0.85 ± 0.11 f	0.77 ± 0.07 d	3.1 ± 0.50 c	4.5 ± 0.64 e
Salt stress	Columbine	NS	0.9 ± 0.12 a	0.71 ± 0.11 e	0.34 ± 0.04 a	1.3 ± 0.40 a	4.7 ± 0.61 e
Salt stress	Columbine	SS	1.3 ± 0.41 b	0.21 ± 0.02 b	0.47 ± 0.03 b	6.2 ± 0.80 e	3.2 ± 0.55 a
Salt stress	Allstarter	NS	0.8 ± 0.13 a	0.32 ± 0.04 c	0.39 ± 0.03 ab	2.5 ± 0.49 b	2.9 ± 0.45 a
Salt stress	Allstarter	SS	1.1 ± 0.31 b	0.31 ± 0.04 b	0.45 ± 0.04 b	3.5 ± 0.44 c	3.1 ± 0.41 a
Salt stress	Esquire	NS	1.3 ± 0.42 b	0.28 ± 0.03 b	0.50 ± 0.03 b	4.6 ± 0.74 d	3.2 ± 0.39 a
Salt stress	Esquire	SS	1.2 ± 0.31 b	0.20 ± 0.02 b	0.49 ± 0.04 b	6.0 ± 0.81 e	2.9 ± 0.31 a

Data are means ± SEM ($n = 5$). Treatments represent all combinations of salinity (Control and Salt Stress), cultivar ('Columbine', 'Allstarter', 'Esquire'), and soil (NS, natural soil and SS, sterile soil). Chl *a*: chlorophyll *a*; Chl *b*: chlorophyll *b*; Car: carotenoids. Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey's HSD test ($p < 0.05$).

Soil treatment again influenced the magnitude of pigment depletion. Plants grown in natural soil showed cultivar-dependent differences in chlorophyll content and pigment stability between natural and sterile soil treatments, although the buffering effect was less uniform than under water deficit conditions. Among cultivars, 'Esquire' exhibited smaller reductions in chlorophyll content, suggesting a relatively greater stability of the photosynthetic machinery under saline stress, whereas 'Columbine' and 'Allstarter' showed more pronounced pigment loss.

When individual experimental factors were analyzed separately (Figures A10 and A11), salinity emerged as the dominant driver of both shoot dehydration and chlorophyll decline. Soil effects were expressed primarily through improved maintenance of shoot water status and chlorophyll concentration, rather than through major alterations in root hydration or carotenoid content. Overall, the combined patterns indicate that salinity stress disrupts

plant performance at both the hydraulic and photosynthetic levels, with soil biotic context and genotype modulating, but not overriding, the primary osmotic constraint.

3.2.3. Biochemical Stress Markers and Osmoprotectants Under Salinity Stress

Salinity stress elicited a pronounced biochemical reconfiguration in *Lolium perenne*, characterized by the simultaneous activation of osmoprotective mechanisms and intensification of oxidative stress (Table 12; Figure A12). Compared with water deficit, salinity imposed a stronger oxidative constraint, as reflected by the consistent elevation of malondialdehyde and hydrogen peroxide across treatments. This confirms that, beyond osmotic imbalance, ionic toxicity under salt stress amplifies membrane lipid peroxidation and reactive oxygen species (ROS) accumulation.

Table 12. Effects of salinity stress, soil treatment (natural vs. sterile soil), and cultivar on osmoprotectants, antioxidant compounds, and oxidative stress markers in *Lolium perenne*.

Treatment			Pro ($\mu\text{mol g}^{-1}$ DW)	TSS (mg eq. g^{-1} DW)	GB ($\mu\text{mol g}^{-1}$ DW)	TPC (mg eq. GA g^{-1} DW)	TFC (mg eq. C g^{-1} DW)	MDA (nmol g^{-1} DW)	H_2O_2 ($\mu\text{mol g}^{-1}$ DW)
Stress	Cultivar	Soil							
Control	Columbine	NS	1.7 $\pm$ 0.2 a	56.9 $\pm$ 4.2 a	34.0 $\pm$ 2.2 bc	2.5 $\pm$ 0.2 a	2.9 $\pm$ 0.3 b	82.4 $\pm$ 5.7 ab	38.2 $\pm$ 2.4 bc
Control	Columbine	SS	1.2 $\pm$ 0.1 a	84.0 $\pm$ 5.6 c	40.7 $\pm$ 3.1 a	3.0 $\pm$ 0.3 b	2.9 $\pm$ 0.3 b	69.3 $\pm$ 6.3 a	18.2 $\pm$ 1.5 a
Control	Allstarter	NS	2.0 $\pm$ 0.3 a	65.2 $\pm$ 4.9 b	20.4 $\pm$ 2.6 d	3.4 $\pm$ 0.3 c	3.3 $\pm$ 0.3 c	96.6 $\pm$ 7.6 cb	52.3 $\pm$ 2.4 d
Control	Allstarter	SS	1.8 $\pm$ 0.2 a	67.1 $\pm$ 4.7 b	12.3 $\pm$ 1.2 c	2.6 $\pm$ 0.2 a	2.2 $\pm$ 0.2 a	72.2 $\pm$ 4.2 a	39.3 $\pm$ 1.8 c
Control	Esquire	NS	2.8 $\pm$ 0.3 a	83.7 $\pm$ 6.1 c	23.7 $\pm$ 2.4 d	3.6 $\pm$ 0.3 c	3.5 $\pm$ 0.3 c	154.0 $\pm$ 8.7 d	54.0 $\pm$ 2.7 d
Control	Esquire	SS	2.1 $\pm$ 0.2 a	93.9 $\pm$ 5.9 d	30.4 $\pm$ 3.0 f	4.7 $\pm$ 0.4 d	4.7 $\pm$ 0.4 d	109.2 $\pm$ 5.3 c	78.1 $\pm$ 2.9 f
Salt stress	Columbine	NS	35.7 $\pm$ 2.4 c	67.9 $\pm$ 4.7 b	33.1 $\pm$ 3.2 b	3.5 $\pm$ 0.6 c	2.8 $\pm$ 0.6 b	87.4 $\pm$ 5.9 b	27.2 $\pm$ 2.8 b
Salt stress	Columbine	SS	28.8 $\pm$ 2.2 c	80.4 $\pm$ 5.9 c	27.5 $\pm$ 2.8 bc	3.1 $\pm$ 0.4 b	3.0 $\pm$ 0.5 c	92.0 $\pm$ 6.1 b	35.7 $\pm$ 3.2 bc
Salt stress	Allstarter	NS	58.7 $\pm$ 3.8 d	68.1 $\pm$ 4.8 b	15.1 $\pm$ 1.5 d	2.5 $\pm$ 0.3 a	2.7 $\pm$ 0.4 b	92.8 $\pm$ 6.2 b	54.6 $\pm$ 5.1 d
Salt stress	Allstarter	SS	26.5 $\pm$ 2.4 c	58.7 $\pm$ 5.1 a	15.8 $\pm$ 1.4 c	2.6 $\pm$ 0.5 a	2.4 $\pm$ 0.3 a	94.4 $\pm$ 6.3 b	41.5 $\pm$ 4.7 c
Salt stress	Esquire	NS	64.0 $\pm$ 4.1 d	94.4 $\pm$ 5.9 d	11.2 $\pm$ 1.3 c	3.0 $\pm$ 0.4 b	3.2 $\pm$ 0.6 c	99.7 $\pm$ 6.5 cb	40.7 $\pm$ 4.2 c
Salt stress	Esquire	SS	16.0 $\pm$ 1.7 b	91.5 $\pm$ 5.8 d	19.9 $\pm$ 1.6 e	3.7 $\pm$ 0.6 c	3.4 $\pm$ 0.4 c	82.5 $\pm$ 5.9 ab	65.4 $\pm$ 4.6 e

Data are means $\pm$ SEM ($n = 5$). Treatments represent all combinations of salinity (Control and Salt Stress), cultivar ('Columbine', 'Allstarter', 'Esquire'), and soil (NS, natural soil and SS, sterile soil). Pro: proline; TSS: total soluble sugars; GB: glycine betaine; TPC: total phenolics content; TFC: total flavonoids content; MDA: malondialdehyde; H_2O_2 : hydrogen peroxide. Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey's HSD test ($p < 0.05$).

Soil microbial context significantly modulated these responses. Plants grown in natural soil showed cultivar-dependent differences in oxidative stress marker accumulation between natural and sterile soil treatments, indicating partial mitigation of salt-induced oxidative damage. This buffering effect was particularly evident for hydrogen peroxide, whereas malondialdehyde accumulation showed stronger genotype dependence. The pattern suggests that soil biota may enhance antioxidant regulation or reduce ROS overproduction under saline conditions, although the magnitude of this effect varied among cultivars.

Osmoprotectant accumulation followed a distinct and robust trend. Proline increased markedly across cultivars, whereas total soluble sugars showed variable responses depending on cultivar and soil treatment, indicating active osmotic adjustment. In several cultivar–soil combinations, proline accumulation exceeded levels observed under water stress, emphasizing its central role in salinity tolerance. In contrast, soluble sugar responses were more genotype-dependent, suggesting differential carbon reallocation strategies under ionic stress. Glycine betaine displayed moderate variation, contributing to osmotic balance but not emerging as the dominant compatible solute in this system.

Cultivar-specific biochemical strategies became evident when osmolyte accumulation was considered alongside oxidative markers. 'Esquire' and 'Allstarter' generally combined strong proline induction with comparatively moderate increases in malondialdehyde and hydrogen peroxide, particularly in natural soil. This coordinated response indicates a more efficient coupling between osmotic adjustment and oxidative protection. Conversely, 'Columbine' exhibited less consistent osmolyte accumulation and relatively higher oxidative

stress marker levels in sterile soil, suggesting greater biochemical sensitivity to salinity in the absence of microbial buffering.

Antioxidant-related compounds, including total phenolics and flavonoids, contributed to this differential response pattern. Although their variation was less dramatic than that of proline, plants grown in natural soil tended to maintain higher antioxidant compound levels, supporting enhanced ROS scavenging capacity under saline conditions.

When analyzed as unilateral effects (Figure A12), salinity emerged as the dominant driver of biochemical alteration, significantly increasing proline, soluble sugars, malondialdehyde, and hydrogen peroxide across cultivars. Soil effects were primarily expressed through attenuation of oxidative damage rather than through drastic shifts in osmolyte pools. Overall, the data indicate that salinity stress in *L. perenne* involves a tightly interconnected response network, where osmotic adjustment, antioxidant metabolism, and membrane stability are simultaneously regulated, and where genotype and soil biotic context modulate the efficiency (but not the direction) of this stress adaptation strategy.

3.2.4. Mineral Ion Accumulation and Ionic Balance Under Salinity Stress

Salinity stress induced profound disturbances in mineral ion homeostasis in *Lolium perenne*, leading to marked accumulation of Na^+ and Cl^- in both leaves and roots and a concomitant reduction in K^+/Na^+ selectivity (Tables 13 and 14; Figures A13 and A14). Unlike water deficit, where osmotic limitation predominated, salinity imposed an additional ionic constraint that became the dominant physiological driver, largely overriding moderate genotype-dependent variation.

Table 13. Effects of salinity stress, soil treatment (natural vs. sterile soil), and cultivar on leaf mineral ion concentrations and K^+/Na^+ selectivity in *Lolium perenne*.

Treatment			Na^+ ($\mu\text{mol g}^{-1}$ DW)	K^+ ($\mu\text{mol g}^{-1}$ DW)	Ca^{2+} ($\mu\text{mol g}^{-1}$ DW)	Cl^- ($\mu\text{mol g}^{-1}$ DW)
Stress	Cultivar	Soil				
Control	Columbine	NS	2175.2 $\pm$ 292.3 c	7111.6 $\pm$ 792.3 d	143.4 $\pm$ 7.8 a	606.6 $\pm$ 25.2 a
Control	Columbine	SS	1645.0 $\pm$ 254.4 b	6955.8 $\pm$ 712.8 d	149.1 $\pm$ 7.9 a	674.9 $\pm$ 27.1 a
Control	Allstarter	NS	1113.5 $\pm$ 150.6 a	5131.0 $\pm$ 602.1 b	133.3 $\pm$ 5.6 a	454.9 $\pm$ 20.7 a
Control	Allstarter	SS	1845.0 $\pm$ 291.3 b	6222.5 $\pm$ 701.3 c	140.6 $\pm$ 6.1 a	680.9 $\pm$ 28.8 a
Control	Esquire	NS	2550.2 $\pm$ 272.6 c	9064.3 $\pm$ 852.6 e	129.3 $\pm$ 5.8 a	811.3 $\pm$ 29.7 a
Control	Esquire	SS	1746.2 $\pm$ 227.5 b	7341.3 $\pm$ 772.8 d	102.7 $\pm$ 4.5 a	674.1 $\pm$ 26.2 a
Salt stress	Columbine	NS	6476.1 $\pm$ 701.6 e	4532.2 $\pm$ 425.6 b	465.7 $\pm$ 36.5 c	2355.2 $\pm$ 344.2 cd
Salt stress	Columbine	SS	6834.9 $\pm$ 658.5 e	5448.4 $\pm$ 439.2 c	439.9 $\pm$ 32.2 c	2053.8 $\pm$ 301.2 c
Salt stress	Allstarter	NS	9267.8 $\pm$ 841.3 g	2970.1 $\pm$ 275.6 a	479.4 $\pm$ 34.9 c	2345.9 $\pm$ 311.2 cd
Salt stress	Allstarter	SS	4421.2 $\pm$ 425.2 d	3856.0 $\pm$ 322.1 ab	276.4 $\pm$ 9.8 b	1390.8 $\pm$ 45.1 b
Salt stress	Esquire	NS	7839.9 $\pm$ 688.2 f	4727.0 $\pm$ 429.2 b	473.0 $\pm$ 35.4 c	2452.4 $\pm$ 255.1 cd
Salt stress	Esquire	SS	9917.1 $\pm$ 856.2 g	5839.1 $\pm$ 425.2 c	254.0 $\pm$ 9.7 b	1272.9 $\pm$ 34.2 b

Data are means $\pm$ SEM ($n = 5$). Treatments represent all combinations of salinity (Control and Salt Stress), cultivar ('Columbine', 'Allstarter', 'Esquire'), and soil (NS, natural soil and SS, sterile soil). Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey's HSD test ($p < 0.05$).

In leaf tissues, all cultivars exhibited a strong increase in Na^+ and Cl^- concentrations under salt stress, accompanied by a consistent decline in K^+ levels. Although absolute concentrations differed among genotypes, the uniform direction of change indicates a limited capacity to restrict Na^+ translocation to shoots. The resulting decline in K^+/Na^+ balance reflects impaired ionic selectivity and competitive inhibition of K^+ uptake, with direct consequences for enzymatic activity and stomatal regulation. Plants grown in natural soil showed cultivar-dependent variation in leaf Na^+ accumulation between natural and sterile soil treatments, suggesting partial modulation of shoot ionic balance by soil biotic context; however, this buffering effect remained quantitative rather than qualitative.

Root ion profiles revealed intense ionic pressure at the soil–root interface. Under salinity, root Na^+ and Cl^- concentrations increased sharply, while K^+ levels declined in

most cultivar–soil combinations. Genotypic differences were more visible at the root level: ‘Esquire’ showed comparatively lower Na^+ accumulation in certain treatments, whereas ‘Columbine’ exhibited higher Na^+ loads, and ‘Allstarter’ displayed intermediate behavior. Nevertheless, even cultivars with relatively moderate Na^+ accumulation experienced substantial ionic imbalance, confirming the severity of the imposed stress.

Table 14. Effects of salinity stress, soil treatment (natural vs. sterile soil), and cultivar on root mineral ion concentrations of *Lolium perenne*.

Treatment			Na^+	K^+	Ca^{2+}	Cl^-
Stress	Cultivar	Soil	($\mu\text{mol g}^{-1} \text{DW}$)	($\mu\text{mol g}^{-1} \text{DW}$)	($\mu\text{mol g}^{-1} \text{DW}$)	($\mu\text{mol g}^{-1} \text{DW}$)
Control	Columbine	NS	2724.2 $\pm$ 351.2 a	2403.0 $\pm$ 324.2 cd	135.3 $\pm$ 6.5 b	448.3 $\pm$ 37.7 a
Control	Columbine	SS	4147.1 $\pm$ 558.5 a	2204.8 $\pm$ 311.2 c	146.9 $\pm$ 6.9 b	373.9 $\pm$ 18.9 a
Control	Allstarter	NS	3815.6 $\pm$ 421.2 a	2995.2 $\pm$ 371.1 e	159.7 $\pm$ 6.7 b	554.0 $\pm$ 44.2 a
Control	Allstarter	SS	3410.4 $\pm$ 400.8 a	2629.7 $\pm$ 321.2 d	99.5 $\pm$ 4.5 a	341.0 $\pm$ 20.7 a
Control	Esquire	NS	3759.6 $\pm$ 422.5 a	2627.2 $\pm$ 326.4 d	91.3 $\pm$ 3.9 a	422.9 $\pm$ 50.5 a
Control	Esquire	SS	2306.5 $\pm$ 311.2 a	2582.0 $\pm$ 357.2 d	71.5 $\pm$ 4.5 a	331.1 $\pm$ 42.6 a
Salt stress	Columbine	NS	12,198.0 $\pm$ 1652.2 d	1441.9 $\pm$ 245.2 b	275.9 $\pm$ 15.3 c	1264.7 $\pm$ 159.3 b
Salt stress	Columbine	SS	4882.7 $\pm$ 498.2 b	1115.2 $\pm$ 152.2 a	290.5 $\pm$ 15.9 c	1300.0 $\pm$ 162.3 b
Salt stress	Allstarter	NS	8234.5 $\pm$ 752.2 c	1189.0 $\pm$ 144.2 a	410.1 $\pm$ 34.6 d	2006.4 $\pm$ 193.2 d
Salt stress	Allstarter	SS	7510.6 $\pm$ 688.3 c	2098.8 $\pm$ 325.6 c	303.8 $\pm$ 32.1 c	1542.6 $\pm$ 169.3 c
Salt stress	Esquire	NS	25,305.4 $\pm$ 321.2 e	2387.5 $\pm$ 365.2 cd	341.2 $\pm$ 36.2 c	1865.0 $\pm$ 178.5 d
Salt stress	Esquire	SS	5274.7 $\pm$ 445.2 b	2717.2 $\pm$ 329.3 de	416.6 $\pm$ 34.1 d	1589.3 $\pm$ 164.2 c

Data are means $\pm$ SEM ($n = 5$). Treatments represent all combinations of salinity (Control and Salt Stress), cultivar (‘Columbine’, ‘Allstarter’, ‘Esquire’), and soil (NS, natural soil and SS, sterile soil). Different lowercase letters within a column indicate statistically significant differences among treatment combinations according to Tukey’s HSD test ($p < 0.05$).

Soil treatment influenced root ionic composition in a direction similar to that observed in leaves. Plants grown in natural soil tended to maintain lower Na^+ concentrations and more stable K^+ levels compared with those in sterile soil. This suggests that soil microbial communities may contribute to improved ion regulation, possibly through modulation of rhizosphere chemistry or enhanced root membrane functionality. However, these effects did not fully counteract the strong ionic influx under high salinity.

When ionic traits were analyzed as unilateral effects (Figures A13 and A14), salinity clearly emerged as the overriding factor, consistently driving Na^+ and Cl^- accumulation and depressing K^+/Na^+ ratios in both shoots and roots. Soil and cultivar effects were expressed mainly through relative differences in ion magnitude rather than through changes in response direction. Collectively, these results demonstrate that under saline conditions, disruption of ionic homeostasis constitutes the central physiological constraint in *L. perenne*, interacting with osmotic stress, oxidative imbalance, pigment degradation, and altered biomass allocation described in the preceding sections.

3.3. Multivariate Integration of Trait Responses Under Water and Salinity Stress

Hierarchical clustering analysis based on Euclidean distance provided an integrated overview of plant responses to water stress (Figure 1a). The dendrogram and associated heat map revealed a clear separation between control and water-stressed plants, indicating a coordinated shift in morphological, physiological, biochemical, and ionic traits under water deficit conditions. Parameters related to osmolyte accumulation and oxidative stress tended to cluster together, whereas growth- and water-status-related traits formed distinct groups, highlighting stress-specific trait associations. Cultivar- and soil-dependent patterns were also evident, suggesting differential integration of response mechanisms under water-limited conditions.

Under salinity stress, hierarchical clustering analysis revealed a distinct multivariate response pattern compared with water stress (Figure 1b). Treatments were clearly separated according to salinity regime, with strong clustering driven by ion-related traits, particularly

Na^+ and Cl^- accumulation and K^+/Na^+ ratio. Biochemical stress markers and osmoprotectants formed tightly associated clusters, reflecting the pronounced ionic and oxidative constraints imposed by salinity. Compared with water stress, salinity stress induced a more compact clustering structure, indicating stronger coupling among stress-response traits.

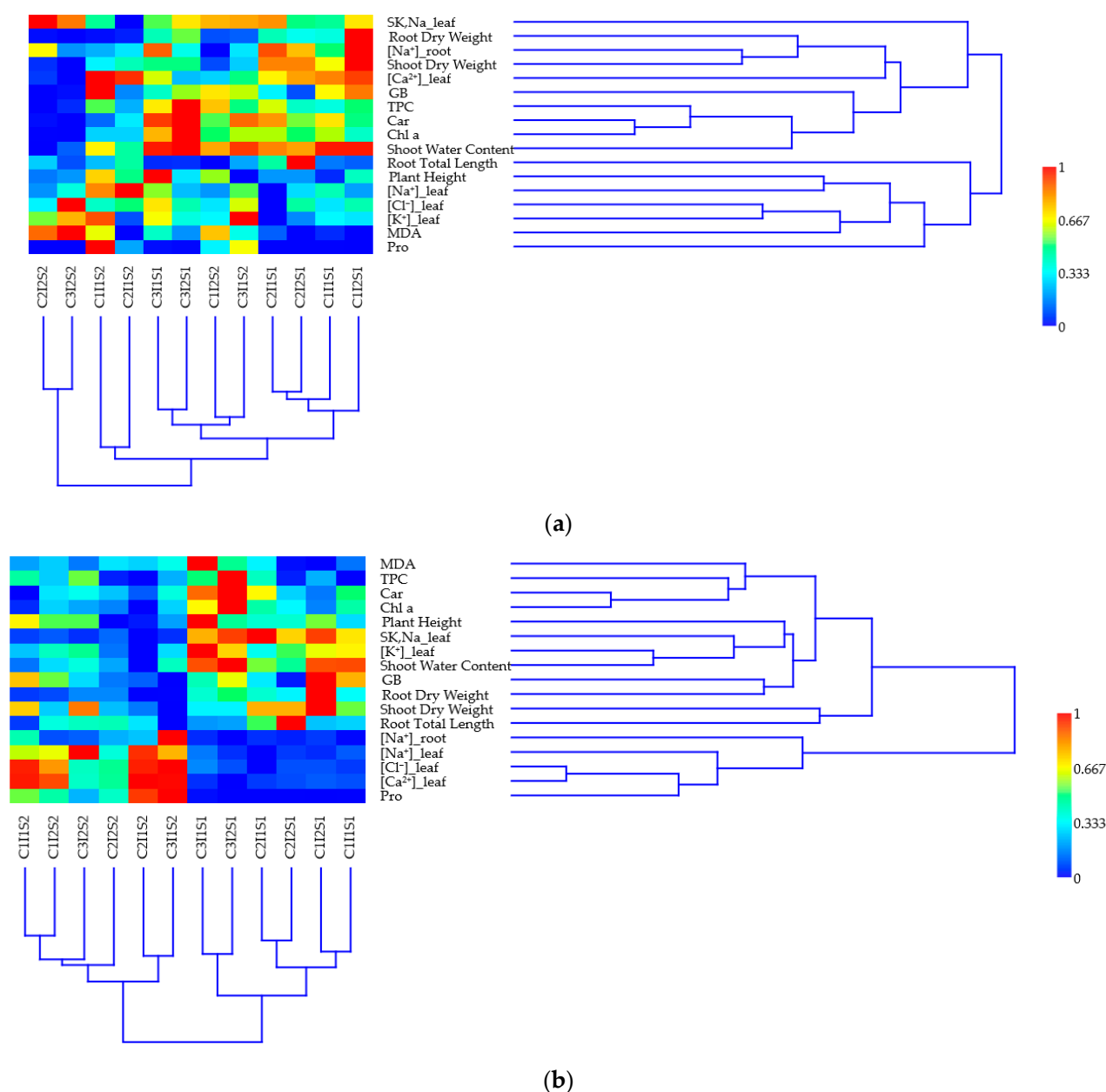


Figure 1. Hierarchical clustering (UPGMA) and heat map based on Euclidean distance illustrating multivariate trait associations and treatment grouping of *Lolium perenne* under (a) water stress and (b) salinity stress. Data were normalized prior to analysis. Abbreviations: Pro, proline; GB, glycine betaine; TPC, total phenolic content; MDA, malondialdehyde; Chl a, chlorophyll a; Car, carotenoids; Na^+ , sodium; K^+ , potassium; Ca^{2+} , calcium; Cl^- , chloride. K/Na leaf, leaf K^+/Na^+ ratio.

4. Discussion

The results reveal that although both water deficit and salinity imposed significant constraints on plant growth and physiology, the underlying drivers and the degree of trait integration differed fundamentally between the two stress types. Water stress primarily exerted an osmotic limitation that affected biomass accumulation and plant water status, while salinity introduced an additional ionic component that amplified oxidative imbalance

and disrupted ion homeostasis. Across trait categories, ionic disequilibrium under salinity emerged as the central constraint linking Na^+ and Cl^- accumulation with reduced K^+ selectivity, enhanced reactive oxygen species production, pigment destabilization, and altered biomass allocation. In contrast, responses to water deficit reflected a more plastic adjustment involving root architectural modulation and osmoprotectant accumulation. Soil treatment and cultivar identity consistently modulated the magnitude of these responses, but not their overall direction, highlighting differences in regulatory efficiency rather than in stress perception. These findings suggest that salinity imposed a multi-layered constraint, where ionic disequilibrium acted as a primary driver amplifying osmotic and oxidative stress components.

4.1. Comparative Plant Responses to Water Stress and Salinity Stress

Building upon the integrative patterns described above, the comparison between drought and salinity responses in *L. perenne* highlights clear differences in the hierarchy and coordination of stress-related traits. Although both stresses reduced growth and altered physiological performance, salinity induced a more complex response pattern due to the additional impact of ionic toxicity alongside osmotic limitation [22,57,58].

4.1.1. Growth Limitation and Biomass Allocation Under Contrasting Stresses

Both water deficit and salinity significantly reduced shoot and root growth; however, the patterns of biomass reduction revealed important differences. Under water stress, shoot growth was generally more affected than root growth, leading to altered biomass allocation patterns and, in some cases, increased root-to-shoot ratios. This response is commonly interpreted as an adaptive strategy aimed at enhancing soil exploration and water uptake under limited water availability [59–61].

In contrast, salinity caused a more uniform suppression of both shoot and root growth, with pronounced reductions in total biomass accumulation. Unlike drought, salinity imposes not only osmotic constraints but also ionic toxicity, which limits the effectiveness of compensatory biomass allocation strategies [62,63]. The more severe restriction of root system development observed under salt stress suggests a reduced capacity for belowground adjustment when ionic stress co-occurs. The stronger growth limitation under salinity compared with drought likely reflects the combined metabolic cost of osmotic adjustment and ion detoxification mechanisms.

4.1.2. Root System Architecture as a Key Differentiator Between Stresses

Root system architectural traits responded differently to water deficit and salinity. Under drought, reductions in root length, surface area, and branching were evident but relatively moderate, particularly in plants grown in natural soil. This pattern suggests structural plasticity that allows partial maintenance of root exploration capacity under limited water availability [64,65].

In contrast, salinity imposed stronger constraints on root system development, with pronounced decreases in root length, surface area, and tip number across cultivars. Beyond osmotic limitation, ionic toxicity at the root apex may impair meristematic activity and lateral root initiation, thereby restricting both elongation and branching processes [62,66,67]. These contrasting architectural responses emphasize that root trait plasticity under drought primarily reflects adaptive reallocation, whereas under salinity, it is constrained by disrupted ionic homeostasis.

4.1.3. Water Status Regulation Under Water Stress Versus Salinity

Despite superficial similarities in reduced shoot water content under both stress types, the underlying mechanisms and physiological implications differ substantially. Under

drought, declines in shoot water content were accompanied by relatively stable root hydration, suggesting coordinated regulation of water uptake and internal redistribution [68–70]. This pattern reflects classical acclimation processes, including stomatal control and osmotic adjustment. In contrast, salinity resulted in a sharper decline in shoot water content, while root hydration showed greater variability. Beyond osmotic effects at the root–soil interface, ionic imbalance may impair hydraulic conductivity and disrupt water transport pathways [62,63]. The limited capacity to sustain shoot hydration under salinity further supports the interpretation that ionic stress amplifies the physiological burden beyond that imposed by water deficit alone.

4.1.4. Photosynthetic Pigment Stability and Stress Specificity

Photosynthetic pigment responses further distinguished drought from salinity. Under water deficit, chlorophyll reductions were moderate and frequently accompanied by relatively stable carotenoid levels, resulting in adjusted pigment ratios indicative of photoprotective acclimation rather than structural damage [71,72].

In contrast, salinity caused more pronounced chlorophyll decline and stronger shifts in pigment ratios, reflecting greater destabilization of the photosynthetic apparatus. While carotenoid stability under both stresses supports their protective role, the sharper chlorophyll loss under salinity likely results from the combined effects of ionic imbalance and enhanced oxidative pressure [62,63,71,72], increasing the susceptibility to photoinhibition.

4.1.5. Biochemical Adjustment and Oxidative Stress: Drought Versus Salinity

Both stresses triggered the accumulation of osmoprotectants such as proline and soluble sugars, consistent with their roles in osmotic adjustment and stress mitigation [63,73–75]. However, salinity was associated with a stronger increase in oxidative stress markers, including malondialdehyde and hydrogen peroxide, compared with drought.

This divergence indicates that salinity imposes a higher oxidative burden, likely due to ion-induced disruption of cellular homeostasis and enhanced reactive oxygen species production [62,76]. The concomitant increase in antioxidant compounds under high salinity suggests that osmotic adjustment alone is insufficient to counteract stress effects, requiring intensified redox regulation relative to water deficit.

4.1.6. Ionic Homeostasis as the Defining Feature of Salinity Stress

The most striking contrast between water deficit and salinity emerged at the level of mineral ion accumulation. While drought caused only moderate shifts in ion concentrations, high salinity resulted in substantial Na^+ and Cl^- accumulation in both roots and leaves, accompanied by a pronounced decline in K^+/Na^+ ratios.

Maintenance of ionic homeostasis, particularly K^+ retention and Na^+ exclusion, is widely recognized as a critical determinant of salt tolerance [62,77,78]. The cultivar-dependent differences observed in leaf and root ion profiles therefore reflect variation in ionic regulation capacity, a mechanism that remains marginal under water deficit but becomes central under salinity. Disruption of ionic balance not only constrains nutrient selectivity but also amplifies osmotic stress and oxidative pressure, reinforcing its role as the defining feature of salinity-induced damage [79].

4.1.7. Effects of Soil Treatment (Natural vs. Sterile) Across Stress Types

Across both water stress and salt stress, plants grown in natural soil consistently exhibited improved performance in terms of growth, water status, biochemical balance, and ionic regulation. However, the nature of this benefit differed between stresses. Under water stress, natural soil primarily enhanced root development and water status maintenance,

whereas under salinity stress, it played a crucial role in limiting Na^+ accumulation and preserving K^+/Na^+ ratios [65,80,81].

These findings are consistent with a potential contribution of soil-associated biotic factors to stress modulation, as inferred from the contrasting responses between natural (NS) and sterile (SS) soil treatments [82,83].

4.2. Integration of Traits and the Need for Multivariate Analysis

While univariate analyses provided clear insights into individual trait responses, the complexity of plant responses to water and salinity stress underscores the need for multivariate approaches [84–86]. Hierarchical clustering analysis provided an integrated overview of coordinated trait responses associated with stress tolerance, cultivar differentiation, and the contrasting soil treatments (NS vs. SS). The separation of treatments and trait groupings observed in the dendrograms highlights contrasting multivariate configurations under water stress and salinity stress, emphasizing the differential contribution of morphological, physiological, biochemical, and ionic traits to stress adaptation. These multivariate patterns complement the univariate analyses by summarizing complex trait interactions without introducing additional analytical assumptions.

The present study was conducted under controlled greenhouse conditions to enable precise differentiation between osmotic (drought) and combined osmotic–ionic (salinity) stress hierarchies. While independent temporal repetition or multi-season field validation may further strengthen extrapolation to broader agronomic contexts, the replicated design applied here provides mechanistically robust inference within the defined experimental framework.

5. Conclusions

Water deficit and salinity induced distinct but partially overlapping response patterns in *Lolium perenne*. While drought primarily imposed osmotic limitation affecting biomass accumulation and plant water status, salinity introduced an additional ionic component that amplified oxidative stress and disrupted mineral homeostasis. Ionic imbalance, reflected by Na^+ accumulation and reduced K^+/Na^+ ratios, emerged as the defining feature of salinity-induced damage and a central determinant of stress severity.

Across stress types, coordinated changes in morphological, physiological, biochemical, and ionic traits revealed hierarchical response patterns, with salinity driving tighter integration among ion regulation, redox balance, and growth limitation. Soil microbial context modulated the magnitude of stress effects, particularly by improving root development under drought and ionic regulation under salinity, without altering the overall direction of plant responses.

Genotypic differences were expressed primarily in regulatory efficiency rather than in stress-specific pathways, highlighting intraspecific variability in adaptive capacity. These findings provide an integrated physiological framework for understanding stress adaptation in turfgrasses and support targeted cultivar selection and soil management strategies under increasing drought and salinity pressures. The results provide mechanistic insight under controlled greenhouse conditions and establish a foundation for future multi-environment validation in turfgrass systems.

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O.V. and A.F.S.; funding acquisition, L.C., M.B. and A.F.S. All authors have read and agreed to the published version of the manuscript.

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Appendix A. Interaction Plots and Unilateral Effects

The following figures present detailed interaction plots and unilateral effects supporting the tabulated results discussed in the main text.

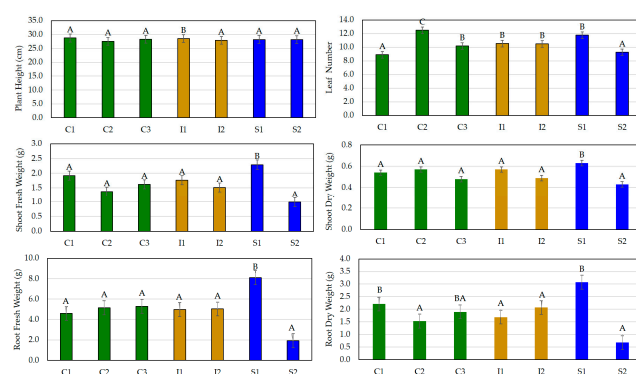


Figure A1. Unilateral effects of cultivar, soil treatment, and water stress on the analyzed traits of *Lolium perenne*. Bars represent mean values $\pm$ SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and water regime (Control S1 vs. Water Stress S2) on the analyzed parameters. Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

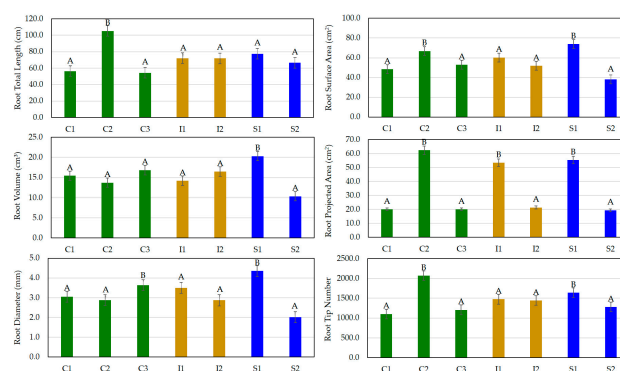


Figure A2. Unilateral effects of cultivar, soil treatment, and water stress on root architectural traits of *Lolium perenne*. Bars represent mean values $\pm$ SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and water regime (Control S1 vs. Water Stress S2) on selected root architectural parameters. Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

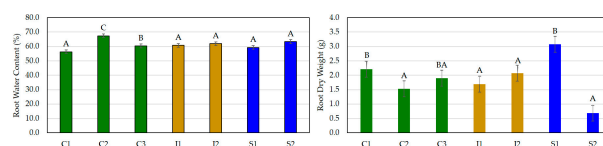


Figure A3. Unilateral effects of cultivar, soil treatment, and water stress on root parameters of *Lolium perenne*. Bars represent mean values $\pm$ SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and water regime (Control S1 vs. Water Stress S2) on root parameters. Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

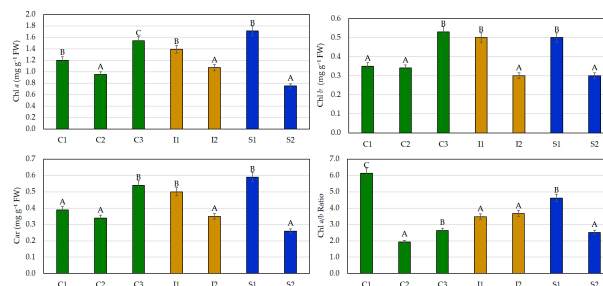


Figure A4. Unilateral effects of cultivar, soil treatment, and water stress on photosynthetic pigments of *Lolium perenne*. Bars represent mean values $\pm$ SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and water regime (Control S1 vs. Water Stress S2) on photosynthetic pigment parameters, including chlorophyll a, chlorophyll b, total carotenoids, and chlorophyll a/b ratio. Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

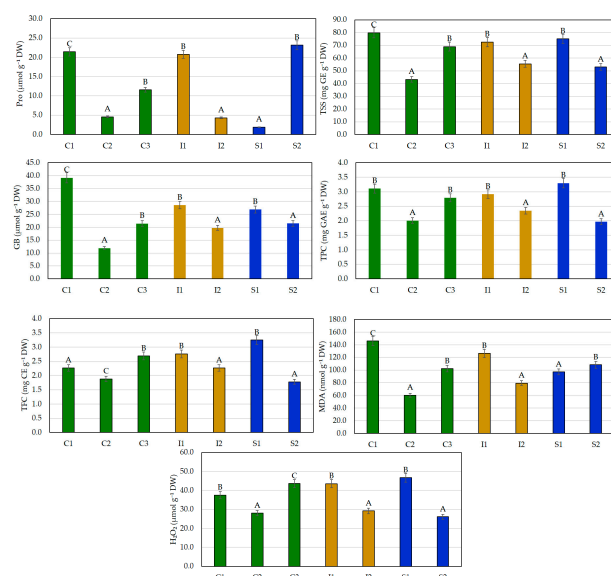


Figure A5. Unilateral effects of water stress, soil treatment, and cultivar on osmoprotectants, antioxidant compounds, and oxidative stress markers of *Lolium perenne*. Bars represent mean values $\pm$ SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and water regime (Control S1 vs. Water Stress S2) on biochemical stress-related parameters, including proline (Pro), total soluble sugars (TSS), glycine betaine (GB), total phenolic content (TPC), total flavonoid content (TFC), malondialdehyde (MDA), and hydrogen peroxide (H₂O₂). Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

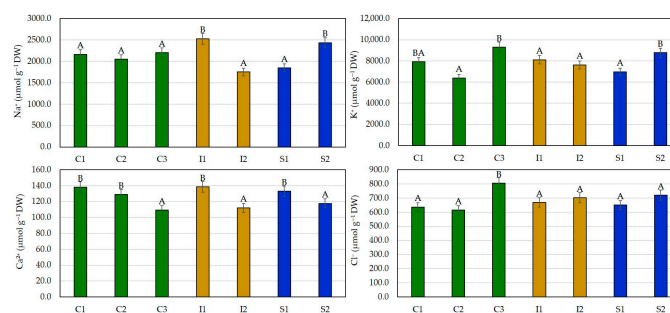


Figure A6. Unilateral effects of cultivar, soil treatment, and water stress on mineral ion concentrations in leaves of *Lolium perenne* cultivars. Bars represent mean values ± SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and water regime (Control S1 vs. Water Stress S2) on leaf Na⁺, K⁺, Ca²⁺, and Cl⁻ concentrations. Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

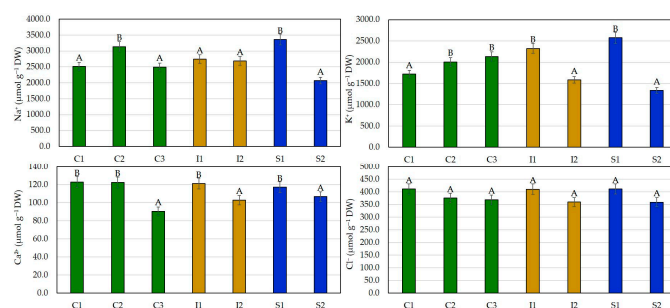


Figure A7. Unilateral effects of water stress, soil treatment, and cultivar on root mineral ion concentrations of *Lolium perenne*. Bars represent mean values ± SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and water regime (Control S1 vs. Water Stress S2) on root Na⁺, K⁺, Ca²⁺, and Cl⁻ concentrations. Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

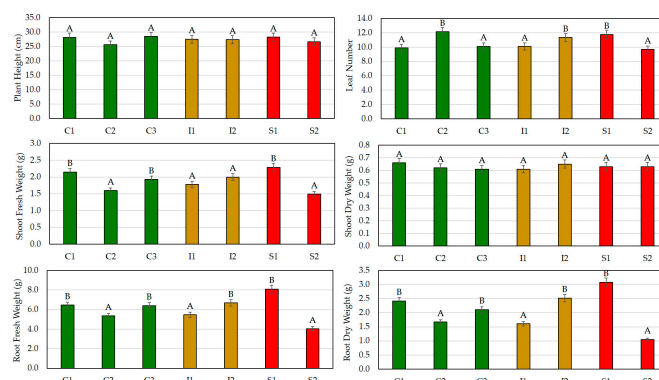


Figure A8. Unilateral effects of salinity stress, soil treatment, and cultivar on shoot and root traits of *Lolium perenne*. Bars represent mean values ± SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and salinity (Control S1 vs. Salt Stress S2) on shoot and root parameters, including plant height, leaf number, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight. Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

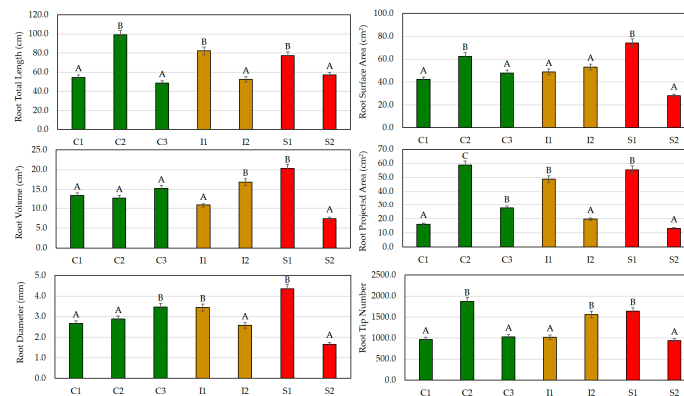


Figure A9. Unilateral effects of salinity stress, soil treatment, and cultivar on root system architectural traits of *Lolium perenne*. Bars represent mean values $\pm$ SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and salinity (Control S1 vs. Salt Stress S2) on root system architectural parameters quantified by image analysis, including root total length, root surface area, root volume, root projected area, root average diameter, and root tip number. Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

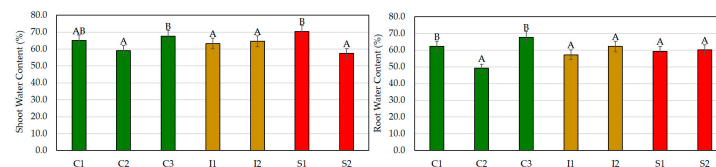


Figure A10. Unilateral effects of salinity stress, soil treatment, and cultivar on shoot and root water content of *Lolium perenne*. Bars represent mean values $\pm$ SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and salinity (Control S1 vs. Salt Stress S2) on shoot water content (SWC) and root water content (RWC). Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

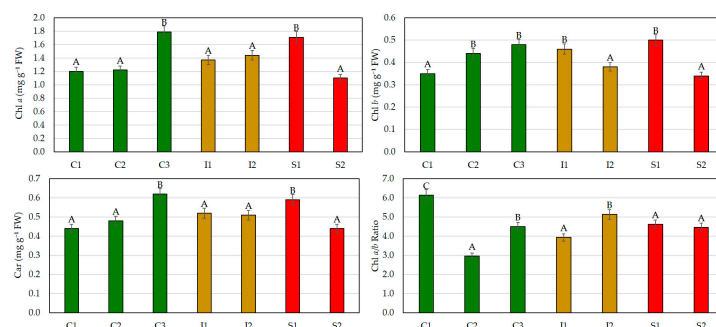


Figure A11. Unilateral effects of salinity stress, soil treatment, and cultivar on photosynthetic pigment content and pigment ratios of *Lolium perenne*. Bars represent mean values $\pm$ SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and salinity (Control S1 vs. Salt Stress S2) on photosynthetic pigment parameters, including chlorophyll *a*, chlorophyll *b*, total carotenoids, and chlorophyll *a/b* ratio. Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

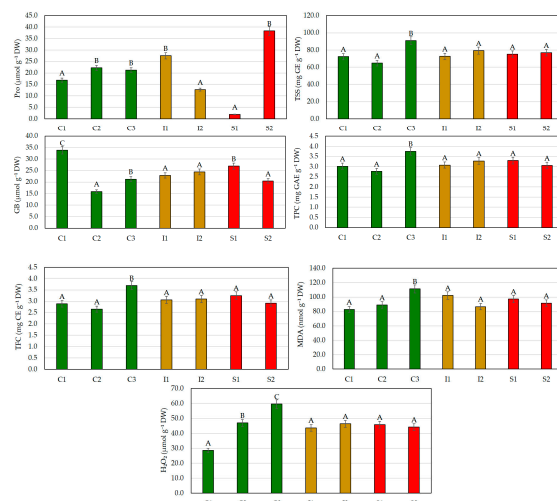


Figure A12. Unilateral effects of salinity stress, soil treatment, and cultivar on osmoprotectants, antioxidant compounds, and oxidative stress markers of *Lolium perenne*. Bars represent mean values $\pm$ SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and salinity (Control S1 vs. Salt Stress S2) on biochemical stress-related parameters, including proline (Pro), total soluble sugars (TSS), glycine betaine (GB), total phenolic content (TPC), total flavonoid content (TFC), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2). Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

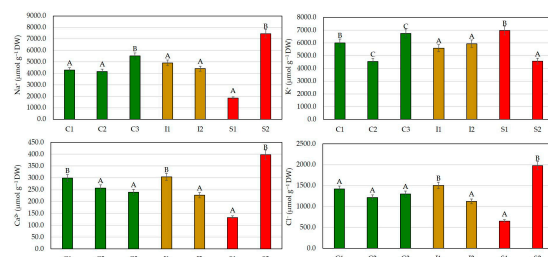


Figure A13. Unilateral effects of salinity stress, soil treatment, and cultivar on leaf mineral ion concentrations of *Lolium perenne*. Bars represent mean values $\pm$ SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and salinity (Control S1 vs. Salt Stress S2) on leaf Na^+ , K^+ , Ca^{2+} , and Cl^- concentrations. Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

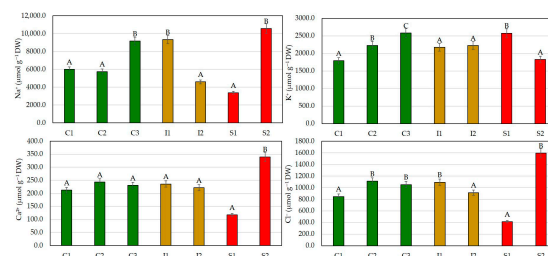


Figure A14. Unilateral effects of salinity stress, soil treatment, and cultivar on root mineral ion concentrations of *Lolium perenne*. Bars represent mean values $\pm$ SEM ($n = 5$). The figure illustrates the main effects of cultivar ('Columbine', C1; 'Allstarter', C2; and 'Esquire', C3), soil (Natural soil, I1; Sterile soil, I2), and salinity (Control S1 vs. Salt Stress S2) on root Na^+ , K^+ , Ca^{2+} , and Cl^- concentrations. Experimental factors and their levels are indicated by bar grouping and symbols within each panel. Different uppercase letters above bars indicate statistically significant differences among levels of the same factor according to Tukey's HSD test ($p < 0.05$).

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