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Article

Comparative Analysis of Lettuce Morphological and Physiological Traits: Effects of Cultivar, Biofertiliser, and Seasonal Variations in Different Soil Types

Milica Stojanović [1,*], Zoran Dinić [2], Jelena Dragišić Maksimović [1], Vuk Maksimović [1], Zorica Jovanović [3], Đorđe Moravčević [3] and Slađana Savić [4]

[1] Institute for Multidisciplinary Research, University of Belgrade, 11030 Belgrade, Serbia; draxy@imsi.bg.ac.rs (J.D.M.); maxivuk@imsi.bg.ac.rs (V.M.)
[2] Institute of Soil Science, 11000 Belgrade, Serbia; zoran.dinic@soilinst.rs
[3] Faculty of Agriculture, University of Belgrade, 11080 Belgrade, Serbia; zocaj@agrif.bg.ac.rs (Z.J.); djordjemor@agrif.bg.ac.rs (Đ.M.)
[4] Institute for Plant Protection and Environment, 11040 Belgrade, Serbia; bonita.sladja@gmail.com
* Correspondence: milica.stojanovic@imsi.bg.ac.rs; Tel.: +381-642937556; Fax: +381-11-3055289

Abstract

A multi-factor analysis of cultivar, biofertiliser, and growing season was conducted to optimise lettuce agronomic and quality traits in diverse soil conditions. The goal was to identify soil differences and offer practical recommendations to improve lettuce traits and quality for farmers and the processing industry. The study employed a complete block design with four treatments, three involving biofertilisers, applied to six lettuce cultivars grown in two contrasting soil types- Mollic Gleysol (Calcaric)-GL and Hortic Anthrosol (Terric, Transportic)-AT, across three consecutive greenhouse seasons (autumn, winter, and spring). Biofertilisers were applied to the soil before transplanting and foliarly during the growing cycle, with four of the following treatments: control (no fertilisation), a fertiliser containing beneficial microorganisms, a *Trichoderma*-based fertiliser, and a combination of both. In GL soil, all biofertiliser treatments increased rosette height, leaf number, and stem length, whereas in AT soil, all morphological parameters declined significantly. The green cultivars 'Aquino' and 'Kiribati' showed superior morphological performance, particularly in spring and winter. Rosette fresh weight, a key indicator of plant biomass, reached 236.4 g in 'Aquino' grown in GL soil, and 208.6 g in 'Kiribati' grown in AT soil. Dualex™ leaf sensor measurement indicated that 'Aquino' exhibited the highest nitrogen balance index (NBI), while the red cultivar 'Gaugin' recorded the highest chlorophyll, flavonoid, and anthocyanin contents. Combined fertilisers increased NBI by 6.3% and chlorophyll by 6.8% in GL soil. *Trichoderma* fertiliser alone raised NBI by 6.8% in GL soil, whereas in AT soil, plants accumulated more flavonoids and anthocyanins (by 9.2% and 8.5%). Optical parameters were highest in autumn. The three-factor experiment demonstrated that cultivar, biofertiliser, and growing season significantly influenced the majority of measured traits. Correlation analysis revealed that rosette fresh weight was positively associated with NBI but negatively correlated with quality-related traits. Based on these findings, cultivars 'Aquino', 'Kiribati', and 'Gaugin' are recommended for both farmers and the processing industry to improve lettuce production quantity and quality. Overall, cultivar, biofertiliser, and season strongly influenced the measured parameters, underscoring the importance of tailoring biofertiliser application to soil type and season to achieve optimal production outcomes.

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1. Introduction

Lettuce (*Lactuca sativa* L.) is an annual leafy vegetable belonging to the family Asteraceae. Its moderate climatic requirements allow year-round cultivation in multiple production systems, including open fields, protected cultivation, and hydroponics, with a growth cycle of 70–100 days. Globally, four countries accounted for 77.1% of the total production of lettuce and chicory in 2023: China, the United States of America, India, and Spain [1]. A wide range of cultivars, differing in colour, texture, size, flavour, and nutritional value, influences both consumer preferences and market price [2]. Lettuce is also an important source of bioactive compounds, including vitamins, phenolic acids, flavonoids, carotenoids, and sesquiterpene lactones, making it a valuable dietary source of natural phytochemicals [3,4].

Lettuce and other salad crops (e.g., arugula, endive, chicory, and lamb's lettuce) have become increasingly popular as ready-to-eat products that are washed, chopped, and packaged for immediate consumption, reflecting lifestyle trends that favour healthy and convenient meal options. During production, the edible plant parts undergo several technological processes to preserve their organoleptic properties until they reach the consumer [5]. However, the growing demand for ready-to-eat salads also increases the risk of vegetable waste during processing, often due to unfavourable morphological traits such as rosette fresh weight, stem length, and number of leaves. These parameters are particularly important for farmers, producers, and breeders. A high proportion of old, damaged, or yellowing leaves, as well as elongated stems, contributes to greater waste, resulting in material and economic losses. Morphological traits are therefore critical for processing efficiency, as they influence shelf life by limiting enzymatic browning, preserving flavour and nutrients, and facilitating quality control during production and storage.

The global population is projected to approach 10 billion people by 2050 [6], creating an urgent demand for increased food production. However, a considerable proportion of food is discarded as waste due to decisions made by consumers, supply chain representatives, societal stakeholders, and retailers, representing a subset of the total food losses [7]. Since the Green Revolution, chemical fertilisers, particularly nitrogen, have played a central role in improving crop growth and yield. Nevertheless, excessive nitrogen application can lead to nutrient imbalances, reduced efficiency, physiological stress, and adverse environmental impact.

Soils account for 95% of global agricultural crop production, far surpassing other production systems [8]. However, approximately one-third of the world's soils are moderately to highly degraded due to factors such as erosion, organic matter loss, nutrient imbalances, reduced biodiversity, salinisation, alkalinisation, acidification, contamination, soil sealing, compaction, and poor water management [9]. Anthropogenic soils, created or altered through human activities, are particularly common in agricultural and urban areas [10]. Microbial remediation strategies, including the use of diverse bacterial species, have been applied to mitigate heavy metal toxicity and support crop growth, as demonstrated in pakchoi and lettuce [11,12]. Moreover, climate change poses an escalating threat to crop stability, with rapid temperature fluctuations, prolonged periods of rainfall or drought, and the emergence or spread of new pests and diseases [13], all of which jeopardise efforts to secure adequate food supplies for a growing global population.

In applied agriculture, biofertilisers are used as inoculants to enhance soil microflora diversity and abundance, accelerate organic matter decomposition, improve nutrient availability, stimulate plant growth and yield, mitigate the negative effects of monoculture, protect against pathogens, and alleviate plant physiological disorders [14,15]. Biofertilisers contain effective microorganisms, either active or dormant, capable of colonising the rhizosphere or internal plant tissues. Among them, *Trichoderma* spp. is a well-known genus of fungi widely applied as a biofertiliser, biopesticide, and soil improver. These avirulent symbionts colonise plant roots, promote root growth, and enhance plant nutrition, productivity, and resistance to various stress factors [16]. Their beneficial effects contribute to environmental protection, soil fertility, pollution prevention, and the enhancement of microflora biodiversity. As cost-effective, non-toxic, eco-friendly, and renewable inputs, biofertilisers provide a sustainable alternative to chemical fertilisers, and can be applied in conventional, organic, and integrated farming systems [17].

In soils with lower organic matter (2.65%), the addition of arbuscular mycorrhizal fungi combined with supplemental nitrogen has been shown to significantly enhance plant biomass [18]. Numerous studies have demonstrated the positive effects of biofertilisers on both the yield and quality traits of lettuce [19,20]. For instance, biofertilisers containing nitrogen-fixing bacteria, phosphorus-mineralising bacteria, and auxins significantly increased lettuce fresh weight in fertile soil [21]. Previous research has established that soil characteristics, including phosphorus availability, organic matter content, and texture, strongly influence lettuce growth, development, and yield, with notable variations in relative growth rates and nutrient uptake observed across soils with different physico-chemical properties [22]. While comparisons between hydroponic and soil cultivation have been explored with respect to morphology and antioxidant capacity [23], fewer studies have examined lettuce performance across multiple soil types under identical environmental conditions. Furthermore, economic and environmental assessments of soil-based versus soilless lettuce production highlight the importance of optimising system selection to ensure both sustainability and product quality [24]. These research gaps underscore the need to directly compare lettuce growth and quality on two distinct soils within the same season, providing clearer insights into soil-specific cultivation practices.

The increasing use of biofertilisers in lettuce cultivation aims to sustainably enhance growth, yield, and nutritional quality while reducing dependence on chemical fertilisers, thereby justifying detailed investigations of their effects in relation to cultivar and season across different soil conditions. Experiments conducted on two distinct soil types with contrasting organic matter content, including one anthropogenic soil, are particularly relevant in the context of global challenges such as food insecurity and land degradation. The inclusion of anthropogenic soils represents an important advantage of this study, as their utilisation can expand arable land, strengthen food security, and provide sustainable options even under intensive vegetable production. This approach offers practical benefits for farmers and the processing industry by informing the selection of cultivars and biofertilisers that optimise production efficiency and product quality.

This study investigates three main aspects: (1) the independent influence of different lettuce cultivars on morphological and physiological traits, encompassing both quantity and quality-related parameters; (2) the effects of biofertiliser application and seasonal variation on lettuce growth and production outcomes; and (3) the specific impacts of two distinct soil types on lettuce morphology and quality.

It is anticipated that individual factors such as cultivar, biofertiliser application, and season will significantly influence the morphological traits and nutritional quality of lettuce (1). Specifically, biofertiliser treatments are expected to enhance lettuce agronomic performance and quality compared to untreated plants (2). Furthermore, seasonal variation is expected to modulate plants' responses, underscoring the importance of tailored cultivation practices to optimise lettuce production (3).

Based on these hypotheses, this study aims to evaluate the individual effects of each factor, emphasising the identification of cultivars with favourable morphological and physiological traits. The findings are intended to support farmers and the processing industry in making decisions about lettuce cultivation and quality management.

2. Materials and Methods

2.1. Plant Material

The experiment included six lettuce cultivars supplied by the vegetable breeding company Rijk Zwaan (De Lier, the Netherlands). Three cultivar types were tested: oak leaf (green 'Kiribati' and red 'Murai'—*L. sativa* var. *crispa*), multi-leaf butterhead Salanova® (green 'Aquino' and red 'Gaugin'—*L. sativa* var. *capitata*), and lollo (green 'Aleppo' and red 'Carmesi'—*L. sativa* var. *crispa*). All lettuce cultivars are suitable for fresh and processed ready-to-eat products, either as whole or chopped leaves.

Seedlings were grown in 4 cm peat cubes under glasshouse conditions. All climatic data reported for seedling production represent averages over their respective periods. Autumn seedling production lasted 20 days, with a daily temperature of 18.1 °C, relative humidity of 61.1%, and cumulative light energy of 1510.3 J cm^{-2}. Winter production spanned 39 days, with a temperature of 13.2 °C, humidity of 81.3%, and 653.1 J cm^{-2} light energy. Spring production lasted 21 days, with a temperature of 15.2 °C, humidity of 57.4%, and 1568.3 J cm^{-2} light energy. The photoperiod averaged approximately 11 h 38 min in autumn, 9 h 2 min in winter, and 13 h 34 min in spring.

2.2. Biofertilisers

Two biofertilisers and their combination were applied in the experiments. EM Aktiv (EM, Candor, EM Tehnologija d.o.o., Valpovo, Croatia) is a liquid formulation produced by the microbiological fermentation of organic matter and sugar cane molasses, containing plant-derived extracts. Vital Tricho (VT, Candor, EM Tehnologija d.o.o., Valpovo, Croatia) is a powdered preparation consisting of *Trichoderma viride* and *Trichoderma asperellum* (5×10^9 CFU mL^{-1}). The third treatment consisted of a simple mixture of these two fertilisers. Fertilisers used in our study are registered and approved on the official list of permitted fertilisers and soil conditioners for organic farming in Serbia [25].

2.3. Experimental Design and Climate Data

To test the hypotheses and research questions previously defined, our experimental plan evaluated the effects of biofertiliser treatments on six lettuce cultivars grown across different soil types and seasons. A complete block design with four treatments was employed under greenhouse conditions. Treatments were applied to the soil and foliarly at recommended doses. Continuous monitoring of environmental factors (temperature, humidity, and photoperiod) along with soil and plant measurements provided data necessary to evaluate the main experimental factors.

Lettuce plants were cultivated at Iceberg Salat Centar (Surčin, Serbia), over three consecutive growing seasons: autumn (11 October–7 December 2016), winter (27 December 2016–5 April 2017), and spring (27 April–3 June 2017). Prior to the first planting, mechanical and chemical soil analyses of the soil were performed. Initial sampling and testing identified two soil types (Table 1). The first was a Mollic Gleysol (Calcaric) (GL-mo.ca) [26], classified as clay loam (ClLo-IUSS soil texture classification) [27]. The second was a Hortic Anthrosol (Terric, Transportic) (AT-ho.tr.tp) [26], formed during the excavation of an ameliorative channel, classified as sandy clay loam (SaClLo-IUSS soil texture classification) [27].

The plants were cultivated in a greenhouse without supplementary heating or lighting. Experimental plots were established in GL soil (256 m^2) and AT soil (144 m^2). Both soil types had been used for intensive vegetable production before this study, with pepper cultivation preceding the lettuce. Intensive production involved the application of mineral fertilisers, while biofertilisers had not been applied to the soil or foliarly prior to this experiment. The experiment followed a complete block design with four treatments: (1) control (C, no fertiliser); (2) EM Aktiv (EM); (3) Vital Tricho (VT); and (4) a mixture of EM Aktiv and Vital Tricho (EM + VT), each replicated three times. In GL soil, plots measured 2 m × 1 m and contained 32 plants each, planted at a spacing of 25 cm × 25 cm. In the AT soil, plots measured 0.75 m × 1 m with 12 plants each, maintaining the same planting density. Distances between replicates and treatments were 50 cm and 100 cm. After soil preparation, biofertilisers were applied directly to the soil in accordance with manufacturer recommendations, keeping the concentration consistent in both soils: for GL soil, the treatments included 150 mL EM, 21 g VT, and a combination of 150 mL + 21 g of EM + VT, respectively, each dissolved in 10 L of water. For AT soil, the treatments included 75 mL EM, 10.5 g VT, and a combination of 75 mL + 10.5 g of EM + VT, respectively, each dissolved in 5 L of water. The soil was then covered with a black mulch film. During the lettuce vegetation period, biofertilisers were applied foliarly four times using a battery sprayer, also keeping the concentration consistent in both soils. For GL soil, the rates were 30 mL EM, 12 g VT, and 30 mL + 12 g of EM + VT, each dissolved in 6 L of water; for AT soil, 15 mL EM, 6 g VT, and 15 mL + 6 g of EM + VT were dissolved in 3 L of water. Standard agronomic practices were applied to both soil types, including hoeing, weeding, greenhouse ventilation, and irrigation. Irrigation was carried out using overhead sprinklers, with the same volume and frequency of water applied across treatments in both soil types. The measurement of air temperature and relative humidity was conducted using two RC-4HC Data Loggers (Elitech Technology Inc., San Jose, CA, USA), with one placed in each soil type. This setup allowed for continuous recording over 24 h throughout the experiment (Figure 1). The photoperiod varied with season, ranging from 11 to 9 h in autumn, 9–13 h in winter, 14–15 h in spring, from transplanting to harvest.

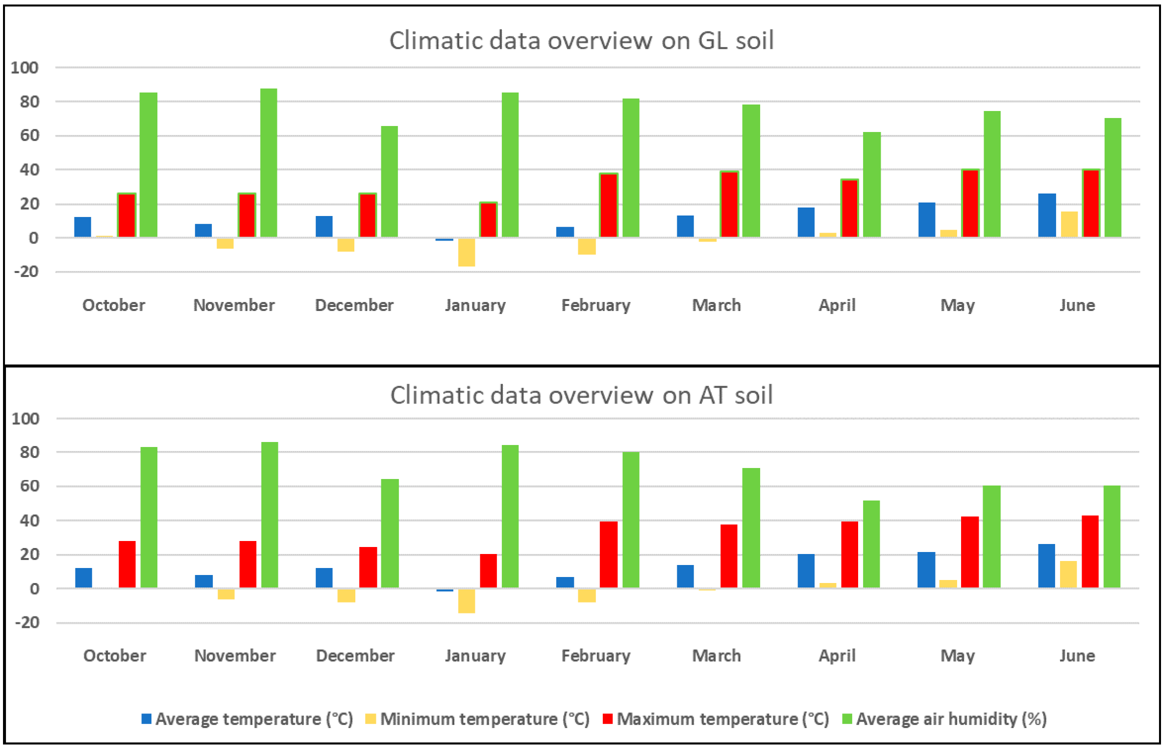


Figure 1. Climatic conditions during lettuce growing cycles (October 2016–June 2017). GL: Mollic Gleysol (Calcaric) soil, AT: Hortic Anthrosol (Terric, Transportic) soil.

Table 1. Initial chemical soil analysis.

Parameter	**N (%)**		**P—AL (mg P_2O_5 10^{-2} g^{-1})**		**K—AL (mg K_2O 10^{-2} g^{-1})**		**SOM (%)**		**Carbonate Content (% $CaCO_3$)**		**pH (H_2O)**		**pH (1 M KCl)**	
Soil type	**GL**	**AT**	**GL**	**AT**	**GL**	**AT**	**GL**	**AT**	**GL**	**AT**	**GL**	**AT**	**GL**	**AT**
	0.22	0.14	58.35	33.92	32.45	18.69	5.02	2.83	9.90	24.67	7.8	8.1	7.5	7.8
Fertility status	medium	low	high		high	medium	high	medium	medium	high	mildly moderately alkaline		alkaline	

GL: Mollic Gleysol (Calcaric) soil, AT: Hortic Anthrosol (Terric, Transportic) soil, N: total nitrogen, P—AL: ammonium acetate lactate extractable phosphorus, K—AL: ammonium acetate lactate extractable potassium, SOM: soil organic matter, Carbonate content—calcium carbonate equivalent ($CaCO_3$), Fertility status—nutrient availability levels, organic matter status, calcium carbonate equivalent status, and soil pH (acidity levels). Units for P—AL and K—AL are expressed as mg P_2O_5 and mg K_2O per 100 g soil, denoted as mg P_2O_5 10^{-2} g^{-1} and mg K_2O 10^{-2} g^{-1} to maintain consistency with the negative exponent notation used throughout the manuscript.

2.4. Soil Analysis and Data Collection of Morphological and Physiological Parameters

Air-dried soil samples were milled and sieved (≤2 mm) to determine soil mechanical composition following the modified International B methods [28]. Chemical analyses included several parameters. Soil pH was measured potentiometrically [29] using a pH metre (SevenCompact S210-K, Mettler-Toledo, Greifensee, Switzerland). Total nitrogen (TN) and total carbon (TC) were determined by dry combustion on a CNS elemental analyser (Model Vario EL III-ELEMENTAL Analysis systems, GmbH, Hanau, Germany) [30,31]. Available phosphorus was measured with a UV-VIS spectrophotometer (UV-160A, Shimadzu Corporation, Kyoto, Japan), and available potassium by flame emission photometry (HINOTEK FP6440 Precise Perfect flame photometer, Ningbo, China), both using the AL method [32]. The carbonate content ($CaCO_3$) was determined volumetrically [33], and soil organic matter (SOM) was calculated from total carbon (TC), soil organic carbon (SOC), and $CaCO_3$ using the following equations:

$$\mathrm{SOC} = \mathrm{TC} - 0.12 \times \mathrm{CaCO_3} \quad (1)$$

$$\mathrm{SOM} = 1.724 \times \mathrm{SOC} \quad (2)$$

where SOM was estimated by multiplying soil organic carbon by the Van Bemmelen factor of 1.724 [34].

Plants were harvested by hand at commercial maturity and marketable size, indicated by rosette (head) compactness, on the same day in both soil types. The vegetation period lasted 58 days in autumn, 100 days in winter, and 40 days in spring. Morphological parameters were measured immediately after the harvest, early in the morning, and reported as the mean of measurements taken from nine plants. Rosette (head) and stem fresh weights were determined with a digital scale and expressed in grams (g). Rosette height was measured with a ruler and expressed in centimetres (cm), while stem length was measured with a calliper, ensuring zero adjustment before measurement, and expressed in millimetres (mm). The Dualex 4 Scientific leaf-clip sensor (FORCE-A, Orsay, Paris, France) is an advanced chlorophyll metre technology that enables real-time, non-destructive, and unitless measurements of leaf chlorophyll content, as well as flavonoid and anthocyanin levels on the leaf surface [35]. In this study, the device was used with its automatic self-calibration function, which activates each time it is powered on, ensuring consistent measurement accuracy throughout data collection. Chlorophyll content (Chl) was determined based on the absorption of far-red light by chlorophyll and the transmittance of near-infrared light as a reference. Flavonoid (Flav) and anthocyanin (Anth) contents were calculated from different ratios of chlorophyll fluorescence in the leaf epidermis. The nitrogen balance index (NBI) was derived from the ratio of chlorophyll content to flavonoid content as given by the equation:

$$\mathrm{NBI} = \mathrm{Chl}/\mathrm{Flav} \tag{3}$$

Dualex measurements were conducted in the autumn and spring trials, one day before harvest. Measurements were taken on the abaxial side of leaves (avoiding veins) and presented as mean values for NBI, Chl, Flav, and Anth (n = 9).

2.5. Statistical Analysis

A three-way ANOVA was performed to assess the main and interaction effects of cultivar, fertiliser, and season on the morphological and physiological parameters, with Tukey's test applied for post hoc comparison. Interaction effects are reported in the results tables for completeness and transparency but are not further analysed and discussed, as the primary focus of this study is on main effects. Statistical significance was set at $\alpha = 0.05$. Pearson's correlation coefficient was used to examine potential relationships between the measured parameters. All analyses were conducted using SPSS Statistics (Version 25.0; IBM Corp., Armonk, NY, USA) and Microsoft Office Excel 2019 (Microsoft Corp., Redmond, WA, USA).

3. Results

3.1. Morphological Parameters

3.1.1. Rosette Height

Rosette height is significantly influenced by cultivar, biofertiliser, and season (Table 2). Cultivars 'Kiribati', 'Murai', and 'Carmesi' exhibited greater rosette heights in both soils, regardless of leaf colour, whereas the green cultivar 'Aquino' consistently recorded the lowest height. In GL soil, the application of all biofertilisers increased height by 3.1–4.2%, while in AT soil, all tested fertilisers reduced height by 5.1–8.1%. Seasonal effects also varied between soils: in GL, rosette height was 13.2% higher in spring compared with autumn, whereas in AT, winter trials resulted in a 10.0% increase over spring.

3.1.2. Rosette (Head) Fresh Weight

Rosette (head) fresh weight was significantly influenced by cultivar and season, while the effect of the fertiliser was not significant in GL soil (Table 2). The green cultivar 'Aquino' achieved the highest head fresh weight (236.4 g), whereas the red cultivar 'Carmesi' recorded the lowest (141.2 g). Seasonal variation was pronounced, with fresh weight in spring increasing by 130.2% compared with autumn.

In AT soil, all three main factors significantly affect rosette (head) fresh weight (Table 2). The green cultivar 'Kiribati' produced the highest rosette fresh weight (208.6 g), whereas the red cultivar 'Gaugin' had the lowest (143.0 g). Compared with the control, the application of all microbiological fertilisers reduced rosette fresh weight by 8.2–13.7%. Seasonal effects were also pronounced, with the highest fresh weight recorded in winter, representing a 94.8% increase compared with autumn.

3.1.3. Number of Leaves

The number of leaves is significantly influenced by cultivar, biofertiliser, and season (Table 2). The green cultivar 'Aquino' produced the highest leaf number in both soil types, whereas the red cultivar 'Carmesi' consistently showed the lowest. In GL soil, the application of all fertilisers increased leaf number by 5.4–7.5% in GL, while in AT soil, VT, and combined fertilisers reduced leaf number by 7.6% and 9.5%. Seasonal effects were evident in both soils: the lowest leaf numbers were recorded in autumn, while increases relative to autumn reached 107.7% in spring for GL soil and 60.4% in winter for AT soil.

Table 2. Main and interaction effects on lettuce morphological parameters in two different soil types.

Parameters	**Rosette Height (cm)**		**Rosette Fresh Weight (g)**		**Number of Leaves**		**Stem Length (mm)**		**Stem Fresh Weight (g)**	
Main Factors	**GL**	**AT**	**GL**	**AT**	**GL**	**AT**	**GL**	**AT**	**GL**	**AT**
Cultivar										
Kiribati	19.4 ± 0.4 e	17.3 ± 0.5 d	225.2 ± 11.2 d	208.6 ± 11.7 b	31.6 ± 1.1 c	33.9 ± 1.1 c	53.8 ± 2.7 d	45.9 ± 2.1 d	26.3 ± 1.9 c	22.9 ± 1.9 c
Murai	18.9 ± 0.5 e	17.8 ± 0.6 d	167.9 ± 8.2 b	153.5 ± 9.0 a	23.8 ± 0.7 b	22.8 ± 0.6 b	47.3 ± 2.1 c	41.3 ± 2.1 c	20.5 ± 1.4 b	19.7 ± 1.6 b
Aquino	12.6 ± 0.3 a	12.1 ± 0.3 a	236.4 ± 9.6 d	197.1 ± 10.7 b	123.8 ± 4.3 e	107.3 ± 4.9 e	37.2 ± 1.4 a	34.1 ± 1.5 a	44.8 ± 3.0 e	38.2 ± 2.8 d
Gaugin	14.8 ± 0.4 b	14.1 ± 0.3 b	161.9 ± 6.0 b	143.0 ± 6.2 a	80.6 ± 3.8 d	80.0 ± 3.2 d	56.7 ± 3.2 d	51.5 ± 2.4 e	36.8 ± 2.0 d	24.6 ± 1.8 c
Aleppo	16.8 ± 0.4 c	16.0 ± 0.5 c	208.6 ± 10.7 c	207.0 ± 11.5 b	25.9 ± 0.8 b	25.0 ± 0.8 b	42.6 ± 2.2 b	37.1 ± 2.3 b	19.6 ± 1.5 b	18.4 ± 1.6 b
Carmesi	18.0 ± 0.4 d	17.6 ± 0.5 d	141.2 ± 7.7 a	144.6 ± 8.9 a	16.4 ± 0.5 a	16.1 ± 0.5 a	34.2 ± 1.9 a	31.9 ± 1.7 a	9.5 ± 0.7 a	10.5 ± 0.9 a
Fertiliser										
Control	16.3 ± 0.4 a	16.6 ± 0.4 c	188.2 ± 9.5	191.4 ± 9.2 c	47.9 ± 1.9 a	49.9 ± 1.7 b	42.7 ± 2.4 a	44.1 ± 2.3 c	25.6 ± 1.7	25.0 ± 2.0 c
EM Aktiv	16.9 ± 0.4 b	15.8 ± 0.4 b	188.7 ± 8.2	175.7 ± 8.6 b	50.5 ± 2.0 b	48.7 ± 1.7 b	46.7 ± 2.0 b	41.1 ± 1.7 b	26.8 ± 1.6	23.0 ± 1.7 b
Vital Tricho	16.8 ± 0.4 b	15.6 ± 0.4 ab	193.4 ± 9.8	170.2 ± 10.5 ab	51.6 ± 2.0 b	46.2 ± 1.9 a	45.3 ± 2.4 b	38.9 ± 2.2 a	26.3 ± 2.0	21.9 ± 1.8 b
EM Aktiv + Vital Tricho	17.0 ± 0.4 b	15.3 ± 0.5 a	190.5 ± 8.2	165.3 ± 10.3 a	51.4 ± 1.6 b	45.2 ± 2.1 a	46.4 ± 2.2 b	37.1 ± 1.8 a	26.1 ± 1.8	19.5 ± 1.5 a
Growing season										
Autumn	15.5 ± 0.4 a	16.0 ± 0.3 b	106.0 ± 5.1 a	112.8 ± 5.6 a	30.6 ± 1.0 a	34.2 ± 1.1 a	23.3 ± 1.1 a	25.6 ± 1.3 a	10.3 ± 0.8 a	11.5 ± 0.9 a
Winter	17.2 ± 0.4 b	16.5 ± 0.5 c	220.7 ± 12.0 b	219.7 ± 13.1 c	56.9 ± 2.3 b	54.9 ± 2.3 b	44.8 ± 2.0 b	45.9 ± 2.0 b	30.1 ± 2.0 b	29.0 ± 2.2 c
Spring	17.5 ± 0.4 b	15.0 ± 0.5 a	243.9 ± 9.7 c	194.4 ± 10.3 b	63.6 ± 2.2 c	53.3 ± 2.1 b	67.8 ± 3.7 c	49.4 ± 2.8 c	38.3 ± 2.5 c	26.7 ± 2.2 b
Significance										
Cultivar (C)	***	***	***	***	***	***	***	***	***	***
Fertiliser (F)	***	***	ns	***	***	***	***	***	ns	***
Growing season (GS)	***	***	***	***	***	***	***	***	***	***
Interaction factors										
C × F	***	**	**	**	***	***	***	***	***	***
C × GS	***	***	***	***	***	***	***	***	***	***
F × GS	***	**	***	***	***	***	***	***	***	***
C × F × GS	***	***	***	**	ns	***	***	***	***	***

The data show the means (n = 9) ± SE. Values followed by the same letter are not significantly different at the 0.05% level of probability according to Tukey's test. Groups of the same factors with no letters are not different from each other. Asterisks indicate significant differences at ** $p \leq 0.01$; *** $p \leq 0.001$; ns, non-significant. GL: Mollic Gleysol (Calcaric) soil, AT: Hortic Anthrosol (Terric, Transportic) soil. Results of mean squares and degrees of freedom for morphological parameters are presented in Table S1.

3.1.4. Stem Length

Stem length is significantly influenced by cultivar, fertiliser, and season (Table 2). The red cultivar 'Gaugin' showed the highest stem length in both soil types, GL and AT (56.7 mm; 51.5 mm, respectively), while the red cultivar 'Carmesi' showed the lowest in both GL and AT soils (34.2 mm; 31.9 mm). In GL soil, the application of all fertilisers increased length by 6.1–9.3%, whereas in AT soil, all tested fertilisers reduced length by 6.9–15.9%. In both soils, the highest stem lengths were observed in spring, representing increases of 191.5% and 92.7% compared with autumn.

3.1.5. Stem Fresh Weight

Stem fresh weight is significantly influenced by all three main factors in AT soil, whereas in GL soil, the effect of fertiliser is not significant (Table 2). In both soils, the green cultivar 'Aquino' produced the highest stem fresh weight, while the red cultivar 'Carmesi' produced the lowest. Biofertilisers did not significantly affect this parameter in GL soil; however, in AT soil, all treatments reduced stem fresh weight by 7.8–21.8%. Autumn season yielded the lowest stem fresh weight, with GL soil achieving its highest in spring, an increase of 273.5%, and AT soil peaking in winter, increasing by 153.1% compared with autumn.

3.2. Physiological Parameters

3.2.1. Nitrogen Balance Index (NBI)

In GL soil, NBI values are significantly influenced by cultivar and fertiliser, while in AT soil, all three factors, including season, are statistically significant (Table 3). The green cultivar 'Aquino' recorded the highest NBI values in both soils (33.7 in GL; 35.0 in AT), whereas the red cultivars 'Murai' and 'Carmesi' showed the lowest values (14.1 in GL; 14.8 in AT). In GL soil, VT and combined fertiliser treatments increased NBI by 6.8% and 6.3%. By contrast, in AT soil, VT application reduced NBI by 7.8%, opposite to its positive effect in GL. Seasonal influence on NBI value was not significant in GL soil, but in AT soil, autumn values were 11.7% higher than those in spring.

Table 3. Main and interaction effects on the lettuce physiological parameters in two different soil types.

Parameters	**NBI**		**Chl**		**Flav**		**Anth**	
Main Factors	**GL**	**AT**	**GL**	**AT**	**GL**	**AT**	**GL**	**AT**
Cultivar								
Kiribati	18.5 ± 1.0 b	20.1 ± 1.3 c	13.2 ± 0.7 a	14.6 ± 1.2 b	0.73 ± 0.04 a	0.74 ± 0.04 a	0.42 ± 0.03 b	0.35 ± 0.04 b
Murai	14.1 ± 0.6 a	14.9 ± 1.0 a	16.3 ± 0.9 b	17.9 ± 1.2 c	1.20 ± 0.08 b	1.23 ± 0.08 b	0.63 ± 0.04 c	0.60 ± 0.04 d
Aquino	33.7 ± 1.1 c	35.0 ± 1.8 d	21.8 ± 0.6 d	23.5 ± 0.7 d	0.66 ± 0.02 a	0.71 ± 0.03 a	0.29 ± 0.02 a	0.27 ± 0.02 a
Gaugin	17.8 ± 1.0 b	17.7 ± 1.1 b	25.4 ± 0.8 e	25.8 ± 0.7 e	1.49 ± 0.07 c	1.53 ± 0.06 c	0.65 ± 0.03 c	0.68 ± 0.03 e
Aleppo	18.8 ± 1.3 b	16.8 ± 0.9 ab	13.8 ± 1.1 a	10.9 ± 0.9 a	0.75 ± 0.07 a	0.67 ± 0.05 a	0.44 ± 0.04 b	0.42 ± 0.03 c
Carmesi	15.3 ± 1.0 a	14.8 ± 0.9 a	17.8 ± 1.1 c	17.9 ± 1.5 c	1.19 ± 0.04 b	1.29 ± 0.08 b	0.63 ± 0.04 c	0.64 ± 0.04 de
Fertiliser								
Control	19.0 ± 1.0 a	20.6 ± 1.4 b	17.6 ± 0.8 a	18.3 ± 0.9	1.00 ± 0.05	0.98 ± 0.07 a	0.52 ± 0.04	0.47 ± 0.04 a
EM Aktiv	19.3 ± 0.9 ab	20.5 ± 1.3 ab	17.5 ± 0.9 a	18.8 ± 1.1	1.01 ± 0.05	1.02 ± 0.04 ab	0.51 ± 0.04	0.49 ± 0.03 ab
Vital Tricho	20.3 ± 0.8 b	19.0 ± 1.0 a	18.2 ± 0.9 ab	18.4 ± 0.9	0.99 ± 0.06	1.07 ± 0.05 b	0.50 ± 0.03	0.51 ± 0.04 b
EM Aktiv + Vital Tricho	20.2 ± 1.2 b	19.3 ± 1.0 ab	18.8 ± 1.0 b	18.2 ± 1.2	1.00 ± 0.05	1.04 ± 0.06 ab	0.51 ± 0.03	0.50 ± 0.03 ab
Growing season								
Autumn	19.9 ± 1.1	21.0 ± 1.6 b	18.9 ± 0.9 b	18.1 ± 0.9	1.03 ± 0.06 b	0.95 ± 0.05 a	0.58 ± 0.04 b	0.55 ± 0.03 b
Spring	19.5 ± 0.9	18.8 ± 0.8 a	17.2 ± 0.9 a	18.7 ± 1.2	0.98 ± 0.05 a	1.11 ± 0.06 b	0.44 ± 0.03 a	0.43 ± 0.03 a
Significance								
Cultivar (C)	***	***	***	***	***	***	***	***
Fertiliser (F)	**	**	**	ns	ns	**	ns	*
Growing season (GS)	ns	***	***	ns	**	***	***	***
Interaction factors								
C × F	**	ns	ns	ns	ns	ns	ns	ns
C × GS	***	***	***	ns	***	***	***	***
F × GS	**	ns	*	ns	**	ns	**	ns
C × F × GS	**	**	*	ns	*	ns	ns	*

The data show the means ($n = 9$) ± SE. Values followed by the same letter are not significantly different at the 0.05% level of probability according to Tukey's test. Groups of the same factors with no letters are not different from each other. Asterisks indicate significant differences at * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, non-significant. GL: Mollic Gleysol (Calcaric) soil, AT: Hortic Anthrosol (Terric, Transportic) soil, NBI: nitrogen balance index, Chl: chlorophyll content, Flav: flavonoids content, Anth: anthocyanins content. Results of mean squares and degrees of freedom for physiological parameters are presented in Table S2.

3.2.2. Chlorophyll Content (Chl)

Chlorophyll (Chl) content is significantly affected by all three main factors in GL soil, whereas in AT soil, only the cultivar has a significant effect (Table 3). The red cultivar 'Gaugin' exhibited the highest Chl content in both soils, while the green cultivars 'Kiribati' and 'Aleppo' had the lowest values. In GL soil, combined fertiliser application increased Chl content by 6.8% compared with the control. Seasonal effects were also evident in GL soil, where autumn values were 9.9% higher than those in spring; however, season did not significantly affect Chl content in AT soil.

3.2.3. Flavonoid Content (Flav)

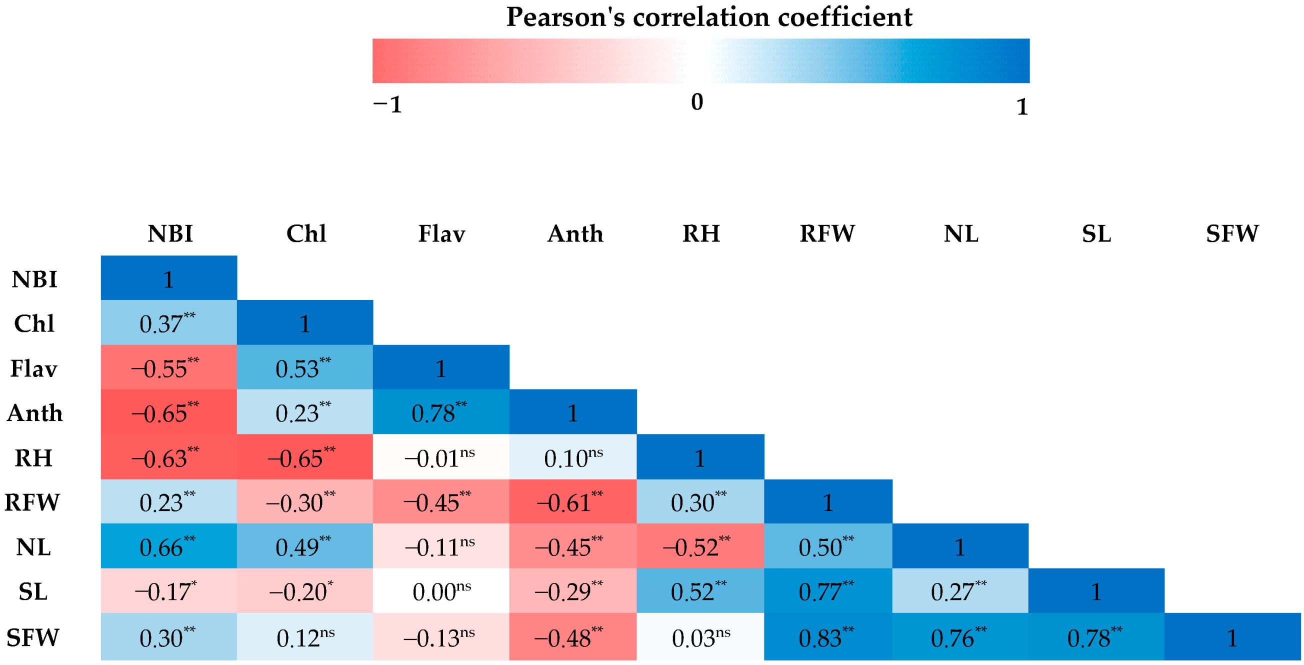
In GL soil, Flav content was significantly influenced by cultivar and season, whereas in AT soil, all three main factors are significant (Table 3). The red cultivar ‘Gaugin’ displayed the highest Flav values in both soils (1.49 in GL; 1.53 in AT), while the green cultivars ‘Aquino’ and ‘Aleppo’ recorded the lowest (0.66 in GL; 0.67 in AT). In the AT soil, VT increased the Flav content by 9.2% compared with the control. Seasonal effects varied between soils: In GL soil, Flav values during autumn exceeded those in spring by 5.1%, whereas in AT soil, spring Flav values were 16.8% higher than those in autumn.

3.2.4. Anthocyanin Content (Anth)

As with Flav content, Anth was significantly influenced by cultivar and season in GL soil, while in AT soil, all three main factors are significant (Table 3). The red cultivar ‘Gaugin’ displayed the highest Anth values in both soils, while the green cultivar ‘Aquino’ recorded the lowest. In the AT soil, the VT application increased Anth content by 8.5% compared with the control. Seasonal effects were consistent across soils: autumn Anth values were higher than spring, with increases of 31.8% in GL and 27.9% in AT.

3.3. Correlations

The results of all tested morphological and physiological parameters were analysed to assess potential correlations in both soils. In GL soil, rosette fresh weight, one of the key yield-related parameters, was positively correlated with all other morphological traits (Figure 2). Specifically, it showed a very strong positive correlation with stem fresh weight (r = 0.83 **), a strong positive correlation with stem length (r = 0.77 **), and a moderate positive correlation with the number of leaves (r = 0.50 **). In contrast, rosette fresh weight exhibited a strong negative correlation with Anth (r = −0.61 **) and a moderate negative correlation with Flav (r = −0.45 **), indicating an inverse relationship with this group of compounds. Additionally, NBI showed a strong negative correlation with Anth (r = −0.65 **) and a negative moderate correlation with Flav (r = −0.55 **).

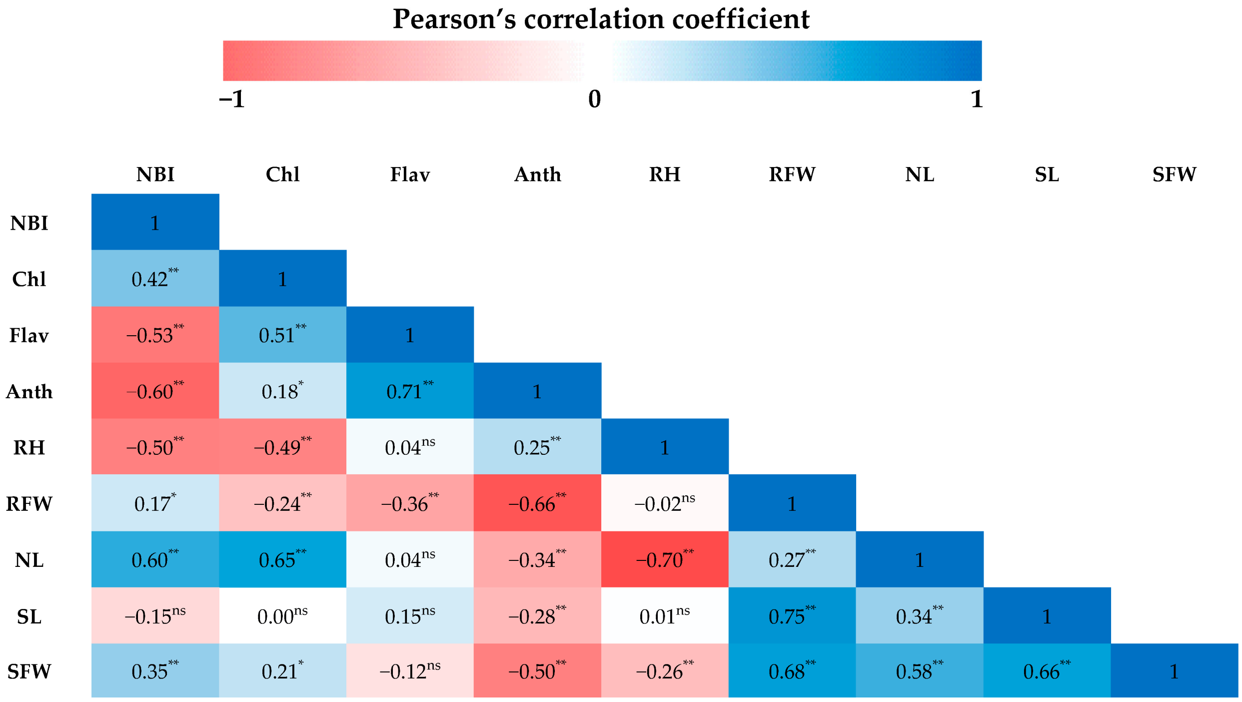


	NBI	Chl	Flav	Anth	RH	RFW	NL	SL	SFW
NBI	1								
Chl	0.37**	1							
Flav	−0.55**	0.53**	1						
Anth	−0.65**	0.23**	0.78**	1					
RH	−0.63**	−0.65**	−0.01^{ns}	0.10^{ns}	1				
RFW	0.23**	−0.30**	−0.45**	−0.61**	0.30**	1			
NL	0.66**	0.49**	−0.11^{ns}	−0.45**	−0.52**	0.50**	1		
SL	−0.17*	−0.20*	0.00^{ns}	−0.29**	0.52**	0.77**	0.27**	1	
SFW	0.30**	0.12^{ns}	−0.13^{ns}	−0.48**	0.03^{ns}	0.83**	0.76**	0.78**	1

Figure 2. The heat map correlation matrix shows correlations among nine lettuce morphological and physiological parameters in GL soil. Their correlated levels were represented with blue (strong

positive relationship) to red (strong negative relationship). Each cell in the correlation matrix is represented by a Pearson's correlation coefficient (r). Asterisks indicate significant differences at * $p \leq 0.05$, ** $p \leq 0.01$, and ns. non-significant. GL: Mollic Gleysol (Calcaric) soil. NBI: nitrogen balance index, Chl: chlorophyll content, Flav: flavonoid content, Anth: anthocyanin content, RH: rosette height, RFW: rosette fresh weight, NL: number of leaves, SL: stem length, SFW: stem fresh weight.

A similar trend between morphological and physiological parameters was observed in AT soil (Figure 3). Rosette fresh weight showed a strong positive correlation with stem length (r = 0.75 **) and stem fresh weight (r = 0.68 **), while exhibiting a strong negative correlation with Anth (r = −0.66 **). Consistent with the findings in GL soil, NBI displayed a negative moderate correlation with Flav (r = −0.53 **) and Anth (r = −0.60 **).



	NBI	Chl	Flav	Anth	RH	RFW	NL	SL	SFW
NBI	1								
Chl	0.42^{**}	1							
Flav	-0.53^{**}	0.51^{**}	1						
Anth	-0.60^{**}	0.18^{*}	0.71^{**}	1					
RH	-0.50^{**}	-0.49^{**}	0.04^{ns}	0.25^{**}	1				
RFW	0.17^{*}	-0.24^{**}	-0.36^{**}	-0.66^{**}	-0.02^{ns}	1			
NL	0.60^{**}	0.65^{**}	0.04^{ns}	-0.34^{**}	-0.70^{**}	0.27^{**}	1		
SL	-0.15^{ns}	0.00^{ns}	0.15^{ns}	-0.28^{**}	0.01^{ns}	0.75^{**}	0.34^{**}	1	
SFW	0.35^{**}	0.21^{*}	-0.12^{ns}	-0.50^{**}	-0.26^{**}	0.68^{**}	0.58^{**}	0.66^{**}	1

Figure 3. The heat map correlation matrix shows correlations among nine lettuce morphological and physiological parameters in AT soil. Their correlated levels were represented with blue (strong positive relationship) to red (strong negative relationship). Each cell in the correlation matrix is represented by a Pearson's correlation coefficient (r). Asterisks indicate significant differences at * $p \leq 0.05$, ** $p \leq 0.01$, and ns, non-significant. AT: Hortic Anthrosol (Terric, Transportic) soil, NBI: nitrogen balance index, Chl: chlorophyll content, Flav: flavonoid content, Anth: anthocyanin content, RH: rosette height, RFW: rosette fresh weight, NL: number of leaves, SL: stem length, SFW: stem fresh weight.

4. Discussion

Agronomic traits such as plant fresh weight, number of leaves, and stem length are essential for maintaining high-quality materials and reducing processing losses of ready-to-eat salads. Lettuce rosette height influences market appeal and perceived quality, while size also affects consumer acceptance and poses risks during transport.

All tested morphological parameters are significantly influenced by cultivar (Table 2). In both soil types, green lettuce cultivars exhibited higher rosette fresh weight, stem fresh weight, and leaf number, compared with red counterparts. Fresh weight is a widely used growth indicator in crops, particularly in lettuce, where shoot fresh weight directly correlates with yield, as both leaves and stems constitute the final harvested product [36]. Optimal fresh weight is therefore critical for marketability and processing efficiency, directly affecting both quality and profitability. On GL soil, the green cultivars 'Aquino' and

'Kiribati' achieved the highest rosette fresh weight, while 'Kiribati' and 'Aleppo' excelled on AT soil. These findings align with a previous study reporting greater fresh weight in green lettuce compared with red [37]. The Salanova cultivars 'Aquino' and 'Gaugin' also produced a higher average number of leaves than the other four cultivars (Table 2). As members of the multi-leaf butterhead group, they are characterised by dense leaf formation and specifically developed for processing, resulting in smaller rosettes composed of numerous leaves. Leaf number is influenced by agrotechnical practices and environmental factors; for example, high temperatures can accelerate the vegetative phase, causing premature flowering and reduced leaf count [38]. From a practical perspective, both farmers and consumers prefer cultivars with a greater number of leaves, since leaves represent the edible portion of the plant, enhance sunlight absorption, affect photosynthesis, contribute to faster growth and earlier maturation, thereby improving their commercial attractiveness [39,40].

For optimal processing of ready-to-eat products, lettuce stem length should remain below 7 cm, whereas lengths of 7–9 cm indicate elongation that reduces usability [41]. Results from both soil types confirmed that all six cultivars fell within the suitable range of 3.2–5.7 cm, with the highest value observed in the red cultivar 'Gaugin' (Table 2). Stem length is closely linked to early flowering sensitivity, which affects leaf number, head size, and compactness, ultimately leading to smaller heads with reduced fresh weight and quality. Salanova cultivars generally exhibited the highest stem values, suggesting that special attention should be given to their selection to minimise the risk of premature bolting. Conversely, the red cultivar 'Carmesi' displayed the lowest stem parameter values, a favourable trait for processing, while also demonstrating resilience to variable climatic conditions that can extend the vegetation period and delay harvest. However, its advantage in stem length was offset by low rosette fresh weight, ranking lowest in GL soil, and second lowest, after 'Gaugin,' in AT soil (Table 2).

Biofertiliser application has the opposite effect on morphological traits in both soil types (Table 2). In GL soil, all fertilisers increased rosette height, number of leaves, and stem length, while in AT soil, biofertiliser application reduced these parameters. Thus, the second hypothesis was validated for GL soil, where fertilisation positively impacted all parameters, but not for AT soil, where fertilisation led to reductions. Microorganisms can produce hormones, vitamins, enzymes, and plant growth-promoting compounds that enhance nutrient availability, nutrient uptake, and influence both plant growth and yield [16]. However, reports on the impact of biofertilisers on plant productivity remain inconsistent. Some studies indicate a positive influence, such as increased fresh weight in lettuce [20,42], whereas others show limited or no beneficial effects. For example, a four-year experiment with potatoes, winter barley, and alfalfa found that continuous application of effective microorganisms did not improve yield, biomass, microbial activity, or soil structure under effective microorganisms' inoculation [43].

The efficiency of beneficial microorganisms, including *Trichoderma* spp., in promoting plant growth and yield depends on several factors: plant species, the ability of *Trichoderma* spp. to colonise roots, soil type, soil organic matter content, and the continuity of application. The application of arbuscular mycorrhiza fungi and seaweed extract, as well as *Trichoderma asperellum* strains (TaMFP1 and TaMFP2) and *Trichoderma harzianum*, increased the number of leaves in lettuce [44,45]. Moreover, treatments with various *Trichoderma* species significantly increased lettuce height, with *Trichoderma asperellum* strains TaMFP1 and TaMFP2 notably enhancing stem length, while *Trichoderma harzianum* did not show the same effect [44].

The negative impact of biofertilisers on morphological parameters in AT soil may be attributed to its lower soil organic matter content compared with GL soil. Reduced organic matter limits nutrient and energy availability for soil microorganisms, thereby diminishing their effectiveness. Additionally, the higher pH of AT soil likely restricts macronutrient availability and microbial activity (Table 1). Water availability represents another limiting factor [46], as the mechanical composition of AT soil promotes rapid drainage, especially during the spring trial. This may result in competition between microorganisms and plants for water and nutrients, ultimately lowering morphological parameter values compared with the unfertilised control.

To address the challenges observed in AT soil, recommended strategies include incorporating organic amendments to enhance soil quality, selecting stress-tolerant microbial strains capable of adapting to soil conditions, applying biofertilisers continuously at higher dosages, and utilising microbial strains with synergistic effects to improve plant production parameters.

Seasonality strongly influenced all morphological traits in both soil types (Table 2). Autumn produced the lowest values across all traits, whereas spring showed the highest values in GL soil and winter in AT soil. These differences can be attributed to the combined effects of photoperiod length and temperature. After considering all three main factors, our first hypothesis was supported, as each significantly influenced morphological parameters across both soils, with the exception of biofertiliser effects on rosette and stem fresh weight in GL soil. Previous studies similarly reported lower lettuce yields in autumn compared with winter and spring, primarily due to short-day effects [47,48]. In this study, winter photoperiods of 9–13 h and spring photoperiods of 14–15 h likely promoted the transition from vegetative to generative phase, resulting in greater stem elongation compared with autumn conditions. Finally, our third hypothesis was confirmed, demonstrating that seasonal variation significantly modulated plant responses. From a processing perspective, smaller and lighter stems are preferred, as they reduce waste and increase the proportion of edible mass [49].

Correlation coefficient analysis on morphological parameters in both soils revealed that increases in these traits were associated with heavier lettuce rosettes (Figures 2 and 3). A similar trend was reported in butterhead lettuce, where positive correlations were observed between plant fresh weight and other morphological parameters under varying photoperiods and light intensities [50]. The positive correlation between stem length and stem fresh weight indicates a risk of premature bolting, which may necessitate earlier harvesting and increase production losses during processing due to a higher proportion of inedible parts. These findings align with the literature data indicating positive correlations between plant fresh weight and stem length in lettuce [51,52].

Optical sensors are effective tools for frequent and rapid monitoring of plant nutrient status, including chlorophyll and flavonoids, and represent a sustainable monitoring alternative to conventional nitrogen fertilisation [53]. Their primary advantage lies in offering a fast, reliable, and non-destructive substitute for traditional wet chemistry analysis by assessing crop status through leaf transmittance, reflectance, or fluorescence. As such, they contribute to sustainable agricultural practices by enabling efficient fertilisation management and maintaining crop quality through real-time physiological and nutritional information [54]. Previous studies have employed optical sensors to investigate lettuce production in controlled environments under varying light and photoperiod treatments [55–57].

Green lettuce cultivars exhibited significantly higher NBI values, whereas red cultivars showed elevated levels of Chl, Flav, and Anth across both soil types (Table 3). The green cultivar 'Aquino' achieved the highest NBI value, the highest head fresh weight in GL soil, and ranked among the heaviest in fresh weight in AT soil (Tables 2 and 3). These parameters were positively correlated, indicating that improved nitrogen status (higher NBI) is associated with increased fresh head weight. A study on rice similarly demonstrated that NBI was strongly correlated with yield, supporting its use as a rapid indicator of nitrogen status and yield prediction [58]. Other studies further confirmed that higher NBI values reflect sufficient nitrogen supply, efficient utilisation, and adequate nitrogen content [53,55]. Chlorophyll content is a key parameter for cultivar selection and phenotyping, as it represents photosynthetic capacity and plant productivity, and can also serve as an indicator of nitrogen deficiency [59]. The positive correlation between NBI and Chl (Figures 2 and 3) highlights that increased nitrogen availability enhances chlorophyll synthesis. Optimal growing conditions increase chlorophyll content and keep flavonoid levels low, whereas nitrogen deficiency conditions affect chlorophyll in a way that supports and elevates flavonoid production [35]. Thus, leaf Chl content is strongly related to plant nitrogen level, supporting its use for non-destructive nitrogen estimation [60]. Among the red cultivars, 'Gaugin' exhibited the highest Chl, Flav, and Anth contents in both soils (Table 3). Previous HPLC analyses confirmed that red lettuce cultivars accumulate higher levels of phenolic acids and flavonoids compared with green cultivars [4]. Correlation coefficients revealed moderate to strong negative correlations between NBI and Flav, as well as between NBI and Anth (Figures 2 and 3), indicating that higher nitrogen availability affected C/N allocation and had an adverse effect on the accumulation of flavonols and anthocyanins. Nitrogen favours protein synthesis over secondary anabolism such as flavonoid biosynthesis (nitrogen-free secondary metabolites), resulting in reduced flavonoid accumulation at higher nitrogen concentrations [55,61].

The effects of biofertilisers varied between the two soil types: in GL soil, VT and combined fertilisers increased NBI, while combined fertilisers also enhanced Chl levels, indicating an improvement in primary metabolism. In contrast, in AT soil, VT application stimulated Anth and Flav accumulation but reduced NBI (Table 3), underscoring the role of *Trichoderma* fertiliser in promoting secondary metabolism. Thus, the second hypothesis was only partially supported: in GL soil, fertilisers increased NBI and chlorophyll levels, whereas in AT soil, they enhanced only flavonoid and anthocyanin content.

Plants in the autumn trial accumulated higher levels of anthocyanins compared with spring, suggesting that elevated temperatures may accelerate anthocyanin degradation (Table 3), a trend also reported in grapes under different temperature regimes [62]. Overall, considering the individual effects of the three main factors, our first hypothesis was largely supported for most physiological parameters, with two exceptions: the effects of biofertilisers on Flav and Anth content, and the effect of season on NBI in GL soil. In AT soil, chlorophyll content was not significantly affected by either fertiliser or season.

Flav content consistently showed a strong positive correlation with Anth content in both soil types (Figures 2 and 3), which can be attributed to their shared biosynthetic pathways and the influence of environmental conditions that promote their simultaneous increase. Overall, seasonal variation played a significant role in modulating plant responses, as reflected in the optical measurement parameters.

Taking the above points into account, our study presents several potential limitations. The contrasting effects observed between GL and AT soils indicate that the biofertilisers tested may not be universally effective across all soil types without careful adaptation. Although the study covered three consecutive growing seasons, potential long-term effects remain unexplored. Additionally, the identification of significant three-way interactions among cultivar, biofertiliser, and season highlights the complexity of these relationships and the need for future research to elucidate their mechanisms and possible formation patterns. Taken together, these factors collectively emphasise the importance of tailoring biofertiliser applications to specific cultivars and soil conditions to optimise agronomic performance.

5. Conclusions

The green lettuce cultivars ‘Aquino’ and ‘Kiribati’ showed the most favourable agronomic performance, with higher rosette fresh weight, leaf number, and stem fresh weight, making them strong candidates for fresh market and processing purposes. The red cultivar ‘Gaugin’ excelled in quality-related traits, including elevated chlorophyll, flavonoid, and anthocyanin levels, suitable for value-added products. Biofertiliser effects were soil-dependent: in GL soil, all tested biofertilisers enhanced most of the growth traits, whereas in AT soil reduced morphological performance. However, *Trichoderma*-based biofertiliser improved quality parameters in AT soil, underscoring the need for soil-specific management strategies. Seasonal variation played a decisive role, with spring and winter favouring morphological growth and autumn enhancing quality parameters.

It is recommended to prioritise green cultivars such as ‘Aquino’ and ‘Kiribati’ for fresh biomass and the red cultivar ‘Gaugin’ for enhanced nutritional quality. Biofertiliser application should be tailored to soil type: *Trichoderma*-based and combined biofertilisers in GL soil, whereas application in AT soil should be approached cautiously. Planting timing should align with seasonal advantages.

Practical strategies combining cultivar choice, tailored biofertiliser application, and seasonal planning can optimise yield and quality. Future research should address long-term application of biofertilisers, optimise biofertiliser application strategies, and explore the complexity of interactions between tested factors across different soil types.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11111372/s1, Table S1: Summary of three-way ANOVA mean squares and degrees of freedom for lettuce morphological parameters in two soil types; Table S2: Summary of three-way ANOVA mean squares and degrees of freedom for lettuce physiological parameters in two soil types.

Author Contributions: Conceptualization, M.S., Z.D., S.S., Z.J. and Đ.M.; methodology, Z.D., Z.J., S.S. and Đ.M.; software, Z.D. and M.S.; validation, S.S., Z.J., V.M., Z.D. and J.D.M.; formal analysis, M.S., Đ.M. and Z.D.; investigation, M.S., Z.D., S.S. and Đ.M.; resources, M.S., Z.D., Z.J., Đ.M. and V.M.; data curation, M.S. and Z.D.; writing—original draft preparation, M.S.; writing—review and editing, M.S., S.S., V.M., J.D.M., Z.J. and Đ.M.; visualisation, M.S.; supervision, Z.J., S.S., Đ.M. and V.M.; project administration, M.S., Z.J., Đ.M. and S.S.; funding acquisition, M.S., V.M., Đ.M. and Z.J. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. For further inquiries, please contact the corresponding authors.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

GL	Mollic Gleysol (Calcaric) soil
AT	Hortic Anthrosol (Terric, Transportic) soil
C	Control
EM	EM Aktiv
VT	Vital Tricho

EM+VT	EM Aktiv +Vital Tricho
TN	Total nitrogen
TC	Total carbon
$CaCO_3$	Calcium carbonate
SOM	Soil organic matter
SOC	Soil organic carbon
Chl	Chlorophyll content
Flav	Flavonoid content
Anth	Anthocyanin content
NBI	Nitrogen balance index
RH	Rosette height
RFW	Rosette fresh weight
NL	Number of leaves
SL	Stem length
SFW	Stem fresh weight
df	Degrees of freedom

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