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Effect of Different Soil Textures and Medicinal Solid Wastes on Growth Performance of Spinach

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ABSTRACT

Synthetic production of essential oils results in significant amounts of solid waste, the disposal of which is associated with environmental risks. The present study evaluated the potential of using mallow (*Althaea officinalis*), thyme (*Thymus vulgaris*), and nettle (*Urtica dioica* L) medicinal plant wastes after oil distillation as organic amendments in different soil textures for spinach cultivation. A complete randomized design with three replications, was used in the experiment to implement a factorial design. Two soil textures of loamy and sandy treated with 1) without organic matter (C), 2) animal manure (M1 and M2), 3) medicinal plant residues including mallow (N1 and N2), 4) thyme (T1 and T2), and 5) nettle (H1 and H2) at 5% and 10% concentrations. Results indicated that loamy soil provided greater nitrogen (N) and iron (Fe) availability. The H2 treatment demonstrated remarkable efficacy in enhancing spinach growth traits in loamy soil, yielding the highest measurements for shoot fresh weight (2.70 g), root fresh weight (0.97 g), and leaf fresh weight (6.25 g). In contrast, the control treatment recorded significantly lower values of 0.28 g, 0.18 g, and 0.99 g for each respective trait. Furthermore, loamy soil exhibited the lowest pH at 6.9, alongside the highest bulk density of 0.63 g/cm^3 and soil basal respiration rate of 116.43 mg CO_2-C g^{-1} soil d^{-1}, particularly noted with the application of M2. Overall, loamy soil, combined with the H2 treatment, is recommended for optimal spinach cultivation, as it significantly improves growth traits and nutrient content, promoting sustainable agricultural practices.

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Aggregate stability; medicinal plant; organic carbon; plant nutrition; soil texture; solid wastes

Introduction

Spinach (*Spinacia oleracea* L.), belongs to the *Chenopodiaceae* family and is indigenous to Western Asia, including regions such as South Turkestan, the Caucasus, Nepal, Iran, and China (Kallo and Bergh 1993). People have been cultivating and consuming spinach worldwide for a long time. Spinach, which is rich in nutrients and has high levels of bioactive compounds such as vitamins A and C and minerals, is a commercially important species due to its consumption of fresh leaves (Nemadodzi et al. 2017; Türkkan and Kibar 2022). While the crop exhibits good growth in several soil types, it prefers sandy loams. Importantly, soil texture significantly influences the growth of spinach, as it affects the retention and drainage of moisture, nutrient availability, and root development. Soils with finer textures, such as loamy soils, typically retain moisture more effectively, promoting healthier spinach growth compared to sandy soils, which drain quickly and may lead to water stress (Lekgoathi, Soundy, and Kgopa 2022; Machado et al. 2020).

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Soil texture is a critical physical property that refers to the different sizes of mineral particles within the soil (Wang et al. 2019). It serves as an important indicator of the soil's potential productivity, as it significantly affects moisture retention, nutrient content, pH, salt distribution, and aeration, which, in turn, influence farming practices (Wang et al. 2021). The moisture content, temperature, pH, and nutrient distribution vary across soils with different textures, ultimately impacting the physiological indices of crop growth, including water and fertilizer retention capacity and water use efficiency. Research indicates that when the sand content of soil is between 30–70%, the addition of organic matter can greatly enhance the soil's water holding capacity (Wang et al. 2021). It is well established that clay soils differ from sandy soils in terms of texture, structure, water holding capacity, nutrient status, and even temperature. An ideal clay content of 10–20% is essential for effective root penetration, as the proportion of clay particles influences the available moisture and the soil's strength. High bulk density combined with elevated soil strength can impede root penetration and elongation, restricting the roots' access to water and nutrients stored in these high-strength layers (Sharma et al. 2017).

The global agricultural industry produces large quantities of bioresidues, which can be solid, liquid, or gaseous, and is considered the most plentiful, profitable, and renewable resource on earth (Santana-Méridas, González-Coloma, and Sánchez-Vioque 2012). Global agricultural production has approximately 1.3 billion tons of organic waste annually, with developed countries producing 670 million tons and developing nations producing 630 million tons (Cavalheiro et al. 2020). The abundance of organic matter and nutrients found in agricultural waste materials, soil structure and productivity can greatly be enhanced (Liu et al. 2022; Muscolo et al. 2019; Williams et al. 2001). Environmentally responsible management and recycling of these residues is crucial, as the neglect of these resources has resulted in resource waste and environmental damage (Li et al. 2022). For instance, Machado et al. (2022) reported that municipal compost and biochar serve as viable alternatives to reduce reliance on coir and peat, thereby enhancing the sustainability of soilless culture in spinach cultivation. Their findings indicated that spinach fresh yield in blends containing 12% (v/v) of selectively collected municipal organic compost or biochar, combined with 10% (v/v) perlite or pine bark, was equal to or exceeded yields obtained with coir. Specifically, blends with municipal compost, perlite, or pine bark increased spinach fresh yield by 28% and 13%, respectively, compared to coir. Additionally, Chau and Diem (2024) demonstrated that a waste treatment mix comprising coffee grounds, cow manure, abalone mushroom residue, and rice husk ash yielded the best growth parameters for spinach. Furthermore, Ros et al. (2020) emphasized that compost extracts can contain biostimulant agents that promote plant growth by enhancing photosynthetic efficiency and stimulating root development.

Therefore, it is important to investigate the potential of converting agricultural waste into organic fertilizer to optimize resource use, reduce waste contamination, and promote soil fertility. For example, the use of certain microorganisms in bio-organic fertilizer derived from agricultural waste materials may have a substantial impact on enhancing microbial diversity and improving the soil environment and quality. This, in turn, accelerates root development and boosts crop output (Ansari and Mahmood 2017). Organic fertilizers have significant positive impacts on the physical environment and productivity of the soil and thus, improve dry matter accumulation, yield components, and crop yields (Ibrahim et al. 2015).

Medicinal plants have the potential to produce a significant quantity of agro- and industrial biomass. For instance, the root of some medicinal plants serves as the economic component and a reservoir of bioactive substances, whereas the above-ground biomass has little commercial utility. The aerial components (stem and leaf) are also significant reservoirs of bioactive chemicals (Jayaprakasam et al. 2003; Sharma et al. 2007). Basak (2017) defines biomass as leftover organic material that can either serve as animal feed or undergo transformation into more valuable products such as charcoal and compost. For plant nutrition, Saha and Basak (2020) state that fresh waste generated during the processing of aromatic and medicinal plants is a cost-effective alternative to rock phosphate and manure. Furthermore, this waste also contains enough nitrogen (N), phosphorus (P), and potassium (K) for producing amendments like compost for efficiently contributing to the preservation and rejuvenation of soil fertility (Filipović et al. 2023; Zaccardelli et al. 2021).

Researchers have studied about 3000 aromatic plants worldwide to extract essential oils (Saha and Basak 2020). The current estimate for the total global output of essential oils is around 104,000 tons (Lubbe and Verpoorte 2011). Saha and Basak (2020) provide an approximation of the substantial amount of residual biomass that the essential oil distillation business might potentially produce based on the essential oil production data. The remaining biomass has a high concentration of polyphenols and several other beneficial chemicals (Sayed Ahmad et al. 2018; Wang et al. 2018). After extracting the bioactive substances, the remaining biomass may be used to produce other high-value products like compost, charcoal, biofuel, biogas, biopesticides, and more (Gomez et al. 2017). Hence, the simultaneous use of distillation biomass not only reduces the cost of final products, but also addresses the issue of disposing of this substantial biomass.

The goal of this study was to evaluate the effects of farmyard manure and solid wastes from medicinal plants (mallow, thyme, and nettle) on the growth characteristics of spinach plants in two different soil textures: loamy and sandy. Mallow, thyme, and nettle were chosen due to their abundant availability in our region, primarily as by-products from the extraction of essential oils. Spinach was selected for this research because it is a fast-growing crop that can provide results in a relatively short time frame. Additionally, spinach is known for its sensitivity to various soil conditions, making it an excellent indicator for assessing soil health and quality. Its growth characteristics are significantly influenced by soil organic matter, and our study aims to address the low organic content typically found in our soils. These medicinal plants produce significant organic waste, which presents both a challenge for waste management and an opportunity for sustainable agriculture. This research is driven by the need to explore sustainable agricultural practices that utilize organic waste materials to enhance soil fertility and crop productivity while addressing the environmental risks associated with synthetic waste disposal. We hypothesize that spinach will demonstrate superior growth traits in loamy soil compared to sandy soil, as indicated by preliminary findings that show loamy soil enhances nitrogen and iron availability, crucial for plant development. Furthermore, we expect that the incorporation of medicinal plant residues will improve spinach growth relative to traditional fertilizers. By optimizing the use of these locally available organic materials, our study aims to provide valuable insights into the effectiveness of these organic amendments in promoting optimal spinach cultivation.

Materials and methods

Experimental site

The experiment was conducted in a greenhouse at Isfahan University of Technology (32° 43’ 4.80” N, 51° 31’ 55.20” E), Isfahan, Iran, during the years 2022–2023. Throughout this period, the average daily temperature was maintained at 25 ± 2 °C, while the average nightly temperature was 17 ± 2 °C.

Experimental design

A complete randomized design (CRD) with three replications, each with one plant, was used in the experiment to implement a factorial design.

Treatments

The treatments included two types of soil textures: loamy and sandy. Additionally, various types of organic matter were utilized: without organic matter (C), animal manure (M), mallow (*Althaea officinalis*) plant residue (H), thyme (*Thymus vulgaris*) plant residue (T), and nettle (*Urtica dioica* L) plant residue (N). Each type of organic matter was applied at two different concentrations: 5% and

Table 1. The information of experiment treatments.

Treatments	Levels	Abbreviation
Soil texture	Loamy	
	Sandy	
Organic matter	Without organic matter	C
	5% animal manure	M1
	10% animal manure	M2
	5% mallow plant residue	H1
	10% mallow plant residue	H2
	5% thyme plant residue	N1
	10% thyme plant residue	N2

10% of the total soil mass. These specific percentages were selected based on our previous research, which indicated that they provided optimal growth results without overwhelming the plants with high concentrations of organic material. Specifically, the treatments were defined as follows: C for the control (without organic matter), M1 for 5% animal manure and M2 for 10% animal manure, H1 for 5% mallow plant residue and H2 for 10% mallow plant residue, T1 for 5% thyme plant residue and T2 for 10% thyme plant residue, and N1 for 5% nettle plant residue and N2 for 10% nettle plant residue (Table 1).

Methodology for soil sample preparation and plant growth experiment

For measuring the physicochemical parameters, soil samples were taken from two different locations of Lavark (33° 40'N and 51° 25' E) with a loamy texture and Badroud (32° 49'N and 50° 26' E) with a sandy texture. Prior to conducting the experiment, we performed soil analysis to assess the initial conditions of the samples (Table 2). The soil samples were then air-dried and passed through a 2 mm sieve. The Barij Essential Pharmaceutical Company in Kashan provided the residue of the medicinal thyme, mallow, and nettle plants. These residues consist primarily of the above-ground parts of the plants, including stems and leaves, which remain after the essential oil extraction process. This biomass is often discarded, representing a significant organic waste that can be repurposed for agricultural use. After processing these plants and prepared essence, the waste was dried and pasteurized in an oven at 50 °C for 24 h, then passed through a 2 mm sieve was used as substrate. Next, the soils of each region were blended with ratios of 5% and 10% by weight from organic materials. The spinach (*Spinacia oleracea* var Shahroudi) seeds were planted in a mixture of soil and organic matter in a greenhouse setting because, while spinach is grown in both greenhouses and open fields in our region, this controlled environment allowed us to standardize growing conditions and eliminate variability from external factors such as temperature and humidity. This ensures that our findings focus solely on the effects of the substrate. Spinach seeds germinated 5–8 day after they were planted. The plants were irrigated manually, with the watering schedule tailored to meet the specific needs of the plants and the characteristics of the different substrates employed in our treatments. The various parameters were measured 40 days after the seeds were planted.

Table 2. Some of the soil physical and chemical characteristics measured in the experiment.

Soil sampling location	Soil texture	Sand (%)	Silt (%)	Bulk density ($mg.m^{-3}$)	FC (%)	pH	EC ($dS\ m^{-1}$)	CEC ($meq\ 100\ g^{-1}$)	Fe (mg kg^{-1})	Zn	Cu	Pb
Lavark 32.56° N, 51.37° E	Loamy	31	24	1.45	13.9	7.7	4.1	4.50	5	2.7	0.4	0.2
Badroud 51.01° N, 33.69° E	Sandy	56	13	1.35	16.5	7.5	2.1	3.60	2.5	1	0.2	–

Measurements and observations of the plants

Plant growth parameters

At the end of the experiment (40 days after the seeds were planted), the plants were harvested and cleaned. Then the shoots, leaves and roots were separated and the fresh weights were determined using an accurate laboratory scale (0.01 balances). A regular ruler was used to measure the length of the shoot and root (mm), the width of the leaves from the widest part of the leaf, and the length of the leaves from the tip to the intersection of the leaf with the stem. The number of leaves was obtained by counting the leaves of each plant.

The leaf's chlorophyll and carotenoid content

Leaf samples weighing 0.2 g were homogenized in 80% acetone. The extract was centrifuged for 5 min at 12,000 rpm while it was at room temperature. The optical density of the supernatant was measured at 663, 647, and 470 nm using a spectrophotometer (Lichtenthaler 1987). Using the following formulas, the chlorophyll concentration which is given in mg 100 g^{-1} fresh weight of leaf was determined: Carotenoids, chlorophyll *a*, and chlorophyll *b* were presented using Lichtenthaler (1987) formula.

Measurement of total N in plant leaves

The Kjeldahl method was used to calculate the total N content of plant leaves. First, test tubes were filled with 0.1 g of leaf samples. Each sample then received the addition of 1 g of catalyst and 7.5 mL of sulfuric acid. The samples were heated to 350 °C after being allowed to stand for 2 h. After preparing the device's solution and reagents, we turned it on and determined the percentage of total N content (Baker and Thompson 1992).

Assessment of nutrients in plant leaves

The leaf tissues were fragmented into minute pieces and subjected to oven-drying at a temperature of 60 °C for a duration of 48 h. It pulverized the leaf samples using a grinding machine, placed them into small plastic bags, and stored them in a dry environment. During the analysis, 1 g of each sample was transferred to a crucible and then subjected to a temperature of 550 °C in a furnace for a duration of 6 h to get the dry ash of the samples. The complete digestion of ashes was carried out by adding drops of 2 N HNO_3 and subsequently 1 N HCl solution. The combination was subjected to heating at a temperature of 70 °C for a duration of 20 min till a solution of a pale hue was achieved. The remaining substance was cleaned and strained into a 50 mL container using purified water and Whatman 4 filter paper. The N content was quantified using automated Kjeldahl equipment (V40, Iran) using the methodology described by Wang et al. (2020). The concentration of several heavy metals, including copper (Cu), manganese (Mn), zinc (Zn), and iron (Fe), in plant tissues was measured using atomic absorption spectroscopy (PerkinElmer AAnalyst 700). Each sample solution was replicated and analyzed using the same process to enhance the credibility of the acquired findings. Cu, Mn, Zn, and Fe standard solutions were created using a stock solution with a concentration of 1000 mg L^{-1} in 2 N HNO_3, sourced from Merck grade chemicals. Additionally, a blank sample was added to enhance the accuracy and precision of concentration determination for all the heavy metals being analyzed. Consequently, calibration curves were created for each element, and the concentration of heavy metals was measured in mg/kg of leaf dry weight (Souri et al. 2018; Souri, Hatamian, and Tesfamariam 2019).

Measurements and observations of the soil

Characterization of soil physical and chemical properties

Samples were collected from the top layer (0–10 cm) for determining the physical and chemical features of the soil. Auger was used to capture disturbed samples. The disrupted samples were allowed to dry naturally and were thereafter set aside for physical and chemical examination. The soil samples underwent sieving using a 4 mm sieve for the purpose of soil structural measurements and a 2 mm sieve for the characterization of other soil attributes. Blake and Hartge (1986) described the cylindrical method for determining bulk density. Soil pH was determined in saturated extracts using the technique described by McLean (1982). Rhoades (1996) outlined the procedure for measuring electrical conductivity (EC). Basal soil respiration was assessed by back-titration with HCl, following the method described by Chen et al. (2000). Nelson and Sommers (1996) outlined the wet oxidation method for determining the organic carbon content.

Stability of aggregates

The stability of aggregates was assessed using a rotating sieve apparatus with a succession of sieves with dimensions of 0.1, 0.25, 0.5, 1, and 2 mm, in accordance with the ASTM standard. The aggregate stability experiment was conducted for a duration of 3 min with a rotating speed of 733 cHz. The mean weight diameter (MWD) was determined using the equation provided by Kemper and Rosenau in 1986.

$$MWD = \sum_{i=1}^{N} W_1 \bar{X}_1$$

The variable wi represents the percentage of stable aggregates in the size class i (measured in mm), whereas X¯i represents the arithmetic mean diameter of the size class i (measured in mm). The weight of gravel or sand particles was subtracted to determine the wi.

Measurement of nutrient in soil

The sample 1 g), which had been dried in the oven, was measured using a top loading balance. The measured sample was then put in separate 250 mL Beakers. Subsequently, 15 mLof aquaregia, consisting of 35% HCL and 70% high-quality HNO_3 in a 3:1 ratio, was added to each beaker. The combination was thereafter heated to 70 °C and allowed to digest until the solution achieved transparency. The solution obtained was passed through a Whatman filter paper no. 42 and collected in a 50 mL volumetric flask. The flask was then filled up to the mark with deionized water. The resulting sample solution was analyzed for the concentrations of Cu, Zn, Cd, Ni, Mn, Cr, and Pb using an atomic absorption spectrophotometer (model AAnalyst700 from PerkinElmer Company USA) (Maurya, Kesharwani, and Mishra 2018).

Statistical analysis

The research used a factorial experimental design, using a CRD with three replications. The data were examined using Statistix 8 software, developed in Tallahassee, FL, USA. The data underwent a two-way analysis of variance (ANOVA), and the significance of the means was evaluated using the least significant difference (LSD) test at a significance threshold of $p < .05$. Principal Component Analysis (PCA) was conducted using Statgraphics Centurion, version XVI. A heat map graph was produced using the website https://discover.nci.nih.gov/cimminer/oneMatrix.do.

Results and discussion

The results of the analysis of variance and the main effect of treatments were presented in the Tables (S1-S6) of the supplementary materials.

The interaction effects of the treatments on spinach growth characteristics

In general, loamy soil yielded higher fresh weights for shoots than sandy soil, with the H2 treatment in loamy soil exhibiting the greatest fresh weight relative to other organic elements (Figure 1a). In loamy

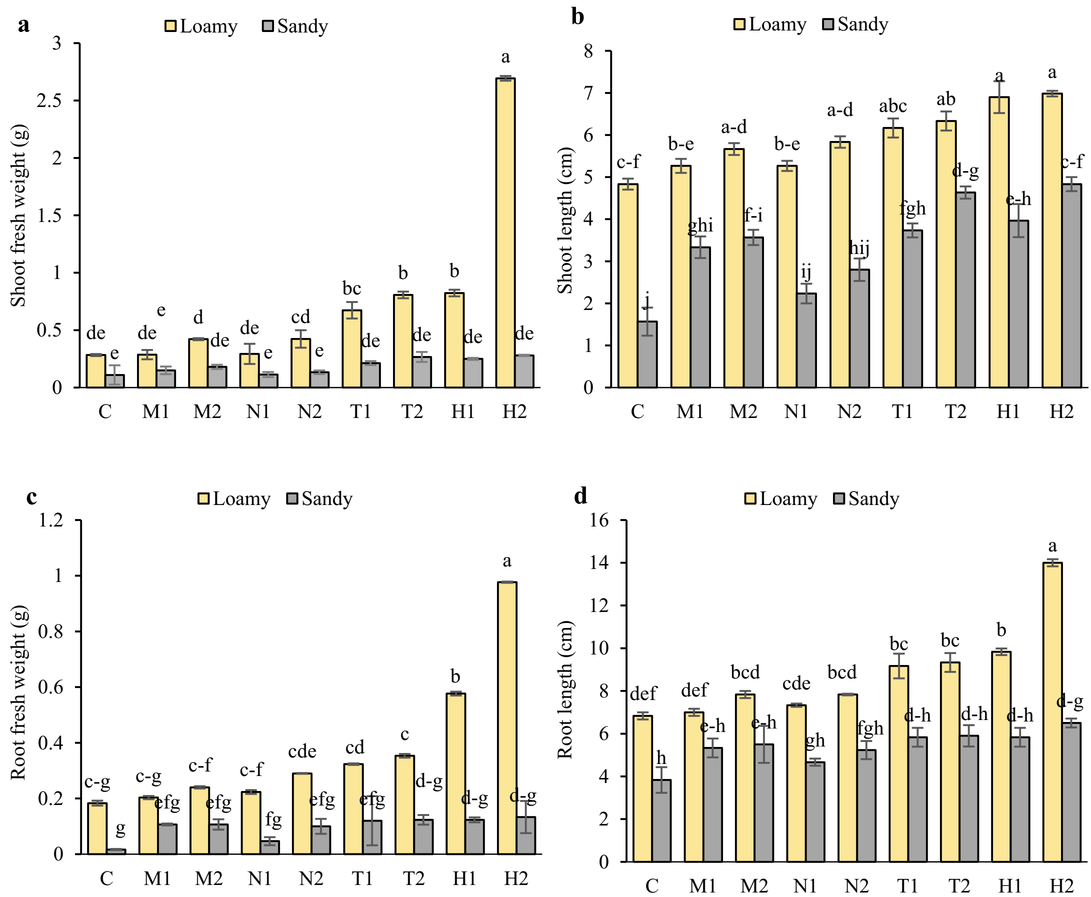

Figure 1. Interaction effects of soil textures (loamy and sandy) and types of organic matter on shoot fresh weight (a), shoot length (b), root fresh weight (c) and root length (d) of spinach. The treatments included: without organic matter (c), animal manure (M), mallow plant residue (H), thyme plant residue (T), and nettle plant residue (N). The numbers 1, and 2 corresponded to 5% and 10% of each organic matter in the bed. Significant differences between the treatments were denoted by different letters at a significance level of $p < .05$ using the least significant difference test (LSD) method. Errorbars were included in the graphs to show the standard error of the mean, indicating the uncertainty associated with the mean values.

soil, the length of shoots was larger compared to sandy soil. The H1, H2, T1 and T2 treatments had the greatest values (Figure 1b). Furthermore, the roots exhibited increased fresh weight and length in loamy soil compared to sandy soil. The H2 treatment in loamy soil showed the greatest measurements among the various organic components (Figure 1c,d).

In loamy soil, the fresh weight of leaves was greater than in sandy soil, with the highest amount observed in the H2 treatment compared to other organic materials. However, we found no significant differences among the various organic treatments in sandy soil (Figure 2a). The number of leaves was also higher in loamy soil than in sandy soil, with the greatest counts recorded in the M1, M2, N1, N2, T1 and T2 treatments. No significant differences were noted among the organic treatments in sandy soil (Figure 2b). Leaf length was greater in loamy soil than in sandy soil, with the highest lengths found in the T1, T2, H1 and H2 treatments compared to other organic materials (Figure 2c). Conversely, leaf length in sandy soil was greater than in loamy soil, with the longest leaves observed in the C, M2, and N1 treatments compared to other organic materials (Figure 2d).

Research has shown that using organic fertilizers, such as municipal solid waste compost residue from Chinese herbs, significantly enhances plant growth. For example, Machado et al. (2021) found that using municipal solid waste compost significantly enhanced the fresh weight of spinach. Similarly,

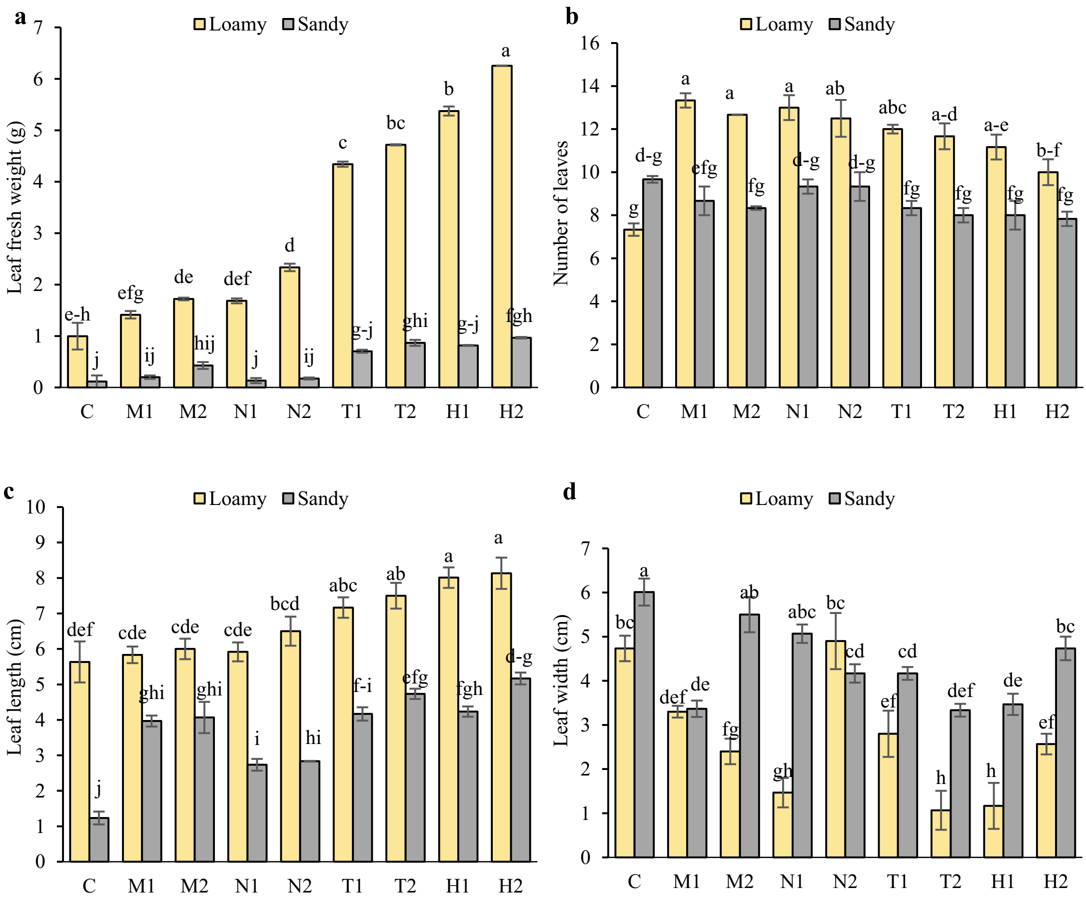

Figure 2. The interaction effects of soil textures (loamy and sandy) and types of organic matter on leaf fresh weight (a), number of leaves (b), leaf length (c) and leaf width (d) of spinach. The treatments included: without organic matter (c), animal manure (M), mallow plant residue (H), thyme plant residue (T), and nettle plant residue (N). The numbers 1, and 2 corresponded to 5% and 10% of each organic matter in the bed significant differences between the treatments were denoted by different letters at a significance level of $p < .05$ using the least significant difference test (LSD) method. Error bars were included in the graphs to show the standard error of the mean, indicating the uncertainty associated with the mean values.

tomato and cabbage plants' dry matter content increased with the addition of MAP residues (Zhou et al. 2023). Organic manures have the dual effect of increasing soil microbial populations as well as improving soil organic matter and structural stability (Sutrisno and Yusnawan 2018). Together, these factors result in improved growth and biomass production. The use of H2 treatment significantly enhanced the fresh weight of spinach shoots in loamy soil, with the maximum leaf fresh weight being 6.25 g.

The involvement of root systems in nutrient absorption is vital, and the release of root exudates may enhance the availability of nutrients in the soil, leading to an increase in crop yield (Burton, Brown, and Lynch 2013). Organic fertilizers stimulate root development, resulting in an increase in both the overall surface area and length of the roots (Chen et al. 2021). This, in turn, enhances the absorption of nutrients and water (Kaien et al. 2012). In addition, the availability of P has a beneficial impact on both root architecture and development (Niu et al. 2012). The results of our study showed that adding N to loamy soil resulted in a 105% increase in root length compared to the control, and that spinach produced better yields in clay soil compared to sandy soil (Rezk et al. 2007). This was due to the improved cation exchange capacity and nutrient availability in clay soil. Our research has shown that spinach growth and yield were significantly better in loamy soil as opposed to sandy soil, thereby emphasizing the advantageous impact of organic fertilizers on plant development.

The interaction effects of the treatments on the plant photosynthesis pigments

Loamy soil had higher levels of chlorophyll *a* than sandy soil, with the control treatment showing the lowest levels. However, the organic materials in loamy soil showed no significant differences (Figure 3a). The T2 and H2 treatments in sandy soil recorded higher concentrations of chlorophyll *b* compared to other organic materials. We found no significant differences among the organic treatments for chlorophyll *b* in loamy soil, as all were at a similar high level (Figure 3b). Carotenoids were also more abundant in loamy soil than in sandy soil, with the lowest amounts detected in the control treatment. However, no significant differences were observed among the organic treatments (Figure 3c).

A plant's ability to carry out photosynthesis strongly correlates with its chlorophyll concentration (Kiarostami, Mohseni, and Saboora 2010). Applying vegetable waste dramatically raised the levels of chlorophyll *a* and *b* in okra plants (Anwar et al. 2021). They proposed that the increased length and

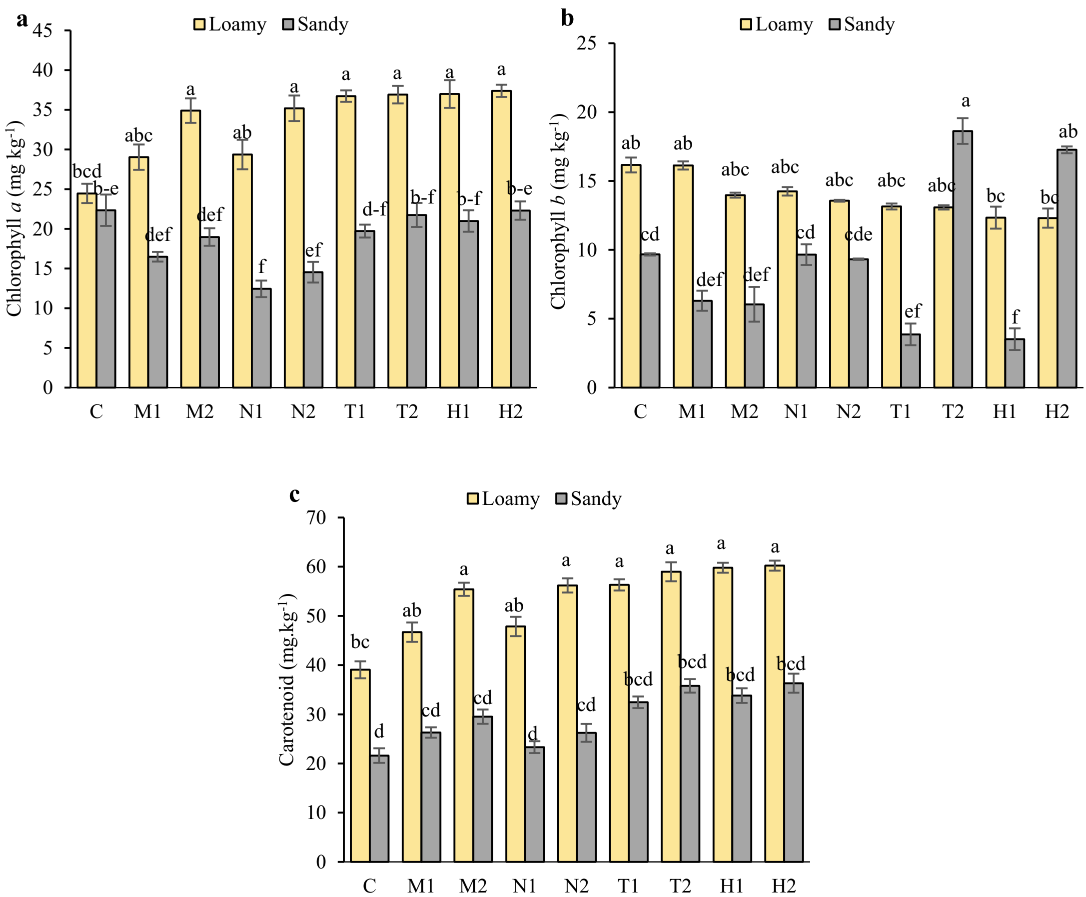

Figure 3. The interaction effects of soil textures (loamy and sandy) and types of organic matter on chlorophyll *a* (a), chlorophyll *b* (b) and carotenoid (c) of spinach. The treatments included: without organic matter (c), animal manure (M), mallow plant residue (H), thyme plant residue (T), and nettle plant residue (N). The numbers 1, and 2 corresponded to 5% and 10% of each organic matter in the bed. Significant differences between the treatments were denoted by different letters at a significance level of $p < .05$ using the least significant difference test (LSD) method. Error bars were included in the graphs to show the standard error of the mean, indicating the uncertainty associated with the mean values.

biomass of the roots, which improve water absorption and relative water content, might be responsible for this improvement. Plants treated with 35 tons of municipal solid waste compost per hectare exhibited greater levels of chlorophyll *b* than plants treated with other treatments in acidic soils (Machado et al. 2021). However, they also observed that increased compost rates ultimately led to a drop in total chlorophyll. The results of our study indicate that soil texture has a greater influence on photosynthetic pigments compared to the kind of organic matter used. Photosynthetic pigments showed a significant increase in loamy soil compared to sandy soil. However, there were no significant variations found in terms of the kinds and amounts of organic matter. Chlorophyll production is dependent on a number of nutrients, including critical micronutrients (Marschner and Marschner 2002). A shortage in these nutrients may have a negative impact on the structure and functionality of the photosynthetic apparatus (Kalaji et al. 2014). While clay soils frequently contain abundant nutrients, their inadequate drainage may restrict plant access to nutrients and water (Lekgoathi, Soundy, and Kgopa 2022). Our research revealed that loamy soil had significantly higher concentrations of photosynthetic pigments in comparison to sandy soil. This indicates that loamy soil has superior nutrient and moisture retention capabilities with organic waste addition, which consequently enhances the chlorophyll and carotenoid content in plants.

The interaction effects of the treatments on nutrition elements of spinach

The N concentration in plants was higher in loamy soil compared to sandy soil, with the lowest levels found in the control and M1 treatments. We observed no significant differences in N concentration among the other organic materials. Similarly, in sandy soil, there were no significant differences between the organic treatments and the control (Figure 4a). Loamy soil, specifically the N1 treatment, recorded the highest Zn concentration compared to other organic materials. We found no significant differences between the organic treatments and the control in sandy soil (Figure 4b). Fe concentration was greater in loamy soil than in sandy soil, with the highest amount found in the H2 treatment compared to other organic materials. Once again, we observed no significant differences between the organic treatments and the control in sandy soil (Figure 4c). The M1 and M2 treatment observed the highest Mn levels in loamy soil compared to other organic materials. Cu concentration was also higher in loamy soil than in sandy soil, with the highest levels in loamy soil found in the H2 treatment compared to other organic materials (Figure 4d).

Given the prevalence of heavy metals in this region, our focus on micronutrients such as Zn, Mn, and Cu was intentional to investigate their potential relationship with heavy metal uptake. By prioritizing these specific micronutrients, we aimed to understand how organic materials might influence the absorption and accumulation of heavy metals in plants, especially in loamy soil conditions where higher concentrations were observed.

According to Zhou, Selvam, and Wong (2016), herbal residues may contain cellulose, protein, and polysaccharides that can provide soil with N, P, and K after the organic matter decomposition (Wang et al. 2010). This was also evidenced in the MAP-enriched growing media in the Chrysargyris et al. (2023) study, as the N, K, Mg, and P levels were significantly increased with increasing ratios of waste in the media. Machado et al. (2021) found that when the treatment of municipal solid waste compost was below the recommended threshold of 2 to 4%, the leaf N concentrations in spinach plants increased. Surprisingly, neither the soil nor compost treatments significantly influenced the levels of Fe and Cu. The researchers found that the rise in soil pH did not decrease the amount of Fe in the plant shoots. Humic acids present in the compost likely bind the Fe (Bocanegra, Lobartini, and Orioli 2006; Chen, Clapp, and Magen 2004). In addition, the introduction of compost resulted in a reduction in shoot Mn concentrations, likely due to the elevated pH levels. This, in turn, decreased the amount of exchangeable Mn available in the rhizosphere. These findings suggest that the addition of municipal solid waste compost (MSWC) could potentially mitigate the negative effects of Mn toxicity, a prevalent issue in this specific soil type (Carvalho, Goss, and Teixeira 2015). Our study findings showed that applying T and N at concentrations of 5% and 10% led to the least amount of Mn in loamy soil

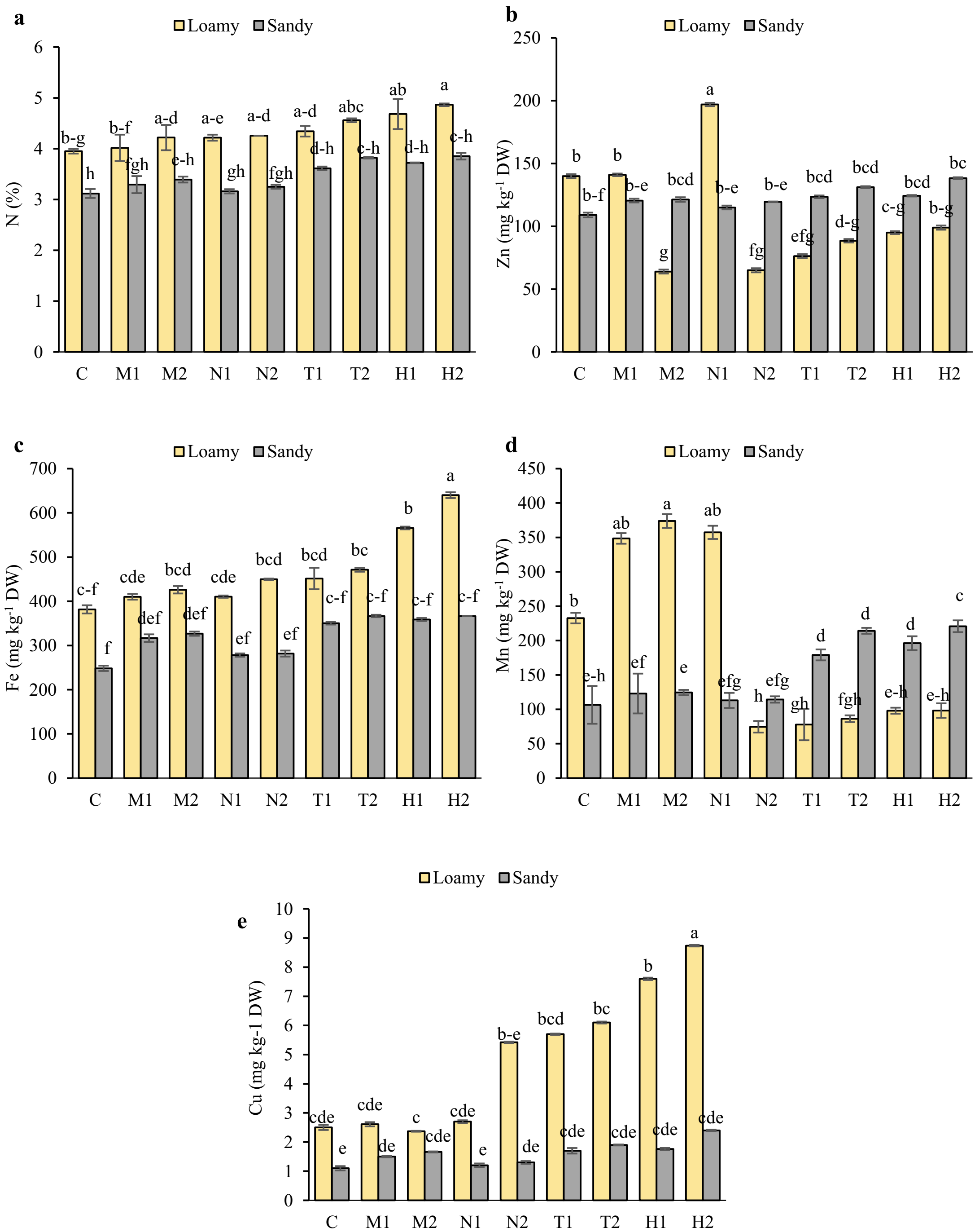

Figure 4. The interaction effects of soil textures (loamy and sandy) and types of organic matter on nutrition elements of spinach. The treatments included: without organic matter (c), animal manure (M), mallow plant residue (H), thyme plant residue (T), and nettle plant residue (N). The numbers 1, and 2 corresponded to 5% and 10% of each organic matter in the bed. Significant differences between the treatments were denoted by different letters at a significance level of $p < .05$ using the least significant difference test (LSD) method. Error bars were included in the graphs to show the standard error of the mean, indicating the uncertainty associated with the mean values.

compared to other organic materials. In addition, Machado et al. (2021) found that the application of MSWC resulted in an increase in both soil pH and shoot Zn concentrations. However, the increased rates of MSWC did not lead to a further elevation in shoot Zn levels, likely due to the increasing pH effect on Zn accessibility. MSWC treatment increased shoot Zn in tomatoes (Rajaie and Tavakoly 2016). Similarly, both tomatoes and lettuce showed an elevation in shoot Zn when treated with MSWC (Giannakis et al. 2014). Our investigation revealed that the application of treatment N1 in loamy soil resulted in a significant 40% increase in the Zn content of spinach leaves, as compared to the control.

The interaction effects of the treatments on the soil characteristics

Overall, the physical and chemical properties of loamy soil were superior to those of sandy soil. In loamy soil, the H2 treatment exhibited the highest levels of organic carbon, as well as elevated concentrations of Fe and Cu compared to other organic materials. The M2 treatment demonstrated the highest bulk density compared with control treatment (Table 3).

Incorporated mint distillation wastes into the soil to positively impact mustard (*Brassica juncea*) production and enhance the physicochemical qualities of the soil (Patra, Anwar, and Chand 2000). In neutral soils, adding compost did not have a significant impact on soil pH. However, in acidic soils, increasing levels of compost led to a considerable rise in pH (Machado et al. 2021). In general, the addition of MSWC resulted in higher soil pH values compared to the levels before the planting (Machado et al. 2021). Our study findings indicate that treatment M2 resulted in the lowest pH value of 6.91 when compared to the control in the loamy soil. The original pH of the soil, CEC, and the enhanced soil resistance to pH changes due to MSWC may contribute to the rise in pH variation. The humic acids included in MSWC enhance the CEC and buffering ability (García-Gil et al. 2004). Nevertheless, these increased pH levels could have a detrimental effect on plant nutrition by decreasing the availability of nutrients in the soil solution (Rousk, Brookes, and Baath 2009). Sandy soils treated with organic residues had elevated pH levels compared to untreated soils (Sukitprapanon et al. 2020). The rise in soil pH after the use of organic residues is probably affected by the alkalinity of ash produced from these residues, which may effectively elevate soil pH (Noble, Zenneck, and Randall 1996).

According to Machado et al. (2021), the soil's EC rose as the rates of MSWC treatment increased. Despite the rise, the EC values remained low, not surpassing 0.40 dS m^{-1}, which is far below the threshold that would hinder the development of spinach (2 dS m^{-1}) or most other vegetable and fruit crops (Machado et al. 2021). Studies including spiny chicory (Papafilippaki, Paranychianakis, and Nikolaidis 2015) and spinach (Machado et al. 2020) also found similar results, with the addition of compost with high EC at comparable rates. Our investigation found that using medicinal plant wastes resulted in greater EC compared to the control. The maximum EC value of 7.47 dS m^{-1} was seen in loamy soil treated with H2, which is much over the ideal EC level for spinach development. The increase in EC is attributed to the addition of organic residues used in our experiments. These plant residues, particularly from sources like animal manure and medicinal plants, contain various soluble salts and nutrients that contribute to higher ionic concentrations in the soil, thereby elevating the EC values.

Hao et al. (2008) discovered that the application of organic manures with lower levels of N, P, and K might increase the activity of microorganisms and the availability of nutrients. This ultimately leads to significant improvements in plant growth and biomass production. Organic additions have a beneficial impact on soil quality by stimulating the release of nutrients and improving their accessibility for plants (Birkhofer et al. 2008). Furthermore, these supplements help to reduce soil bulk density by increasing the quantity of biopores, expanding soil aeration, promoting soil organic matter, and stabilizing soil aggregates. Consequently, these supplements enhance the soil's water storage capacity and porosity, resulting in improved growth and biomass production (Gangwar et al. 2006; Papini et al. 2011). Our research found that applying treatment M1 to sandy soil resulted

Table 3. The interaction effects of soil textures (loamy and sandy) and types of organic matter on soil characteristics.

Soil	Organic matters	pH	EC (dS m^{-1})	Bulk density (g cm^{-3})	Organic carbon (%)	Basal soil respiration (mg CO_2-C g^{-1} soil d^{-1})	Aggregate stability (g)	Fe soil (mg kg^{-1} DW)	Zn soil (mg kg^{-1} DW)	Cu soil (mg kg^{-1} DW)
Loamy	C	7.796 a	2.831 efg	0.286 efg	0.764 de	107.140 ab	1.676 def	9.507 def	28.393 a	0.826 abc
	M1	7.813 a	2.932 ef	0.4267 b	0.772 d	110.710 ab	0.600 j	13.56 c-f	3.073 ef	0.906 ab
	M2	6.911 i	4.620 cd	0.636 a	0.998 bc	116.430 a	1.946 bcd	27.040 bcd	4.753 def	0.953 ab
	N1	7.363 efg	6.348 ab	0.270 fgh	0.786 cd	112.860 ab	1.726 c-f	21.427 b-e	4.504 def	0.906 ab
	N2	7.204 gh	7.476 a	0.263 ghi	1.000 bc	85.710 a-e	1.163 gh	31.173 bc	6.047 def	0.993 ab
	T1	7.577 bcd	5.380 bc	0.246 hij	1.009 b	85.710 a-e	1.410 efg	36.587 b	6.393 c-f	1.073 ab
	T2	7.749 ab	6.168 ab	0.260 ghi	1.128 b	89.290 a-d	0.940 hij	36.680 b	6.627 c-f	1.073 ab
	H1	7.769 ab	3.563 de	0.310 e	1.492 a	96.430 abc	0.716 ij	37.000 b	6.780 c-f	1.080 ab
	H2	7.360 efg	5.063 bc	0.363 d	1.577 a	98.210 abc	2.310 ab	56.787 a	7.260 c-f	1.146 a
Sandy	C	7.719 abc	1.167 i	0.300 e	0.278 h	50.000 e	1.07 ghi	2.760 f	1.380 f	0.473 d
	M1	7.797 a	1.494 f-i	0.230 j	0.454 fgh	75.000 b-e	1.130 ghi	12.093 def	13.520 bcd	0.560 cd
	M2	7.763 ab	1.549 f-i	0.250 hij	0.554 efg	75.000 b-e	2.136 abc	12.787 c-f	15.587 bc	0.746 bcd
	N1	7.506 de	2.112 e-i	0.400 bc	0.385 gh	75.000 b-e	1.923 bcd	3.133 ef	1.567 ef	0.533 cd
	N2	7.259 fgh	2.692 e-h	0.296 ef	0.454 fgh	75.000 b-e	1.910 bcd	5.307 ef	10.713 b-e	0.553 cd
	T1	7.452 def	1.765 f-i	0.360 d	0.654 def	50.000 e	2.120 abc	13.253 c0f	18.293 b	0.793 bcd
	T2	7.461 def	2.373 e-i	0.373 cd	0.717 de	57.140 de	1.823 cde	6.147 ef	18.500 b	0.813 abc
	H1	7.143 h	1.257 hi	0.380 cd	0.702 de	105.450 ab	2.413 a	14.520 c-f	18.340 b	0.800 bcd
	H2	7.527 cde	1.379 ghi	0.236 ij	0.750 de	60.710 cde	1.330 fgh	9.008 def	2.653 ef	0.820 abc

The treatments included: without organic matter (C), animal manure (M), mallow plant residue (H), thyme plant residue (T), and nettle plant residue (N). The numbers 1, and 2 corresponded to 5% and 10% of each organic matter in the bed. Significant differences between the treatments were denoted by different letters at a significance level of $p < .05$ using the least significant difference test (LSD) method.

in a 23% decrease in soil apparent density compared to the control. This demonstrates the efficacy of organic manures to improve soil structure.

Soil aggregate stability is a critical parameter for evaluating the development, degradation of soil structure (Six et al. 2004). Recent data has shown a favorable correlation between the use of organic fertilizers in agricultural systems and the increase in soil aggregate stability (Du et al. 2020). According to Wang et al. (2017), organic fertilizers include binding agents that improve the stability of soil aggregates. Furthermore, a study has shown that both organic amendments and mineral fertilizers have a beneficial impact on aggregate stability (Adnan et al. 2020). Different data emphasized that the application of organic amendments enhances the stability of soil structure by increasing the percentage of water-stable aggregates (Du et al. 2020). Chen et al. (2021), also found that adding both organic amendments and mineral fertilizers together made both the average weight diameter and the amount of water-stable aggregates much higher than when they were used alone. Our research found that applying treatment H1 to sandy soil led to a significant 125% improvement in aggregate stability compared to the control.

Microaggregates, with a size ranging from 0.053 to 0.25 mm, play a crucial role in storing organic carbon. On average, they account for 41% of the total organic carbon (Sukitprapanon et al. 2020). Notably, soils treated with tamarind leaf litter had the greatest carbon storage rate in these microaggregates, reaching 49%. The chemical composition of organic residues, including total N, lignin, and polyphenols, is essential for the accumulation of organic carbon in soils (Palm et al. 2001). Treatments H1 and H2 resulted in elevated amounts of organic carbon in loamy soils compared to the control. Adding MSWC led to soil organic matter contents exceeding 3.5% in neutral soils and 2% in acidic soils (Machado et al. 2021). Conversely, when applied to acidic soil, the same amounts of MSWC led to very modest increases of just 0.45% and 0.90%, respectively. The changes in soil parameters, such as bulk density or the rates at which organic matter decomposes, may account for these discrepancies (Machado et al. 2021).

The overall concentration of heavy metals in the soil is anticipated to stay constant or maybe rise when organic matter is added. A comprehensive 11-year field study was conducted to investigate the impact of different organic wastes on soil heavy metal content. The results revealed that applying organic amendments increased the total amount of heavy metals in the soil compared to the control. However, the availability of these heavy metals did not consistently increase (Wierzbowska et al. 2018). Our investigation found that applying treatment H2 to loamy soil led to elevated concentrations of Fe and Cu in comparison to the control. Existing studies suggest that the pH of the soil and the amount of organic matter rise. This is due to the formation of carbonate or hydroxyl complexes, as well as stable complexes with humic substances (Colombo et al. 2014). Researchers have linked the application of more manure to an increase in soil pH and a decrease in extractable heavy metal levels like Cu and Zn (Ramzani et al. 2016). Higher organic matter concentration in soils has also been associated with lower Zn and Cu availability (Ramzani et al. 2016).

The PCA analysis and heat map of the effect of the treatments on plant and soil parameters

Overall, the PCA plot results showed a correlation between plant growth traits and the levels of Fe, N, and Cu in the plants, with the highest concentrations observed in loamy soil under the H1, H2, T2 and T2 treatments. Bulk density and microbial respiration were also correlated with leaf number and Mn levels, with the M1 and M2 treatments showing the highest values (Figure 5a).

The heat map analysis revealed that the treatments primarily influenced the levels of plant elements, photosynthetic pigments such as chlorophyll *a* and carotenoids, and microbial respiration among the studied traits. Among the plant elements, Fe, Mn, and Zn were more affected by the treatments, with Fe clustering separately. The highest Fe levels were found in loamy soil under the H1, H2, T1 and T2 treatments, while the lowest levels were in sandy soil under the control treatment. The highest Mn concentration in plants was observed in loamy soil under the M1 and M2 treatment, with

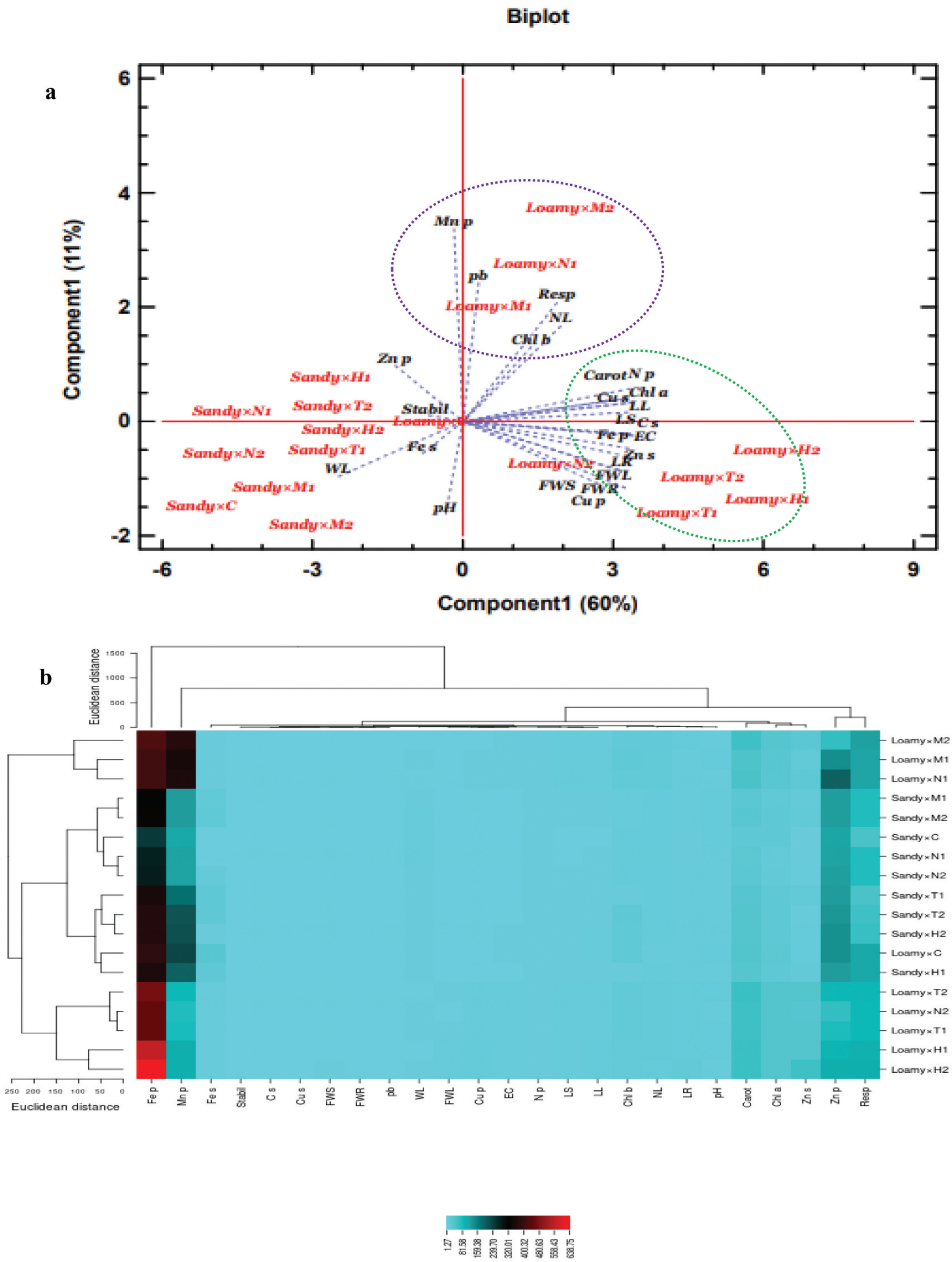

Figure 5. PCA analysis (a) and heat map graph (b) of the interaction effect of type texture of soil and different organic matter on the spinach plant and soil bed. Fresh weight shoot (FWS), length of shoot (LS), fresh weight root (FWR), length of root (LR), fresh weight leaf (FWL), number of leaves (NL), length of leaf (LL), width of leaf (WL), chlorophyll *a* (chl a), chlorophyll *b* (chl b), carotenoid (carot), nitrogen of plant (N p), zinc of plant (zn p), iron of plant (fe p), magnesium of plant (mn p), copper plant (cu p), pH soil (pH), EC soil (EC), bulk density (pb), organic carbon (C s), basal soil respiration (resp), aggregate stability (Stabil), zinc of soil (zn s), iron of soil (fe s), copper soil (cu s).

the lowest levels in loamy soil. In loamy soil, microbial respiration and photosynthesis pigments were greater than in sandy soil (Figure 5b).

Conclusions

This study emphasizes the advantages of loamy soil over sandy soil in promoting spinach development, particularly highlighting the outstanding results of the 10% mallow plant residue treatment. This specific treatment not only improved the growth traits of spinach but also significantly increased the Fe content in the spinach leaves, demonstrating a positive impact on plant health and nutrient absorption. The combination of loamy soil with the 10% mallow plant residue treatment resulted in the highest levels of soil Fe, organic carbon content, and soil stability, making it an excellent option for spinach cultivation. Overall, the combination of loamy soil and the 10% mallow plant residue treatment is recommended for optimal spinach cultivation, as it significantly enhances growth traits and nutrient content.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

All data are available upon request to the corresponding author, Maryam Haghighi (mhaghighi@cc.iut.ac.ir).

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