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High Soil pH and Plastic-Shed Lead to Iron Deficiency and Chlorosis of Citrus in Coastal Saline–Alkali Lands: A Field Study in Xiangshan County

Yili Zang [1,2,†], Yu Huang [3,†], Xiaoyan Chang [1,2], Jiuzhou Chen [1,2], Tianchi Jiang [1,2], Zhiying Wu [1], Lingli Lu [1,2] and Shengke Tian [1,2,*]

1 Key Laboratory of Environment Remediation and Ecological Health, Ministry of Education, College of Environmental & Resource Science, Zhejiang University, Hangzhou 310058, China
2 Zhejiang Provincial Key Laboratory of Subtropic Soil and Plant Nutrition, Zhejiang University, Hangzhou 310058, China
3 Xiangshan County Agricultural and Rural Bureau, Ningbo 315700, China
* Correspondence: tiansk@zju.edu.cn; Tel.: +86-571-8898-2515; Fax: +86-571-8898-2907
† These authors contributed equally to this work.

Abstract: Citrus are one of the most important fruit crops in the world, and the citrus hybrid cv. Beni-Madonna (BM) is widely cultivated in Xiangshan County in China because of its profitability. However, due to the lack of technical guidance and management, nutritional unbalances of trees occur frequently and are a major constraint for fruit quality and yield. In this study, the soil and fruit nutritional status of 62 citrus orchards in three diverse landforms (mountain, flat, and coastal saline–alkali land) located in Xiangshan County has been investigated. Higher pH, calcium, and magnesium content but lower soil organic matter, nitrogen, iron, and boron content were observed in orchards of the coastal saline–alkali land compared with the mountain and flat lands. Compared to Citrus x unshiu, another major hybrid citrus cultivar in Xiangshan county, the Fe deficiency in fruits of BM in coastal saline–alkali lands was more severe, leading to chlorosis symptoms. In addition, long-term cultivation under plastic housing increased soil salinization and affected the absorption of Fe and other nutrients by BM. In conclusion, organic fertilizers, acidic fertilizers, and micronutrient fertilizers should be applied in citrus orchards located in coastal saline–alkali lands. Especially, BM orchards should be supplemented with iron fertilizer and not rely excessively on protection with plastic films.

Keywords: citrus cv. Beni-Madonna; coastal saline–alkali soil; chlorosis; iron deficiency; plastic house

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1. Introduction

Citrus is one of the most important fruit crops in the world, and China is the first producer country in the world [1]. The citrus cultivar Beni-Madonna (BM), also known as Ehime Kashi No. 28, is largely appreciated by customers because of its excellent taste and long shelf-life [2,3]. In China, BM was first introduced in Xiangshan County, in the eastern coastal area of Zhejiang Province, which has become a typical production area. Now it is cultivated in many provinces across China [4].

However, high quality BM also has very high requirements for growing conditions. Its high quality generally needs to be achieved through protected cultivation [5]. Adverse growing conditions can lead to a decline in tree vigor, jeopardizing fruit quality and yield [6]. In recent years, chlorosis and shedding of leaves has been observed in many citrus orchards [7]. BM trees in Xiangshan County often show obvious trace element deficiency symptoms, especially in the coastal saline–alkali land, resulting in a decline in citrus yield and quality.

Iron (Fe) is an essential nutrient and plays a key role in important biochemical processes, such as respiration and photosynthesis [8]. Fe deficiency not only leads to chlorosis [9], but also causes a decline of fruit yield and quality [10]. In particular, citrus are very sensitive to iron deficiency [11]. However, many fruit orchards worldwide are planted on calcareous and alkaline soils [12], where the availability of Fe is quite low [13], which favor the occurrence of Fe chlorosis. Hence, it urges to find out the condition and cause of citrus nutrient deficiency to improve citrus quality.

In this study, Xiangshan County, a typical coastal saline–alkali land, and the citrus cv. Beni-Madonna were taken as a model system to investigate the relationship between soil type and tree nutritional status. The objective was to provide theoretical basis and technical guidance for nutrient management of citrus orchard in calcareous and alkaline soils.

2. Materials and Methods

2.1. Geographical Location

Xiangshan County is located in the southeast of Ningbo city, Zhejiang Province, China (28°51′18″ N–29°39′42″ N, 121°34′03″ E–122°17′30″ E). This county mainly consists of low hills and coastal tidal flats, with a small portion in non-coastal plains. The annual rainfall of Xiangshan is more than 1400 mm and the local mean yearly temperature is 16–17 °C.

2.2. Sample Collection in Xiangshan County

Overall, soil samples were collected in 62 citrus orchards in Xiangshan County in September 2017 (as noted in Figure 1). In each orchard, eight citrus trees with uniform age and size were selected according to a z-shaped sampling scheme, and about 1.0 kg surface layer (0–20 cm) of soil samples were collected from each tree. The soil samples were then air-dried and screened through 20 mesh and 100 mesh for subsequent determination of physical and chemical properties. Then in November 2017, among the orchards mentioned above, 31 orchards were further selected for fruit sampling. Citrus varieties included the cv. Beni-Madonna (BM) and *Citrus unshiu*. In each orchard, eight trees of each cultivar with the same age and size were selected, and from each tree, 10 mature fruits with similar size were picked up. Fruits from the same tree were regarded as a single sample. The soil and fruit samples were all stored in an ice box during the delivery.

2.3. Sample Collection of Selected Beni-Madonna Orchards

To further explore the cause of nutrient deficiency in citrus, tree body, and fruit of citrus orchard in coastal saline–alkali in Xiangshan County, five representative cv. Beni-Madonna (BM) orchards were selected among the 62 orchards. The five sites are shown in Table S1. Three of them were located in coastal saline–alkali land (Dongwei, DW; Dayang, DY; Xiaotang. XT) and another two were located in non-coastal alkali land (Maoyang, MY; Qiangtou, QT) as control. The intermediate rootstock of BM is *Citrus unshiu*, and the basic rootstock is *Citrus aurantium* L. Soil samples and fruits samples were collected as described above. In addition, in each BM orchard leaves, branches, and fine roots (diameter less than 3 mm) were collected from both trees with chlorotic and normal leaves as well as from both trees covered and non-covered with plastic film. The leaf and branch samples were separated into young leaves, mature leaves, old leaves, young stems, mature stems, and old stems, respectively.

2.4. Soil Analysis

The properties of soil samples including pH, soil organic carbon (SOM), total nitrogen (TN), total phosphorus (TP), total potassium (TK), available iron (AvFe), available manganese (AvMn), available boron (AvB), and total content of calcium (Ca), magnesium (Mg) were analyzed. For soil samples collected from saline–alkali land and non-saline–alkali land, pH, soil electrical conductivity (EC), soil organic carbon (SOM), alkali-hydrolysable nitrogen (AN), Olsen phosphorus (OP), available potassium (AK), exchangeable Ca (ExCa), exchangeable Mg (ExMg), AvFe, available Zn (AvZn), AvMn, AvCu, and the total content of N, K, Ca, Mg, Zn, Fe, Mg, and Cu were measured. Specific methods can be found in the Supplementary Materials.

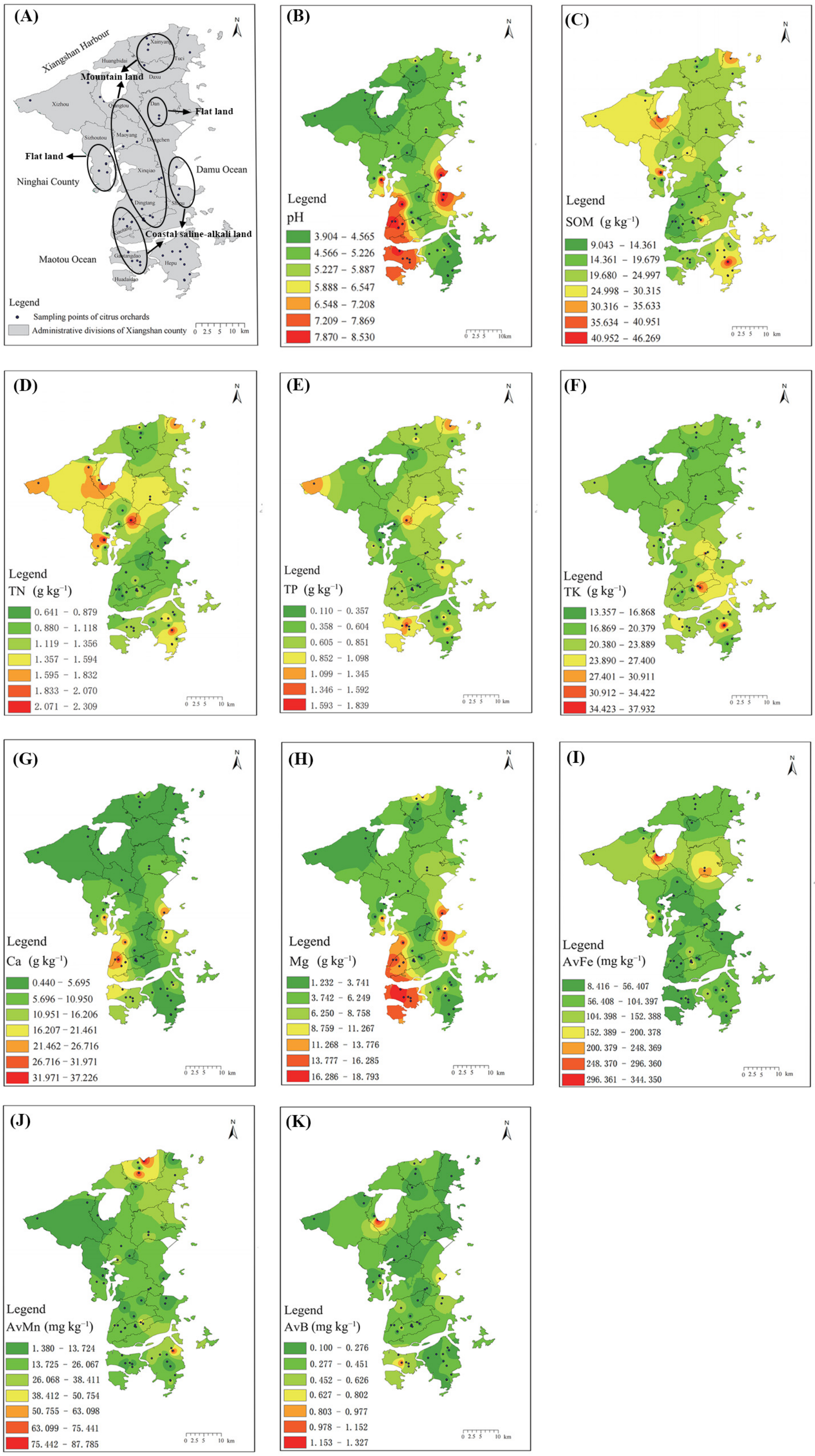

Figure 1. (**A**) Sampling points of citrus orchards in Xiangshan county. Spatial distribution of soil pH (**B**), soil organic matter (SOM) (**C**), the content of total N (TN) (**D**), total P (TP) (**E**), total K (TK) (**F**), total Ca (**G**), total Mg (**H**), available Fe (AvFe) (**I**), available Mn (AvMn) (**J**), available B (AvB) (**K**).

2.5. Measurement of Mineral Nutrient Contents in Plants

Leaves and fruits were oven-dried at 65 °C for 72 h and ground using a stainless-steel mill. About 0.1 g plant samples were digested with 5.0 mL HNO_3-H_2O_2 (4:1, *v*/*v*). The contents of mineral elements (i.e., Fe, Zn, Cu, Mn, Ca, K, Mg, B) were analyzed using inductively coupled plasma mass spectroscopy (ICP-MS, Plasma Quant 9000 Elite, Analytik Jena, Jena, Germany).

2.6. Determination of Fruit Quality

Each fruit was weighted by electronic balance. Fruit shape index was determined by calculating the ratio of longitudinal diameter to transverse diameter. Total soluble solid (TSS) was determined using a portable refractometer (PAL-1, ATAGO, Fukaya-shi, Japan). Vitamin C (VC) content was measured using 2,6-dichlorophenol titration method [14]. Each replicate is the average of ten fruits.

2.7. Statistical Analysis

All data were statistically analyzed using the SPSS package (Version 20.0). Significant differences were detected by Duncan's test ($p < 0.05$). Spearman was used for correlation analysis.

3. Results

3.1. Distribution of Soil Properties in Citrus Orchards in Xiangshan County

3.1.1. Soil pH

The physical and chemical properties of soil in the 62 citrus orchards in Xiangshan County varied considerably in different citrus orchards (Figure 1). The soil pH of citrus orchards located in saline–alkali land ranged from 6.30–8.60 (Figure 1A), while the optimum pH value for citrus growth ranges from 5.5 to 6.5 [15]. Conversely, the soil pH of citrus orchards in plain land and mountainous land, ranged from 3.90 to 6.30 (Figure 1A). Furthermore, 20.97% of the investigated citrus orchards were located in the strongly alkaline coastal saline–alkali land, with soil pH > 7.50 (Table S1). The soil pH of 32.26% of the investigated orchards was lower than 4.50 (Table S1). Among the investigated 62 citrus orchards, only 9.68% met optimum soil pH requirement for citrus growth (Table S1).

3.1.2. Soil Organic Matter

As shown in Figure 1B, citrus orchards with low organic matter content were mainly located in coastal saline–alkali areas, while citrus orchards with high organic matter content were mainly distributed in mountainous and flat areas in the north and south of Xiangshan County, showing a similar pattern with soil pH (Figure 1A). The citrus orchard with the lowest organic matter content was (only 8.98 $g \cdot kg^{-1}$) was located in the coastal saline–alkali area (Table S2). This value was far below the optimum requirement (15–30 $g \cdot kg^{-1}$) for citrus growth [15].

3.1.3. Total Contents of Nutrients in Soil

The spatial distribution of soil total nitrogen (TN), total phosphorus (TP), and total potassium (TK) in Xiangshan Citrus orchards is shown in Figure 1D–F. Soil TN was higher in the north and lower in the south of Xiangshan County (Figure 1D). In coastal saline–alkali land, the content of TN was in conditions of deficiency or extreme deficiency (Table S3). In terms of spatial distribution of soil TP in citrus orchards was low in general (Figure 1E). In terms of citrus orchards in the mountain, flat land, and coastal saline–alkali regions, they were all in TP deficiency status (Table S3). The spatial distribution of soil TK in citrus orchards was lower in the north and central part of the county, but higher in the southeast part (Figure 1F). Differently from TN and TP, the content of TK in citrus orchards in coast saline–alkali was most in the appropriate range (Table S3).

The spatial distribution of calcium (Ca) and magnesium (Mg) showed an overall low trend, and in the southwest and southeast coastal saline–alkali land tended to be high

(Figure 1G,H), similar to the distribution pattern of soil pH (Figure 1B). The total Ca in citrus orchards in coastal saline–alkali was about 19.81 g kg^{-1}, which was four to six times higher than mountains and flat land (Table S4). The total Mg in the citrus orchard in coastal saline–alkali was about 15.16 g kg^{-1}, more than three times of mountain and flat land (Table S4).

3.1.4. Available Contents of Nutrients in Soil

The content of AvFe and AvMn in Xiangshan County varied greatly (Figure 1I,J), ranging from 7.30 mg·kg^{-1} to 259.00 mg·kg^{-1} (Table S5), while the distribution of AvB in the county was relatively even (Figure 1K and Table S5). Citrus orchards with low AvFe were concentrated in the coastal saline–alkali area, and their spatial distribution was similar to that of organic matter, the lowest was only 10.5 mg·kg^{-1}, which could easily lead to iron deficiency in citrus (Table S5). The content of AvFe in mountain and citrus orchard was high, which could lead to iron poisoning (Table S5). The distribution of AvMn was uneven, and values were extremely low in some orchards and excessively high in others (Table S5). The shortage of AvB was extremely serious, and all 46 citrus orchards in mountains and plane were suffering B deficiency (Table S5).

3.2. Nutrient Contents of Citrus Trees

3.2.1. Fruit Nutrient Contents in Different Landforms

The content of Ca in the whole county was between 109.00 and 430.00 mg·kg^{-1}, with an average value of 255.81 mg·kg^{-1} (Table 1). The distribution pattern of fruit Ca content in citrus orchards was similar to that of the soil, and the coastal saline–alkali land had the highest Ca content with 289.83 g·kg^{-1} (Table 1). The Mg content of fruit varied between 74.50 and 134.00 mg·kg^{-1}, with an average of 105.30 mg·kg^{-1}, and it was slightly lower in coastal saline–alkali compared with orchards in mountains, in contrast with the distribution pattern of Mg content in soil (Table 1). The spatial distribution of fruit Zn and Fe in citrus orchards was consistent. The average Fe content of fruits was 3.78–3.97 mg·kg^{-1}, and the average Zn content was 1.33–1.38 mg·kg^{-1}, with no significant difference (Table 1). However, the Mn content in the fruits varied greatly. The Mn content varied between is 0.39 and 3.19 mg·kg^{-1}, with a variation range of 2.80 mg·kg^{-1}. The content of Mn in fruits collected in citrus orchards on the mountains was higher than that in the flatland and coastal saline–alkali land (Table 1). The average content of Cu in fruit varied between 0.26 and 0.32 mg·kg^{-1}, and was slightly higher in mountain orchards (Table 1). The average B content varied between 1.39 and 1.77 mg·kg^{-1}, and in coastal saline–alkali land was higher than in mountain and flat land (Table 1).

Table 1. The contents of mineral nutrients in citrus fruits in Xiangshan County.

Element	Landform	Range	Mean	Variation Coefficient
Ca (mg·kg^{-1})	Whole county	109.00–439.00	255.81	31.33
	Mountain land	109.00–396.00	266.44	30.34
	Flat land	134.00–328.00	205.40	25.62
	Coastal saline–alkali land	198.00–439.00	289.83	26.79
Mg (mg·kg^{-1})	Whole county	74.50–134.00	105.30	19.04
	Mountain land	87.00–133.00	117.78	17.04
	Flat land	74.50–130.00	101.87	18.25
	Coastal saline–alkali land	79.80–134.00	98.81	16.91
Zn (mg·kg^{-1})	Whole county	0.51–2.50	1.36	35.32
	Mountain land	0.77–2.48	1.33	35.65
	Flat land	0.51–2.23	1.38	34.20
	Coastal saline–alkali land	0.75–2.50	1.36	35.92

Table 1. *Cont.*

Element	Landform	Range	Mean	Variation Coefficient
Fe ($mg \cdot kg^{-1}$)	Whole county	2.67–5.79	3.85	20.61
	Mountain land	2.79–5.32	3.80	20.53
	Flat land	3.04–4.71	3.97	14.11
	Coastal saline–alkali land	2.67–5.79	3.78	24.91
Mn ($mg \cdot kg^{-1}$)	Whole county	0.32–3.19	1.60	61.32
	Mountain land	0.87–3.19	2.27	53.79
	Flat land	0.32–2.50	1.41	44.55
	Coastal saline–alkali land	0.35–2.19	1.25	58.40
Cu ($mg \cdot kg^{-1}$)	Mountain land	0.15–0.52	0.28	29.25
	Flat land	0.17–0.52	0.32	33.50
	Coastal saline–alkali land	0.15–0.40	0.28	25.96
	Whole county	0.15–0.39	0.26	23.26
B ($mg \cdot kg^{-1}$)	Mountain land	0.88–2.58	1.64	21.90
	Flat land	0.88–2.05	1.39	26.90
	Coastal saline–alkali land	1.29–2.58	1.70	19.84
	Whole county	1.26–2.13	1.77	14.59

3.2.2. Fruit Nutrient Contents of Different Citrus Varieties

The mineral nutrient contents of the two cultivars (Beni Madonna and *Citrus unshiu*) in mountainous, flat, and coastal saline–alkali land in Xiangshan County are shown in Table 2. The Ca and Fe contents of BM in coastal saline–alkali citrus orchards were significantly lower than those of *C. unshiu* (Table 2). In addition, the Mn content of *C. unshiu* in flat land was significantly higher than that of BM, and the Zn content of *C. unshiu* in mountain areas was also significantly higher than that of BM (Table 2).

Table 2. The nutrient contents of the citrus hybrid Beni-Madonna and *Citrus unshiu* in Xiangshan County. The significant differences at $p < 0.05$ was indicated by small letters. The significant differences between the two varieties were indicated by asterisks (* $p < 0.05$; ** $p < 0.04$). a, a′ was used for the significant difference among landforms of Beni-Madonna and *Citrus unshiu*, respectively.

Element	Beni-Madonna			*Citrus unshiu*		
	Mountain Land	Flat Land	Coastal Saline–Alkali Land	Mountain Land	Flat Land	Coastal Saline–Alkali Land
Ca ($mg \cdot kg^{-1}$)	283.50 ± 50.77 a	178.20 ± 30.80 b	251.14 ± 31.90 a	253.00 ± 41.67 ab′	208.78 ± 32.30 b	344.00 ± 89.51 a′*
Mg ($mg \cdot kg^{-1}$)	104.23 ± 12.16 a	96.23 ± 8.23 a	89.12 ± 6.29 a	128.62 ± 18.49 a′	121.00 ± 5.92 a′**	104.20 ± 16.92 a′
Zn ($mg \cdot kg^{-1}$)	0.95 ± 0.14 b	1.56 ± 0.33 a	1.42 ± 0.35 ab	1.24 ± 0.09 a′*	1.22 ± 0.14 a′	0.98 ± 0.18 a′
Fe ($mg \cdot kg^{-1}$)	3.81 ± 0.52 bc	4.12 ± 0.43 a	3.19 ± 0.35 c	3.65 ± 0.59 a′	3.61 ± 0.51 a′	4.84 ± 0.81 a′**
Mn ($mg \cdot kg^{-1}$)	1.18 ± 0.40 a	1.39 ± 0.26 a	1.88 ± 0.60 a	1.77 ± 0.34 a′	2.07 ± 0.52 a′	0.85 ± 0.18 b′
Cu ($mg \cdot kg^{-1}$)	0.32 ± 0.11 a	0.34 ± 0.06 a*	0.25 ± 0.03 a	0.30 ± 0.03 a′	0.25 ± 0.02 a′	0.25 ± 0.03 a′
B ($mg \cdot kg^{-1}$)	1.11 ± 0.24 b	1.58 ± 0.10 a	1.77 ± 0.28 a	1.50 ± 0.24 a′	1.64 ± 0.24 a′	1.76 ± 0.22 a′

3.3. Correlation between Fruit Nutrients of Different Cultivars and Soil Properties

The correlation analysis between soil nutrients in BM fruits and soil showed that Fe content in fruits was significantly negatively correlated with soil pH, total Mn, and total Ca, and significantly positively correlated with SOM and TN (Table S6). In addition, the B content of BM fruit was significantly positively correlated with soil pH, AvB, total K, and total Mg (Table S6). There was a significant negative correlation between the Mn content in *C. unshiu* fruits and soil pH, total calcium, and total magnesium (Table S6), indicating that soil pH, total Ca, and total Mg had a significant antagonistic effect on the Mn content in citrus fruits. Mg in fruit was negatively correlated with soil pH, while iron was negatively correlated with soil total phosphorus (Table S6). The physical and chemical properties of soil showed different correlation between BM and *C. unshiu*, which further indicated different varieties of citrus were affected differently by soil nutrients. The contents of Fe, B, and other trace elements in BM citrus fruits were more affected by soil nutrients.

3.4. Soil Properties of Representative BM Orchards

From the above 62 orchards, five representative BM orchards were selected to investigate the nutrient deficiency of BM in coastal saline–alkali land (Figures S1 and S2). The total content of C and N in coastal saline–alkali orchards (noted as DW, DY and XT) was significantly lower than that of non-saline–alkali orchards (noted as MY and QT) (Table S7). The total content of Ca, Mg, Fe, and Mn in saline–alkali orchards was 2.52, 3.61, 2.16, and 2.82 times of that in non-saline–alkali orchards, respectively (Table S7). In terms of available nutrients, non-saline–alkali soil showed higher AN, OP, and lower ExCa and ExMg (Table S8). However, although the total content of Fe and Mn in saline–alkali soil were higher, AvFe and AvMn were even lower than those in non-saline–alkali soil. Higher pH and EC were also found in saline–alkali orchards, as expected (Table S8).

3.5. Nutrients Contents of Citrus in Different BM Orchards

3.5.1. Nutrient Contents of Citrus Trees

Among all mineral elements, Fe content in leaves was significantly different between the two types of soil. The Fe content in leaves of non-saline–alkali orchards was 32.15% lower than that of saline–alkali orchards (Figure 2). The content of other trace elements, including Zn, Mn, and Cu, in the leaves of saline–alkali orchards was also low (Figure S3). Fe content of leaves with different degrees of chlorosis was also determined (Figure 2). We classified the samples into healthy, light chlorosis, and severe chlorosis according to the degree of leaf chlorosis (Figure 2). Iron content in leaves was inversely correlated with the severity of chlorosis symptoms (Figure 2). Severe chlorosis resulted in a reduction of 43.41% in Fe content compared with healthy control (Figure 2).

3.5.2. Nutrient Contents of Fruits

The content of microelements (including Fe, Mn, Zn) was much higher in fruit grown in non-saline–alkali orchards (Table 2), while K content was significantly higher in saline–alkali orchards (Table 2). Fruits grown in saline–alkali orchards had greater biomass and a more oblate shape compared with those in non-saline–alkali orchards (Table 3). The content of vitamin C was higher too (Table 3). No significant difference was observed for total soluble solid (Table 3).

Table 3. Fruit quality and mineral nutrient concentration of the citrus hybrid cv. Beni-Madonna, the significant difference of different citrus orchards at $p < 0.05$ was indicated by small letters.

Fruit Nutrition Index	BM Orchards		
	MY	DW	DY
Weight (g)	255.49 ± 29.2 b	316.25 ± 29.33 a	274.74 ± 29.30 ab
Shape index	0.92 ± 0.01 a	0.86 ± 0.03 b	0.87 ± 0.01 b
TSS (%)	11.86 ± 0.42 a	11.60 ± 0.05 a	11.41 ± 0.55 a
VC ($mg \cdot 100\ g^{-1}$)	26.75 ± 1.90 c	36.34 ± 1.84 a	33.23 ± 2.52 b
K ($g \cdot kg^{-1}$)	5.94 ± 0.30 b	6.25 ± 0.34 a	6.46 ± 0.30 a
Ca ($mg \cdot kg^{-1}$)	946.54 ± 75.09 a	923.28 ± 56.18 a	879.19 ± 83.68 a
Mg ($mg \cdot kg^{-1}$)	571.43 ± 34.71 a	548.05 ± 24.07 a	549.98 ± 46.36 a
Zn ($mg \cdot kg^{-1}$)	29.17 ± 2.52 a	12.52 ± 0.72 c	22.66 ± 2.45 b
Fe ($mg \cdot kg^{-1}$)	21.49 ± 1.47 a	14.88 ± 1.73 b	9.25 ± 0.77 c
Mn ($mg \cdot kg^{-1}$)	14.26 ± 1.43 a	1.93 ± 0.08 b	2.47 ± 0.28 b
Cu ($mg \cdot kg^{-1}$)	3.20 ± 0.29 a	3.14 ± 0.16 a	1.96 ± 0.26 b
B ($mg \cdot kg^{-1}$)	13.32 ± 1.50 a	10.40 ± 1.27 b	13.10 ± 1.26 a

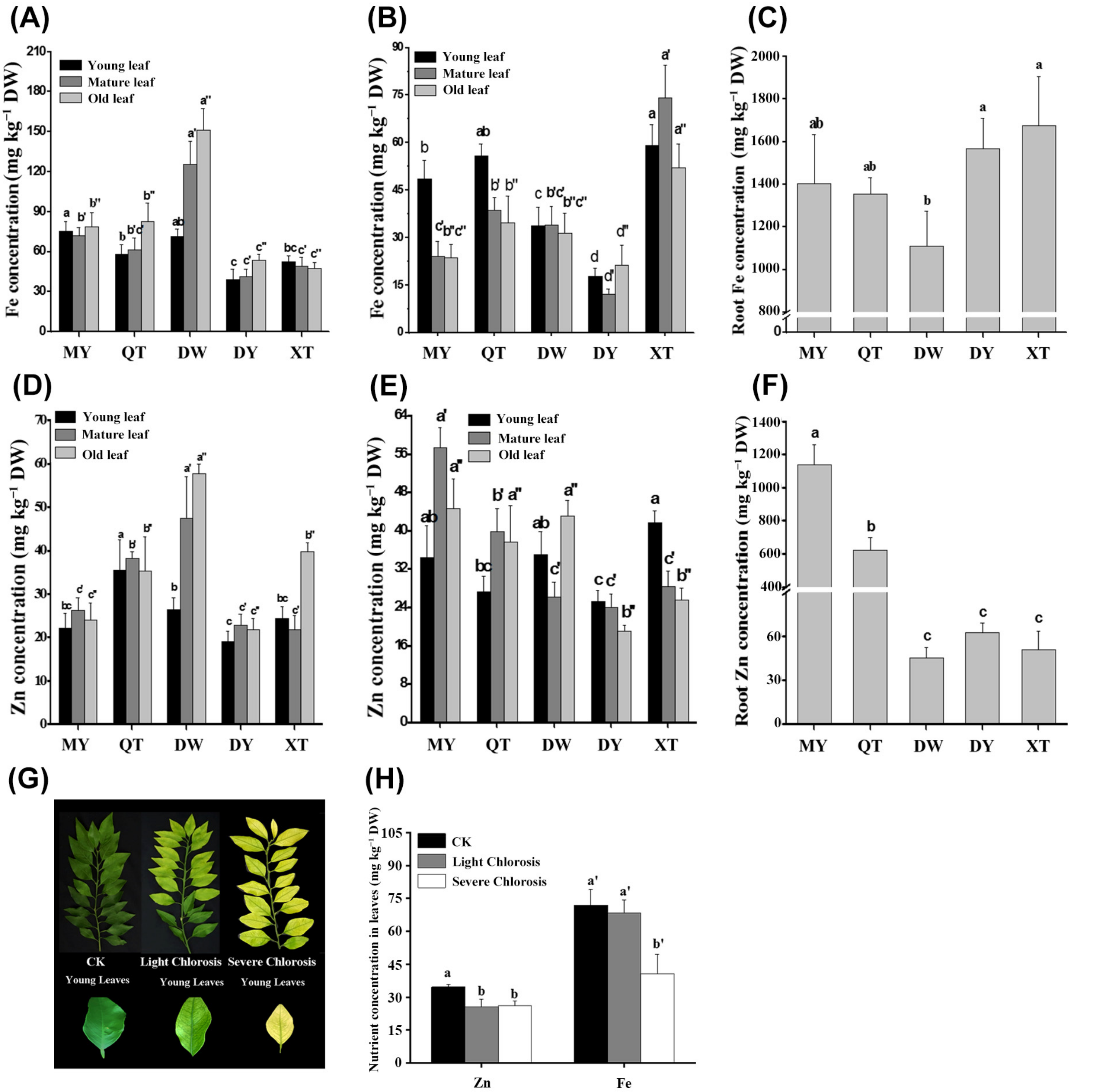

Figure 2. Fe concentration in citrus leaves (**A**), stems (**B**), and roots (**C**) in different orchards. Zn concentration in citrus leaves (**D**), stems (**E**), and roots (**F**) in different orchards. Different letters above the bars indicate significant difference among different orchards ($p < 0.05$), and a, a′, a″ was for young leaf, mature leaf and old leaf, respectively. (**G**) The diverse severity of chlorosis symptoms on citrus leaf. (**H**) Zn and Fe concentration in healthy and chlorotic citrus leaves. a, a′ was used for the significant difference of Zn or Fe concentration, respectively.

3.6. Correlation between Nutrients in Different Parts of the Citrus Tree and Soil Properties

Spearman was used to analyze the correlation between soil properties and plant mineral nutrients. There was a significant negative correlation between Fe and saline–alkali indexes (including pH, EC, ExCa, and ExMg) in both leaves and fruits (Figure 3), indicating that saline–alkali properties inhibited Fe uptake in citrus. Ca content in leaves was positively correlated with soil saline–alkali indexes, but it was the opposite in fruit (Figure 3). Cu content was also negatively correlated with soil saline–alkali indexes (Figure 3). On the other hand, Fe content in leaves and fruits was positively correlated with soil available Fe (Figure 3).

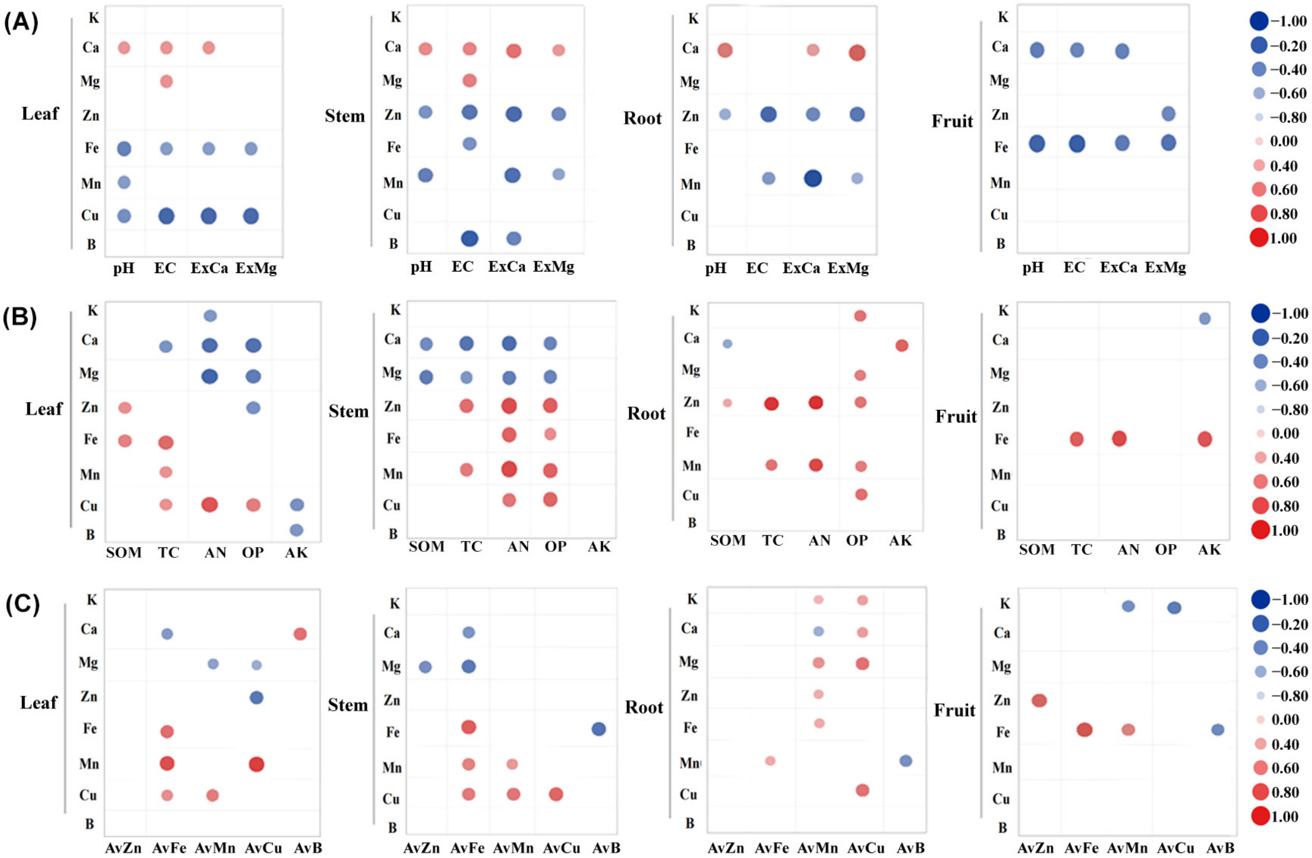

Figure 3. The correlation between nutrients in different parts of citrus and soil salinity indexes (**A**), soil available nutrients of macro-elements (**B**), and soil available nutrients of microelements (**C**), respectively.

3.7. *Effect of Long-Term Film Cover on Soil Properties and Plant Fe Nutrition*

Among the five BM orchards mentioned above, leaf chlorosis and iron deficiency were most severe in the DW orchard (Figure 4), where the trellises were long-term film covered. DW was used as an example to analyze the effect of film cover. Soil profiles of 0–60 cm were investigated at both covered cultivation and uncovered cultivation time points (Figure 4). Fe content in leaves was 23.94% lower in covered period than that in uncovered period (Figure 4; $p < 0.05$). Soil available Fe content was higher at the depth of 10–60 cm in uncovered period (Figure 4). In the profile soil, available Fe content was inversely correlated with the depth (Figure 4). Soil EC reflected the degree of salinization. In the uncovered period, soil EC was directly correlated with the depth (Figure 4). However, during the covered period, the pattern was reversed and EC at the depth of 0–10 cm was 3.58 times of that in uncovered period. The main difference of soil EC between two periods was observed at the depth of 0–30 cm. In the depth of 30–60, there was no significant difference. The high exchangeable Ca content in 0–10 cm soil also indicates the accumulation of salt in soil surface (Figure 4).

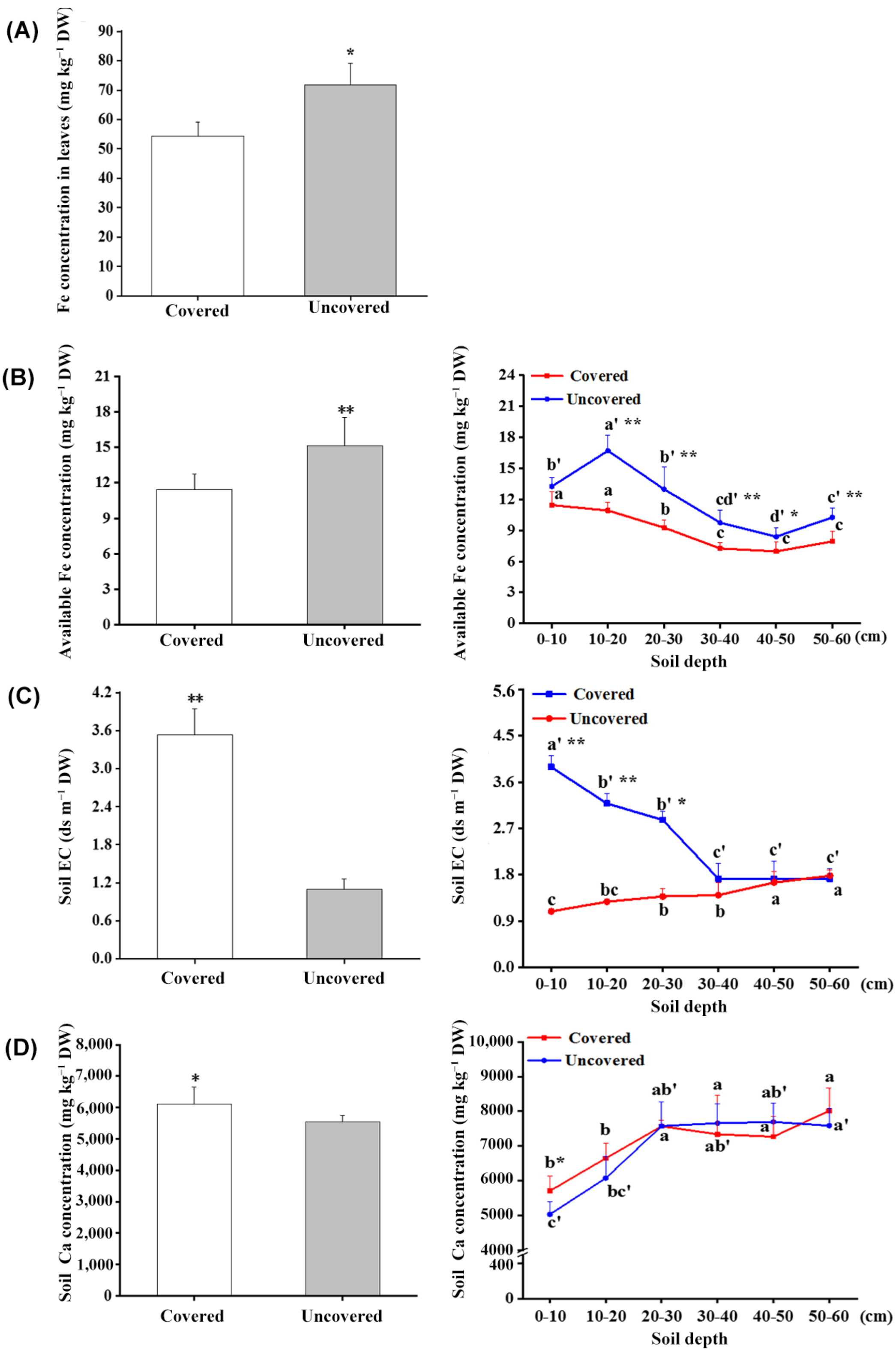

Figure 4. The Fe concentration in leaves (**A**), available Fe concentration in soil (**B**), EC and salt content (**C**), Soil Ca concentration (**D**) in the period of coverture with film and without coverture with film. Asterisks indicate significant difference between coverture and without coverture (* $p < 0.05$; ** $p < 0.01$). Small letters indicate significant differences at $p < 0.05$. Covered: covered with plastic film; Uncovered: uncovered with plastic film. And a, a′ was used for the significant difference of coverture and without coverture, respectively.

4. Discussion

4.1. Analysis of Soil Nutrient Abundance and Deficiency in Citrus Orchards

This study reported that the soil pH of citrus orchards in Xiangshan county varied between 3.9 and 8.6, most of which was not suitable for citrus growth (Figure 1). This depends on the origin of these soils and their chemical properties [16]. According to the survey of Soil Census and Land Planning Committee of Zhejiang Province [17], the soil formation parent material of mountain citrus orchards is mainly acidic solute tuff. The soil of citrus orchards in coastal saline–alkali land is mainly the shallow pond mud field developed from shallow sea sediments, and the soil is alkaline. Moreover, improper fertilization may result in low pH in some orchards. For example, excessive application of nitrogen fertilizer can aggravate soil acidification [18,19].

However, the distribution of soil organic matter was contrary to pH. Organic matter content of alkaline citrus orchards, such as coastal saline–alkali land, was lower, while that of acidic citrus orchards, such as mountainous and terrestrial land, was higher (Figure 1). Soil pH will affect the circulation and accumulation of organic matter [20,21]. In addition, the conversion of coastal wetland to arable land can result in the decline of soil organic carbon storage [22].

Topographic aspect and location are likely to affect the spatial distribution patterns of the soil nutrients. The mean values of TN were in the appropriate range in mountainous orchards and very low in coastal saline–alkali land (Figure 1). It has been shown that high organic matter content and associated vigorous soil microbial metabolism are beneficial for soil N mineralization [23]. The content of total calcium and magnesium in coastal saline–alkali land is much higher than that of mountainous and flat land. This may be related to its soil parent material, shallow sea sediment, which contains a lot of calcium and magnesium [24]. The AvFe level in coastal saline–alkali land was low and close to deficiency, while 50% of mountain and flat land orchards had excess AvFe, which may lead to toxicity. The solubility extent of Fe-bearing minerals is dependent on soil pH and ionic strength [25]. In addition, the TP in Xiangshan county is generally low, probably because of P leaching [26]. In summary, in order to adjust the soil pH and improve soil fertility, coastal saline–alkali orchards should be fertilized with N, P, Fe, B, etc. in addition to acidic fertilizers and organic fertilizers.

4.2. Fruit Nutrient Status Varied in Different Citrus Cultivars in Xiangshan County

Cultivar and geographical location of cultivation may explain the differences in the contents of nutrients in citrus fruits [27]. Compared with *C. unishiu*, the content of various nutrients in the fruit of BM was more easily affected by topographic factors. The calcium and iron contents of BM fruit in coastal saline area were significantly lower than those of *C. unishiu* (Table 2). There was also variability in the effect of soil nutrients in Xiangshan on the fruit nutrients of the two citrus varieties we focused on. Fe and B in BM fruits were significantly correlated with soil physicochemical properties. Fe content in fruit was negatively correlated with soil pH, total amount of calcium and magnesium, and positively correlated with organic matter and total nitrogen. The correlation between the fruit of *C. unshiu* and soil nutrients is weak (Table S6), indicating that the abundance and deficiency of trace nutrients such as iron in BM are more influenced by soil factors and more sensitive to nutrient demand than in *C. unshiu*. The mean weight of fruits of BM was about 250 g each [5] and were generally larger than those of *C. unshiu* (~110 g each) [28], so we hypothesized that BM may require more nutrients than *C. unshiu* to synthesize carbohydrates. In addition, the long fruit setting time of BM may cause the fruit to take away a large amount of nutrients from the tree, resulting in the decline of tree vigor. Especially in saline–alkali soils, where high pH and salinity lead to poor root growth [29] and low soil micronutrient effectiveness [30], which ultimately leads to a reduced ability of citrus trees to obtain micronutrients from the soil.

4.3. Causes of Iron Deficiency in Citrus in Saline–Alkali Land

Citrus is grafted for cultivation. Use of rootstocks tolerating abiotic stresses is critical for successful production [31]. In general, sour orange and rough lemon are considered most tolerant of high pH and intermediate tolerant of salinity [32]. In this study, the basic rootstock of BM is sour orange (*Citrus aurantium* L.), which could alleviate the stress of high salinity and high pH on BM to some extent. However, BM still exhibited strong chlorotic symptoms, indicating that a more suitable rootstock still needs to be found.

The physical and chemical properties of soil will directly affect the nutrient absorption and growth of plants. In this study, Zn and Fe deficiencies were present in coastal saline citrus orchards and were dominated by Fe deficiency (Figure 2, Table S9). Although the content of total iron and total manganese in coastal saline–alkali land citrus orchards were high, the amounts of available iron and manganese were reduced. This is due to the higher soil pH in coastal saline–alkali land. Higher soil pH reduced the solubility of trace elements. For example, although long-term fertilization increased the total amount of iron, the AvFe was still insufficient [33]. Moreover, high soil pH reduced the reduction ability of Fe^{3+} by FCR enzyme on the root surface plasma membrane, leading to a decrease in the amount of Fe^{2+} available for citrus to absorb, resulting in iron deficiency in trees [34].

The higher soil conductivity, and ExCa and ExMg content of citrus orchards in coastal saline–alkali land also affected the iron absorption of citrus (Figure 3). High content of calcium and magnesium in alkaline soil not only reduces the solubility of soil Fe but also inhibits cell elongation and consequently root growth, resulting in decreased root pressure [35,36], thereby affecting the availability of soil iron, limiting the process of iron uptake and transport to the ground by roots, and ultimately resulting in iron deficiency in plants.

In addition, plastic shed cultivation may aggravate iron deficiency in saline–alkali land. In this study, the electrical conductivity and total salinity of the surface soil (0–20 cm) of the orchards covered with plastic film in the coastal saline lands were significantly higher than those of the deep soil, which had exceeded the critical value (1.0 g kg^{-1}) for plant growth (Figure 4). Easily built, cost-saving, and management-friendly plastic sheds are popular with smallholder farmers [37]. When growing in a greenhouse, the plastic film protects soil from rainfall, resulting in high indoor temperatures and evaporation. Migration of salt ions from the deeper to the surface layer sand and reduced salt leaching by rains exacerbates soil salinization [38], and thus iron deficiency in the soil [39].

5. Conclusions

All together, considering the phenotypic characteristics and the nutrient contents of soils, leaves, and fruits, we concluded that the leaf chlorosis of citrus orchards in coastal saline–alkali lands was mainly caused by iron deficiency. Moreover, a plastic film shed exacerbated iron deficiency. In addition, BM is prone to iron deficiency leading to chlorosis and should be supplemented with iron fertilizers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9040437/s1, Figure S1. Geographical location of Beni Madonna (BM) citrus orchards; Figure S2. Pictures of plastic shed BM orchards in Xiangshan county; Figure S3. K (A), Ca (B), Mg (C), Mn (D), Cu (E) concentration in citrus leaves, stems, and roots in different orchards; Table S1. Distribution of soil pH in citrus orchard of Xiangshan County; Table S2. Distribution of soil matter in citrus orchard of Xiangshan County; Table S3. Macro-elements (N, P, and K) content and percentage for classification of citrus orchard in Xiangshan County; Table S4. The Ca and Mg contents of soil in citrus orchard of Xiangshan County; Table S5. Soil available Fe, Mn, B contents and the percentage for classification of citrus orchard in Xiangshan County; Table S6. The correlation between Beni Madonna and *C.unshiu* fruits and soil nutrients in Xiangshan County; Table S7. The contents of soil total nutrients in different citrus orchards; Table S8. The physical and chemical properties of soil in citrus orchards; Table S9. The mineral nutrient levels in leaves of Beni Madonna; Supplementary methods.

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