How Raised Beds and Fe-Chelate Affect Soybean Iron Deficiency Chlorosis and Yield

Lucas C. Holmes[1], Hans J. Kandel[1*], Grant H. Mehring[2], Peder K. Schmitz[1]

[1]Department of Plant Sciences, North Dakota State University, Fargo, ND, USA
[2]WestBred, Bayer Crop Science, Fargo, North Dakota, USA
Email: *hans.kandel@ndsu.edu

How to cite this paper: Holmes, L.C., Kandel, H.J., Mehring, G.H. and Schmitz, P.K. (2021) How Raised Beds and Fe-Chelate Affect Soybean Iron Deficiency Chlorosis and Yield. *Agricultural Sciences*, **12**, 755-768.
https://doi.org/10.4236/as.2021.127049

Received: June 21, 2021
Accepted: July 19, 2021
Published: July 22, 2021

Abstract

Water-logging and the inability to take up sufficient iron (Fe), causing iron deficiency chlorosis (IDC) in soybean (*Glycine max*, L. Merr.), can be major yield reducing factors in certain soils in the northern USA and Manitoba, Canada, soybean growing regions. The objective of this research was to evaluate soybean IDC, biomass production, and yield with seeding on raised beds and seed application of the Fe-chelate compound ortho-ortho-Fe-EDDHA. In six environments, soybean were seeded on raised beds and conventionally prepared seedbeds (flat) and with a factorial arrangement of five cultivars (within adapted maturity group 0.1 to 0.9 and variable IDC tolerance) and seed applied Fe-EDDHA using rates of 0 $kg \cdot ha^{-1}$ and 3.36 $kg \cdot ha^{-1}$. There were no significant interactions between the factors tested. The plant population was 27% higher on the raised beds compared with flat, and yield was 6.3% higher (2893 $kg \cdot ha^{-1}$ vs. 2722 $kg \cdot ha^{-1}$). Total dry plant biomass on raised beds was 9.8% greater compared with flat. The plant population with seed applied Fe-EDDHA was 10.6% lower compared with no application. However, the IDC score was significantly lower 2.2 vs 2.4 (1 = green, 5 = dead) for Fe-EDDHA seed application. Yield and plant biomass were not significantly different between Fe treatments. Raised beds offer an opportunity for soybean growers to reduce the negative influence of excessive water. Further research is needed to determine the long-term effect of raised beds on plant development, IDC expression, and yield. The application of Fe-EDDHA remains a partial solution and should therefore be combined with other methods to reduce IDC. Further research should study other Fe-EDDHA application rates and methods.

Keywords

Soybean, Raised Beds, Iron Deficiency Chlorosis, Fe-Chelate

1. Introduction

Soybean (*Glycine max*, L. Merr.) is a dominant crop in the US Corn Belt, and Northern growing regions of North Dakota, South Dakota, and Minnesota [1]. Soybean is subject to abiotic stresses such as water-logged soil, salinity, and iron-deficiency chlorosis (IDC) which are common in the Northern US growing region as well as Manitoba, Canada [2]. Therefore, methods mitigating these yield reducing stressors need to be investigated. This research focused on management practices to potentially reduce water logging and IDC in soybean.

Raised beds are a form of ridge tillage [3]. Raised beds have a flat and wide surface, thereby enabling seeding directly on top of the bed. Utilizing raised beds intends to reduce crop root exposure to excess water and acts as a beneficial water management practice in areas with poorly drained soils [4] [5]. Although flooding may occur between the beds (including in-furrow irrigation), growing soybean on raised beds nevertheless may improve productivity since the flooding would occur in areas below the elevated root zone during emergence and growth [3]. Raised beds will shed the water off the bed and into the furrows potentially relieving soil saturation caused by flooding. Ideally, the properly drained soils should reach field capacity within 24 - 36 h to minimize excess water stress [5]. Most of the nitrogen (N) needed by soybean plants is from N-fixation in the root nodules. These rhizobacteria are aerobic organisms. Therefore, poorly drained soils at the beginning of the growing season can be low in rhizobacteria, resulting in lower root nodule formation [6].

Raised bed production is not common in the Red River of the North Valley (RRNV), USA and Manitoba, Canada. Hoppe *et al.* [7] was the first to report about raised bed soybean research in the RRNV. Raised beds can increase soil temperatures near the seed due to greater surface area for sunlight interception and because of the lower water content in the top of the bed compared to flat land [7]. Furthermore, raised beds can reduce IDC symptoms compared to flat land seedbeds [7].

Availability of iron (Fe) can also be affected by water. Two forms of Fe that can exist in the soil are Fe(II) and Fe(III). Plants can take up Fe(II) but not Fe-(III). Higher soil water content can change the oxidative state of iron from Fe(II) to Fe(III) [8]. Iron efficient cultivars (*i.e.* IDC tolerant soybean), compared with susceptible cultivars, are better able to excrete reductase chemicals to reduce Fe-(III) to Fe(II) [9].

The application of the Fe-chelate compound ortho-ortho-Fe-EDDHA (henceforth called, Fe-EDDHA) to soybean is used to decrease IDC in soybean plants [10]. The purpose of this Fe-chelate is to provide the plant with readily available Fe(II). Fe-chelate should be applied at a rate between 1.12 to 3.36 $kg \cdot ha^{-1}$, at seeding or with a foliar application, shortly after emergence of the soybean, since IDC typically affects soybean plants early in the growing season [11]. Wiersma [11] used Fe-EDDHA as a seed treatment instead of the more common in-furrow or foliar methods, in Crookston, MN, USA, and found that the 4.5 $kg \cdot ha^{-1}$

and 5.63 kg·ha^{-1} rates of Fe-EDDHA resulted in longer periods that IDC tolerant cultivars were able to take up Fe(II) compared to the 3.36 kg·ha^{-1} rate. In a similar study, Goos and Johnson [2], concluded that Fe seed treatments work better in the greenhouse compared to field conditions.

Products combating IDC are often applied as in-furrow applications or foliar sprays as demonstrated by Gamble *et al.* [12] in a soybean study in Alabama, USA. Visual chlorosis scores were lowered (less IDC) for in-furrow application and foliar spray, but in-furrow application resulted in the most IDC reduction although both methods provided similar yield [12].

Not all producers have liquid fertilizer applicators on their soybean seeding equipment, but most producers are used to seeding soybean seed coated with fungicide, insecticide, or rhizobia bacteria. Adding a compound like Fe-EDDHA would not require additional changes in the seeding procedure. Although research has indicated increased yields with raised beds or application of Fe-EDDHA, no previous research to our knowledge, has evaluated the effect of combining these two methods to increase soybean productivity.

The objective of this research was to evaluate soybean growth and development, IDC incidence, biomass production, and yield response with seeding on raised beds and conventionally prepared land and with and without seed application of the Fe-chelate compound labeled as 6% Fe in the form of ortho-ortho-Fe-EDDHA (iron ethylene diamine-N,N'-bis (hydroxy phenyl)acetic acid) in the field.

2. Materials and Methods

2.1. Location Information

This research was conducted at two locations in 2013 and 2014 with a total of six environments. Fargo, North Dakota, USA, had two experiments each year. One on soil with tile drainage or "drained" and the other without tile or "undrained," as described by Kandel *et al.* [13]. The Casselton, North Dakota, USA, location had one experiment per year. The Fargo location had a "Fargo" fine, smectitic, frigid Typic Epiaquerts soil series and Casselton had a "Bearden" fine-silty, mixed, superactive, frigid Aeric Calciaquolls soil series. Experiment locations and soil test information are provided in **Table 1**. No fertilizer was applied, as N, P, and K soil nutrients were not limited.

2.2. Experimental Design

The experimental design was a randomized complete block design with a split-plot arrangement with four replications. The whole plot was raised beds or conventionally prepared seedbeds (flat) and the sub-plots were a factorial arrangement of five cultivars (maturity and IDC tolerance called proxy cultivar) by two Fe-EDDHA application rates. Proxy cultivar grouping has similar maturity group and IDC tolerance, and was use so that results could be analyzed across environments.

Table 1. GPS location and soil fertility test results for experiment locations at Fargo and Casselton, North Dakota, USA, in 2013 and 2014.

Location	Year	Latitude	Longitude	N	P	K
				- - - - - - - 0 - 15 cm - - - - - - -		
				$kg \cdot ha^{-1}$	$mg \cdot kg^{-1}$	
Fargo[a]	2013	46.932	−96.858	59	25	460
	2014			53	25	460
Casselton	2013	46.878	−97.251	21	16	340
	2014			19	16	340

[a]The Fargo location consisted of two experiments each year.

Data were combined and replication and environment (location-year) were considered random effects, and raised beds, Fe-EDDHA application, proxy cultivar, and these treatment interactions, were considered fixed effects. Analysis of variance was conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) at a probability level of 90% ($\alpha = 0.10$). In addition, a separate analysis was conducted for Fe-EDDHA treatment yield, using established plant population number as a covariate after the main analysis.

2.3. Treatments

Five cultivars were selected ensuring both IDC tolerant and susceptible cultivars were used with a recommended relative maturity for the area (Group zero; with range 0.1 to 0.9) [14]. Two cultivars used in 2013 were unavailable in 2014 and were replaced by cultivars with similar IDC tolerance and relative maturity characteristics. Proxy cultivar numbers (cultivars with similar IDC ratings and relative maturity) were assigned to adjust for seed unavailability between years allowing for proxy cultivar analysis (**Table 2**).

The experimental units were 3 m wide × 7.6 m long with four rows per plot and 76 cm row spacing. A John Deere Model 71 planter (Moline, IA, USA) was used with a seeding depth of 2.5 cm. The middle two rows were used for plant height measurements and harvest for seed yield. The outside rows were used for root and biomass data collection as this involved destructive sampling. A seeding rate of 531,050 seeds ha^{-1} was used targeting an established plant population of 454,000 plants ha^{-1} based on 95% germination and about 10% expected seed to established plant loss [17]. However, the actual achieved plant population was on average about 300,000 plants ha^{-1}, still well within in the normal range for the Northern soybean region [18].

A HR6 Hipper Roller (Pitonyak Machinery Corp., Carlisle, AR, USA) was used to make raised beds 76 cm apart with a height of 30.5 cm in the fall of 2012 and 2013 (**Figure 1**). The raised beds were repaired the following spring using the same equipment. Conventional seedbeds were prepared using a cultivator and are referred to as "flat."

Table 2. Soybean cultivars with relative maturity, and iron deficiency chlorosis tolerance used in Fargo and Casselton, North Dakota, USA, in 2013 and 2014.

Company	Cultivar	Year (s) used	Maturity Group[a]	IDC Score[b]	Proxy Cultivar[c]
Pioneer	A	2013	0.4	2.6	1
Pioneer	B	2014	0.5	2.8	1
Pioneer	C	2013-2014	0.7	1.7	2
Dairyland Seeds Co.	D	2013-2014	0.7	2.6	3
Hyland Seed	E	2013-2014	0.1	1.7	4
NuTech	F	2013	0.8	2.7	5
Channel	G	2014	0.9	2.7	5

[a]Maturity group numbers are provided by companies. [b]IDC = iron deficiency score, chlorosis scores are based on the North Dakota Soybean Variety Trial Results for 2012 and 2013 via the Selection Guide booklet [15] [16]. Scale: 1 = green; 5 = dead tissue. [c]Proxy cultivars with the same number have similar maturity and IDC tolerance.

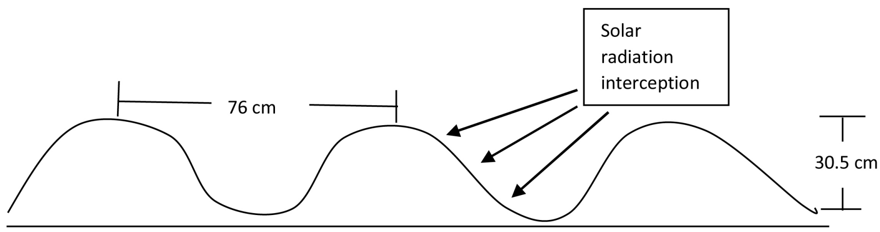

Figure 1. Diagram explaining raised bed layout and interaction with sunlight.

Fe-EDDHA treatments consisted of 0 kg·ha^{-1} and 3.36 kg·ha^{-1}. The product "soygreen" (Soygreen, CHS, Inver Grove Heights, MN, USA) was used. Normally this Fe-chelate is applied in furrow at seeding. In our case, we used the product as a seed treatment. The Fe-chelate product is a soluble powder with 6% Fe in the ortho-ortho-Fe-EDDHA form and applied at a rate of 3.36 kg·ha^{-1} rate based on the highest label recommended rate. The seed was treated using a Gum Arabic sticking agent (Arabic Powder: *Acacia spp.*, Frontier, Natural Products CO-OP, Norway, IA, USA), water, and Fe-EDDHA. The treatment was, mixed as 23% Gum Arabic, 37% water and 40% Fe-EDDHA, ratio similar to Wiersma [11]. The seed treatment was applied to the seed just before seeding.

2.4. Management and In-Season Measurements

Air temperature and rainfall data was obtained from North Dakota Agricultural Weather Network [19] using the Fargo and Prosper, North Dakota locations. Throughout the growing season, Glyphosate [N-(phosphonomethyl) glycine] (Bayer Crop Science, St. Louis, MO, USA) was used for weed control. Plant density was recorded at the V2 (two leaf trifoliolate) stage by counting a 90 cm length in the inner two rows of each plot.

Visual scoring for IDC and vigor was done on the same day once IDC symptoms became visible at the three or four leaf trifoliolate stage. Visual IDC scoring was based on a scale of 1 to 5, with 1 being no IDC symptoms and 5 being severe IDC symptoms (*i.e.* necrosis and dead plants) based on the scale used by Goos and Johnson [2]. Vigor scoring was based on a scale of 1 to 9, with 1 being very poor developed growth structures (*i.e.* leaves, stems, flowers, etc.) and 9 being well-developed growth structures. Measurements and application dates at Fargo and Casselton are presented in **Table 3**. Soybean above and below ground biomass was measured by removing plants from a 90 cm length in a buffer row at the approximate R6 growth stage (pods with fully developed green bean). The plant roots were rinsed of soil and the whole plant was placed in a dryer. First, the whole plant biomass was weighed and then the roots were cut off at the soil line and the above ground mass was weighed. The root mass was calculated by subtraction. Before harvest, plant heights (the distance from the soil surface to the uppermost node), were measured by selecting three plants at random from one of the middle two rows of each experimental unit.

Experimental units were harvested after physiological maturity at about 13% seed moisture content, using a Wintersteiger Classic combine (Winchester Ag, Reid, Austria).

A Dickey John GAC-2100 (Dickey John Corp., Auburn, IL, USA) was used to measure moisture and test weight, and a 1000-kernel sample was counted and

Table 3. Dates of soybean applications and measurements, Fargo and Casselton, North Dakota, USA.

Fargo			
2013 Measurement/Application		2014 Measurement/Application [a]	
31 May	Seeding	23 May	Seeding
1 July	IDC score recorded	25 June	IDC score recorded
1 July	Vigor scores recorded	25 June	Vigor scores recorded
19 June and 8 July	Applied Glyphosate	11 June	Glyphosate applied
20 Aug.	Dug plants for biomass	8 Aug.	Mustang Maxx[b] applied for soybean aphid control
2 Oct.	Plant heights	30 Aug.	Dug plants for biomass
2 Oct.	Harvested trial	26 Sept.	Plant heights recorded
		3 Oct.	Harvested trial
Casselton			
2013 Measurement/Application		2014 Measurement/Application	
13 June	Seeding	23 May	Seeding
8 July	IDC score recorded	8 July	IDC score recorded
8 July	Vigor scores recorded	2 July	Vigor scores recorded
13 Sept.	Dug plants for biomass	20 Aug.	Dug plants for biomass
3 Oct.	Plant heights recorded	26 Sept.	Plant heights recorded
3 Oct.	Harvested trial	30 Sept.	Harvested trial

[a]Dates of observations in 2014 were based on similar soybean growth stages in 2013. [b]Mustang Maxx (*Zeta-cypermethrin*) (FMC, Philadelphia, PA, USA).

weighed. Yield data was corrected to 13% seed moisture content. A Diode Array 7200 NIR Analyzer (Perten Instruments, Springfield, IL, USA) was used to determine protein and oil content.

3. Results and Discussion

3.1. Precipitation

In 2013, the precipitation in Fargo was nearly two times greater in May and June, and two times greater in June in Casselton compared to the 30-year average (Table 4). On average, 2013 had more precipitation and 2014 had less precipitation than the historical total over the same period.

3.2. Raised Beds by Fe-EDDHA

The raised beds by Fe-EDDHA experiments were analyzed across six environments. (Table 5). In general, any interactions between raised beds, Fe-EDDHA, and proxy cultivars were not significant. We selected cultivars with differences in IDC tolerance to see if there would be an interaction between proxy cultivar and raised beds and or Fe-EDDHA seed treatment. In this study, no such interaction was found. Other research also did not find an interaction between Fe-EDDHA and cultivars with differences in IDC tolerance [20].

Most measured observations for the main factor "proxy cultivar" were significantly different as cultivars were selected with known difference in maturity and IDC tolerance, and the significances will not be further discussed. Our primary interest was in the interaction of proxy cultivars with different IDC tolerance with raised beds or Fe-EDDHA; however, the interaction was not significant therefore only the raised beds and Fe-EDDHA effects will be discussed (Table 5).

Table 4. Monthly total rainfall for 2013, 2014, and historical data at Fargo and Casselton, North Dakota, USA.

	Precipitation					
	Fargo			Casselton		
Month	2013	2014	Historical[a]	2013	2014	Historical
	------------------------mm------------------------------					
May	141	50	71	105	52	78
June	199	140	99	193	107	100
July	71	34	83	20	33	88
August	12	37	65	51	61	67
September	106	51	65	93	47	66
October	55	8	53	84	9	62
Total	584	320	436	546	309	461

[a]Historical data represent a 30-year average from 1981-2010 (NDAWN).

Table 5. Probability values for the raised beds, Fe-EDDHA, and proxy cultivar ANOVA for agronomic traits averaged over six environments at Fargo and Casselton, North Dakota, USA, in 2013 and 2014.

Source of Variation	df	Plant population	EV[a]	IDC	Ht	PC	OC	TW	TKW	Yield
					Pr > F[b]					
Raised beds	1	**<0.001**	**0.002**	0.63	0.16	0.88	0.43	0.14	0.77	**0.07**
Fe-EDDHA	1	**0.01**	0.62	**0.06**	0.94	0.70	0.97	0.29	0.95	0.40
Raised beds x Fe-EDDHA	1	0.72	0.65	0.77	0.50	0.58	0.20	0.40	0.16	0.95
Proxy cultivar (proxy)[c]	4	**0.004**	0.36	**0.01**	0.17	**<0.001**	**<0.001**	**0.04**	**<0.001**	**0.01**
Raised beds x proxy	4	0.46	0.96	0.54	0.59	0.27	0.81	0.31	0.93	0.99
Fe-EDDHA x proxy	4	**0.02**	0.80	0.88	0.70	0.15	0.67	0.78	0.70	0.60
Raised beds x Fe-EDDHA x proxy	4	0.54	0.91	0.47	0.93	0.32	0.47	0.45	0.79	0.61

[a]EV, early vigor score; IDC, iron-deficiency chlorosis score; Ht, plant height' PC, protein content; OC, oil content; TW, test weight; and TKW, 1000 kernel weight. [b]Bolded numbers are significant at $p \le 0.10$. [c]Proxy cultivar are soybean cultivars with similar maturity and IDC tolerance.

3.3. Raised Beds Effects

When raised bed observations were averaged across Fe-EDDHA and proxy cultivar treatments, plant population, early vigor score, and yield were significantly different (**Table 5**). In this experiment, oil and protein percent, test weight, and 1000 kernel weight were not significantly different on raised beds compared with flat (**Table 6**). Raised beds are primarily used to dry out the soil in wet years, increase soil temperature overall, and aim to create a root environment that is less compacted and saturated. Soil bulk density has been shown to be lower in raised beds compared to flat land [5] [21]. Raised beds reduce soil waterlogging potential compared to flat since the water table level in the flat land was closer to the soil surface more often than in raised beds areas [5]. Similar observations were made in this experiment in May and June of 2013 with above average precipitation (**Table 4**).

Plant population was 27% higher on raised beds compared to the flat (**Table 6**). The reduced plant population observed on the flat land was due to excessive precipitation during the early season causing reduced emergence. The raised beds directed excess precipitation towards the furrows alongside the beds limiting plant stress and likely improving emergence.

There were no differences between seedbed treatments in the IDC scores. However, another soybean study in the RRNV observed lower IDC symptoms when raised beds were used [22]. Iron chlorosis can be highly environmental dependent and variable [11], and the results may have been different under more severe IDC growing conditions. Greater plant population resulted in a higher vigor score of 5.9 on raised beds versus 4.4 on flat (**Table 6**).

The soybean yield on the raised beds in the combined analysis was 6.3% higher compared with flat (**Table 6**). Bruns and Young [23] also reported higher yields on raised beds compared with flat. Hoppe *et al.* [7] found that there was no yield difference between raised beds and flat in years without excess water.

Table 6. Means for raised beds and Fe-EDDHA across proxy cultivar effects on general agronomic traits averaged across six environments at Fargo and Casselton, North Dakota, USA, in 2013 and 2014.

Treatment	Plant Population	IDC[a]	EV[b]	Ht[c]	PC	OC	TW	TKW	Yield
	plants ha^{-1}	1 - 5	1 - 9	cm	-----------%------------- -		kg·m^{-3}	g	kg·ha^{-1}
				Raised bed effect					
Flat	260,674	2.3	4.4	56	32.5	18.2	741.1	167.0	2722
Raised beds	331,058***	2.3	5.9**	58	32.5	18.2	738.9	167.1	2893‡‡
				Fe-EDDHA[d]					
No Fe-EDDHA	310,735	2.4	5.2	57	32.5	18.2	739.7	167.0	2787
Fe-EDDHA[d]	280,998**	2.2*	5.1	57	32.5	18.2	740.3	167.1	2828

‡‡, *, **, ***Significant at ($p \le 0.10$), ($p \le 0.05$), ($p \le 0.01$), and ($p \le 0.001$) respectively. [a]IDC, Iron Deficiency Chlorosis score was based on a scale of 1 - 5 (1 = no IDC symptoms, 5 = severe IDC symptoms/plant death [2]). [b]EV, early vigor score (July 1, 2013 and June 25, 2014); Vigor score was based on a scale of 1 - 9 (1 = very poor vigor, 9 = excellent vigor). [c]Ht, plant height; PC, protein content; OC, oil content; TW, test weight; TKW, 1000 kernel weight. [d]Fe-EDDHA was seed-applied at a rate of 3.36 kg·ha^{-1}.

Excess water stress significantly decreases stomatal conductance [24]. Oosterhuis *et al.* [24] speculated that soybean root systems resist taking up water in response to flood stress resulting in stomatal closure, increased water saturation of the stomatal area, and decreased photosynthesis. Lower plant population and early season water stress may be factors in the lower yield on flat compared with raised bed.

3.4. Fe-EDDHA Application Effect

When Fe-EDDHA was applied, plant population was significantly lower compared with no Fe-chelate applied to the seed (**Table 6**). Initially, the lower plant population was unexpected given that Fe-chelate has been shown to improve soybean performance [2] [11] [25] [26]. However, after conducting additional germination tests, a delay in germination due to Fe-EDDHA seed treatment was found. We speculate that the Fe-chelate seed coating caused the reduction in established plants compared with seed without Fe-EDDHA applied.

Adverse soil conditions, such as water-logging, high $CaCO_3$ content, and high pH can lead to Fe being plant unavailable [12] [22] [27]. In this study, the IDC scores were significantly lower (less visible chlorosis) with the application Fe-EDDHA. Using Fe-EDDHA as a soil application, researchers found that higher rates of Fe-EDDHA resulted in lower incidences of IDC and lower soybean IDC scores when Fe-EDDDHA was applied to the seed and in the furrow [11] [27].

The lower IDC score did not lead to a significant yield increase (**Table 6**). Since the Fe-EDDHA seed treatment resulted in a lower plant population, we also analyzed yield with plant population as a covariate. Based on the analysis of covariance, the 0 kg·ha^{-1} Fe-EDDHA treatment yield went slightly down from 2787 (**Table 6**) to 2784 kg·ha^{-1} and the 3.36 kg·ha^{-1} Fe-EDDHA treatment yield went slightly up from 2828 (**Table 6**) to 2830 kg·ha^{-1} as result of using plant population as a covariate for yield. However, this yield difference between the two

Fe-EDDHA rates was still not significantly different. The severity of IDC is hard to predict from year to year, even within the same location [11]. The IDC severity in this research was relatively moderate and the soybean plants were able to outgrow the chlorosis and no lingering IDC symptoms where observed during the reproductive stages, which possibly resulted in similar yield with and without Fe-EDDHA.

There was no significant Fe-EDDHA by proxy cultivar interaction, aside from plant population (**Table 5**). All proxy cultivars had lower plant populations with Fe-EDDHA; however, the plant population of proxy cultivar 4 was significantly lower than other proxy cultivars. These results are not relevant to the study objectives.

Previous research concluded that Fe-EDDHA effectiveness was based mostly on cultivar response to application [2] [26] [27].

3.5. Raised Beds Effect on Biomass

Significant differences in total biomass and total root biomass were found for biomass traits on raised beds vs flat (**Table 7**). No significance was observed for Fe-EDDHA application and the raised bed or Fe-EDDHA by proxy cultivar interactions for observed traits and are not discussed further. Although the proxy cultivar showed significant differences, we will not discuss further as it is expected to have differences between cultivars regardless of other treatments.

Total biomass for plants in a 90 cm row length, was 10% greater for raised beds compared to flat (**Table 8**). The total root biomass was 35.1 g and 30.8 g for raised beds compared to flat, respectively. This equates to about 14% greater root mass for plants grown on raised beds (**Table 8**). Since raised beds are known to alleviate excess water stress, greater biomass would be expected as a result to less stress. Greater root mass on raised beds was confirmed by Hoppe [22] and

Table 7. Probability levels for factors in the Analysis of Variance (ANOVA) for raised beds effect on biomass traits averaged over six environments at Fargo and Casselton, North Dakota, USA in 2013 and 2014.

Source of Variation	df	TB[a]	PB	RB
			Pr > F[b]	
Raised beds	1	**0.10**	0.13	**0.01**
Fe-EDDHA	1	0.71	0.84	0.29
Raised beds x Fe-EDDHA	1	0.56	0.48	0.69
Proxy cultivar (proxy)[c]	4	**0.06**	**0.07**	**0.06**
Raised beds x proxy	4	0.55	0.62	**0.07**
Fe-EDDHA x proxy	4	0.95	0.96	0.79
Raised beds x Fe-EDDHA x proxy	4	0.48	0.40	0.79

[a]TB, total biomass per sample size (90 cm row); PB, aboveground biomass per sample; RB, total root biomass per sample. [b]Bolded numbers are significant at $p \le 0.10$. [c]Proxy cultivar are soybean cultivars with similar maturity and IDC tolerance.

Table 8. Means for raised beds effect on biomass traits averaged over six environments at Fargo and Casselton, North Dakota, USA in 2013 and 2014.

	TB[a]	PB	RB
	----------------------------------g-------------------------------		
	Raised beds effect		
Flat	357.7	326.9	30.8
Raised beds	392.6[‡‡]	357.5	35.1***
	Fe-EDDHA		
No Fe-EDDHA	376.8	343.0	33.8
Fe-EDDHA[b]	373.5	341.3	32.2

***Significant at $p \leq 0.001$, and ‡‡denotes significance at $p \leq 0.10$. [a]TB, total biomass per sample (90 cm row); PB, aboveground biomass per sample; RB, total root biomass per sample. [b]Fe-EDDHA was seed-applied at a rate of 3.36 kg·ha^{-1}.

Hoppe *et al.* [7].

Raised beds had a higher plant population than flat (**Table 6**). We also calculated the biomass for individual plants by dividing the biomass numbers by the number of plants per sample (90 cm row length) for each of the experimental units. The total plant mass per plant was 24.5 g plant^{-1} for flat, and significantly ($p \leq 0.05$), greater than the raised bed with 19.3 g plant^{-1} per plant. The individual root mass was 2.0 g plant^{-1} for flat and significantly greater ($p \leq 0.10$) than the raised bed with 1.7 g plant^{-1}. The flat had greater biomass per plant as there were fewer plants in the 90 cm of row.

With the greater plant population on the raised beds and significantly greater total biomass (**Table 6** and **Table 8**), there was likely more light energy interception due to plants being closer together. Soybean in narrow row spacing and higher plant populations have higher light interception compared to wide row spacing and low populations [28] [29]. Other studies have observed that legumes benefit from having neighboring root systems and root structures due to increased rhizosphere activity [6] [25].

There was no significant difference in the total, above ground, or root biomass with or without the application of Fe-EDDHA when 90 cm of the row was harvested (**Table 8**). Schenkeveld *et al.* [27] measured yield in terms of aboveground dry-weight biomass and speculated that better root development played a role in the soybean plant's ability to take up soil-applied Fe-EDDHA more efficiently, thus leading to the disappearance of IDC symptoms over time [27].

4. Conclusions

4.1. Raised Beds

Iron-deficiency chlorosis continues to be a problem in the RRNV. The environmental conditions in the RRNV (*i.e.* high soil saturation early in season, calcareous soil, etc.) are conducive to IDC. When averaged across six environments, plant population, early vigor score, and yield, and biomass were all significantly increased with raised beds compared to flat. Therefore, raised beds can be considered as a management strategy for agricultural producers. Raised beds will have an ongoing effect on crop productivity and can be carried over from season to season via fall and spring maintenance.

Continual raised beds in a field could therefore lead to less soil compaction where the soybean plant grows, better drainage, and as a result, continual reducing of IDC conditions. Raised beds can increase seed yield during years with above normal early season precipitation. In our previous research we found that in a drier year soybean seeded on raised beds yielded equal compared to flat. Further research is needed to determine the long-term effect of raised beds on plant development, IDC expression, and yield.

4.2. Fe-EDDHA Application

The application of Fe-EDDHA via seed treatment in this study significantly reduced plant population. Iron-deficiency symptoms with the seed application of Fe-EDDHA were significantly reduced, when averaged across all environments. Seed-treatment of Fe-EDDHA is a practice that can help reduce IDC incidence but further research needs to focus on the application method or product formulation so no plant population reduction will happen. Fe-EDDHA application effect will only last as a long as the Fe-chelate is actively affecting soil chemistry. Seed treatment may not be the best method of application given the plant population reduction observed in this experiment and other methods such as in-furrow application or foliar sprays should be considered. Further research could evaluate a combination of seed treatment (possibly at a lower rate), in furrow application and or foliar application after emergence. The application of Fe-EDDHA remains a partial solution and should therefore be combined with other methods to reduce IDC. However, adding Fe-EDDHA as a seed treatment does not require any additional steps or equipment if producers already use seed treatments. Our results are similar to others which suggest using a Fe-EDDHA treatment can substantially reduce IDC symptoms, however, selecting IDC-tolerant cultivars remains the best IDC management method.

Acknowledgements

The authors would like to thank Chad Deplazes and undergraduate summer help for their technical assistance.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] USDA-NASS (National Agricultural Statistical Service) (2021) USDA's National Agricultural Statistics Service North Dakota Field Office.

https://www.nass.usda.gov/Statistics_by_State/North_Dakota/index.php

[2] Goos, R.J. and Johnson, B.E. (2000) A Comparison of Three Methods for Reducing Iron-Deficiency Chlorosis in Soybean. *Agronomy Journal*, **92**, 1135-1139. https://doi.org/10.2134/agronj2000.9261135x

[3] Hatfield, J.L., Allmaras, R.R., Rehm, G.W. and Lowery, B. (1998) Ridge Tillage for Corn and Soybean Production: Environmental Quality Impacts. *Soil Tillage Research*, **48**, 145-154. https://doi.org/10.1016/S0167-1987(98)00141-X

[4] Benjamin, J.G., Blaylock, A.D., Brown, H.J. and Cruse, R.M. (1990) Ridge Tillage Effects on Simulated Water and Heat Transport. *Soil Tillage Research*, **18**, 167-180. https://doi.org/10.1016/0167-1987(90)90057-K

[5] Bakker, D.M., Hamilton, G.J., Houlbrooke, D.J. and Spann, C. (2005) The Effect of Raised Beds on Soil Structure, Waterlogging, and Productivity on Duplex Soils in Western Australia. *Australian Journal of Soil Research*, **43**, 575-585. https://doi.org/10.1071/SR03118

[6] Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G. and Zuberer, D.A. (1999) Principles and Applications of Soil Microbiology. Prentice Hall Inc., Upper Saddle River.

[7] Hoppe, A.R., Mehring, G.H. and Kandel, H. (2017) Soybean Productivity as Affected by Raised Beds under Dry Environments. *Open Agriculture Journal*, **11**, 46-59. https://doi.org/10.2174/1874331501711010046

[8] Hansen, N.C., Schmitt, M.A., Andersen, J.E. and Strock, J.S. (2003) Iron Deficiency of Soybean in the Upper Midwest and Associated Soil Properties. *Proceedings of the Agronomy Journal*, *American Society of Agronomy*, **95**, 1595-1601. https://doi.org/10.2134/agronj2003.1595

[9] Zhang, M., Yi, Z., Qiu, Q., Meng, F., Rao, D., Zhao, J., Zhang, W. and Yan, X. (2017) Relationship between Low Iron Stress and Iron Efficiency of Soybean Varieties. *Asian Agricultural Research*, **9**, 37-42.

[10] Kaiser, D.E., Lamb, J.A., Bloom, P.R and Hernandez, J.A. (2014) Comparison of Field Management Strategies for Preventing Iron Deficiency Chlorosis in Soybean. *Agronomy Journal*, **106**, 1963-1974. https://doi.org/10.2134/agronj13.0296

[11] Wiersma, J.V. (2005) High Rates of Fe-EDDHA and Seed Iron Concentration Suggest Partial Solutions to Iron Deficiency in Soybean. *Agronomy Journal*, **97**, 924-934. https://doi.org/10.2134/agronj2004.0309

[12] Gamble, A.V, Howe, J.A., Delaney, D., van Stanten, E. and Yates, R. (2014) Iron Chelates Alleviate Iron Chlorosis in Soybean on High pH Soils. *Agronomy Journal*, **106**, 1251-1257. https://doi.org/10.2134/agronj13.0474

[13] Kandel, H.J., Brodshaug, J.A., Steele, D.D., Ransom, J.K., DeSutter, T.M. and Sands, G.R. (2013) Subsurface Drainage Effects on Soil Penetration Resistance and Water Table Depth on a Clay Soil in the Red River of the North Valley, USA. *Agricultural Engineering International: CIGR Journal*, **15**, 1-10.

[14] Mourtzinis, S. and Conley, S.P. (2017) Delineating Soybean Maturity Groups across the US. *Agronomy Journal*, **109**, 1397-1403. https://doi.org/10.2134/agronj2016.10.0581

[15] Kandel, H. (2012) North Dakota Soybean Variety Trial Results for 2012 and Selection Guide. North Dakota State University, Fargo, ND, USA.

[16] Kandel, H. (2013) North Dakota Soybean Variety Trial Results for 2013 and Selection Guide. North Dakota State University, Fargo, ND, USA.

[17] Schmitz, P.K., Stanley, J.D. and Kandel, H.J. (2020) Row Spacing and Seeding Rate

Effect on Soybean Seed Yield in North Dakota. *Crop, Forage, and Turfgrass Management*, **11**, 605https://doi.org/10.1002/cft2.20010

[18] Gaspar, A.P., Mourtzinis, S., Kyle, D., Galdi, E., Lindsey, L.E., Hamman, W.P., Matcham, E.G., Kandel, H.J., Schmitz, P., Stanley, J.D., *et al.* (2020) Defining Optimal Soybean Seeding Rates and Associated Risk across North America. *Agronomy Journal*, **112**, 2928-2943. https://doi.org/10.1002/agj2.20203

[19] North Dakota Agricultural Weather Network (NDAWN) (2021) Air Temperature and Precipitation. https://ndawn.ndsu.nodak.edu/

[20] Liesch, A.M., Ruiz Diaz, D.A., Martin, K.L., Olson, B.L., Mengel, D. B. and Roozeboom, K.L. (2011) Management Strategies for Increasing Soybean Yield on Soils Susceptible to Iron Deficiency. *Agronomy Journal*, **103**, 1870-1877. https://doi.org/10.2134/agronj2011.0191

[21] Kirnak, H., Gokalp, Z., Dogan, E. and Copur, O. (2017) Soil Characteristics of Soybean Fields as Effected by Compaction, Irrigation, and Fertilization. *Legume Research*, **40**, 691-697. https://doi.org/10.18805/lr.v0i0.8407

[22] Hoppe, A.R. (2013) Soybean Productivity as Affected by Raised Seedbeds and Subsurface Drainage, M.Sc. Thesis: North Dakota State University, Fargo, ND, USA.

[23] Bruns, H.A. and Young, L.D. (2012) Raised Seedbeds for Soybean in Twin Rows Increase Yields over Flat Seedbeds. *Crop Management*, **11**, 1-7. https://doi.org/10.1094/CM-2012-0712-01-RS

[24] Oosterhuis, D.M., Scott, H.D., Hampton, R.E. and Wullschleger, S.D. (1989) Physiological Responses of Two Soybean [Glycine max, (L.) Merr] Cultivars to Short-Term Flooding. *Environmental and Experimental Botany*, **30**, 85-92. https://doi.org/10.1016/0098-8472(90)90012-S

[25] Wiersma, J.V. (2007) Iron Acquisition of Three Soybean Varieties Grown at Five Seeding Densities and Five Rates of Fe-EDDHA. *Proceedings of the Agronomy Journal*, **99**, 1018-1028. https://doi.org/10.2134/agronj2006.0271

[26] Goos, R.J. and Johnson, B. (2001) Seed Treatment, Seeding Rate, and Cultivar Effects on Iron Deficiency Chlorosis of Soybean. *Journal of Plant Nutrition*, **24**, 1255-1268. https://doi.org/10.1081/PLN-100106980

[27] Schenkeveld, W.D.C., Dijcker, R., Reichwein, A.M., Temminghoff, E.J.M. and van Riemsdijk, W.H. (2008) The Effectiveness of Soil-Applied FeEDDHA Treatments in Preventing Iron Chlorosis in Soybean as a Function of the o,o,-FeEDDHA Content. *Plant and Soil*, **303**, 161-176. https://doi.org/10.1007/s11104-007-9496-x

[28] Board, J. (2000) Light Interception Efficiency and Light Quality Affect Yield Compensation of Soybean at Low Plant POPULATIONS. *Crop Science*, **40**, 1285-1294. https://doi.org/10.2135/cropsci2000.4051285x

[29] Taylor, H.M., Mason, W.K., Bennie, A.T.P., Rowse, H.R. (1982) Responses of Soybeans to Two Row Spacings and Two Soil Water Levels. I. An Analysis of Biomass Accumulation, Canopy Development, Solar Radiation Interception and Components of Seed Yield. *Field Crops Research*, **5**, 1-14. https://doi.org/10.1016/0378-4290(82)90002-8