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Organic Nitrogen Fertilizer Selection Influences Water Use Efficiency in Drip-Irrigated Sweet Corn †

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Abstract: Organic farmers often rely on off-farm nitrogen (N) sources for mid-season N. Farmers can also produce cyano-fertilizer on-farm by growing N-fixing cyanobacteria (*Anabaena* spp.) in raceways and applying the cyanobacteria through irrigation systems. A two-year field study was conducted, and blood meal, feather meal, fish emulsion, and cyano-fertilizer were evaluated to determine whether the water use efficiency (WUE) of sweet corn (*Zea mays*) was affected by fertilizer type. Fish emulsion and cyano-fertilizer were supplied in four split applications through drip irrigation, while the blood meal and feather meal were subsurface banded pre-plant. Leaf gas exchange measurements were taken during tasseling. The amounts of phytohormone and Fe applied in organic N fertilizers were correlated with field water use efficiency (fWUE), instantaneous water use efficiency (iWUE), and leaf gas exchange components of sweet corn. A positive relationship was observed between the amount of salicylic acid (SA) applied with both iWUE ($r = 0.71$, $p < 0.05$) and fWUE ($r = 0.68$, $p < 0.01$). The amount of Fe applied was positively correlated with the leaf vapor pressure deficit ($r = 0.54$, $p < 0.01$) and transpiration rate ($r = 0.53$, $p < 0.01$). Cyano-fertilizer had the highest yield and WUE, likely due to the high amount of SA applied, although fish emulsion was comparable in year one. These relationships require further exploration to elucidate the mechanisms impacting WUE.

Keywords: blood meal; cyanobacteria; cyano-fertilizer; feather meal; fish emulsion; organic agriculture; *Zea mays*

1. Introduction

Water use efficiency (WUE) can be measured on various scales from the leaf to the field [1]. WUE is a measurement of the carbon (C) assimilated per unit of water transpired by the plant [2]. It can be measured using either a leaf gas exchange [3] or field-scale approach [4]. Field water use efficiency (fWUE) is defined as the yield produced per unit of water used. Methods of measuring instantaneous WUE (iWUE) using the leaf gas exchange approach are derived from direct measurements of photosynthesis and transpiration [5].

Fertilizer type has the potential to influence WUE. Nitrogen (N) plays a crucial role in the crop growth and yield development of sweet corn (*Zea mays*), and its application affects WUE. Organic growers often use commercial, organic, animal-based fertilizers which vary in their nutrient composition and forms of available N (NH_4^+-N and NO_3^--N) and have high transportation costs to meet the N demand of crops. Organic agriculture often relies on fertilizers and soil amendments from off-farm sources to meet crop N demand [6]. Organic N fertilizers can be classified as solid (e.g., compost, blood meal, and feather meal) or liquid (e.g., fish emulsion) [7]. In organic agriculture, solid fertilizers are often incorporated into the soil before planting, while liquid fertilizers are generally applied season-long through irrigation.

Most commercially available organic fertilizers act as slow-release N sources and are often produced off-farm (such as blood meal, feather meal, and fish emulsion). However, other fertilizer options are being developed that will allow farmers to produce N fertilizer on-farm, such as the cultivation of N-fixing cyanobacteria (e.g., *Anabaena* spp.) for direct use as fertilizer. Cyanobacteria have unique dual properties because they can both fix N from the atmosphere and photosynthesize to produce N fertilizer without fossil fuels. When used as fertilizer, cyanobacteria (also known as cyano-fertilizer) have been shown to be effective in greenhouse studies on kale, maize, and pepper [8] and in field studies, both in drip-irrigated kale [9] and sprinkler-irrigated peach [10]. In addition, cyano-fertilizer can provide additional benefits by solubilizing organic phosphorus (P) sources [11] and improving plant tolerance to abiotic and biotic stresses [12].

Organic N fertilizers have different chemical properties that influence plant growth and development and plant–water relations due to the micronutrients and phytohormones present in these fertilizers. Micronutrients, including iron (Fe) and zinc (Zn), in organic N fertilizers can influence stomatal regulation, photosynthesis, and osmoregulation in plants [13,14]. Phytohormones in organic fertilizers can also influence plant growth and development. Phytohormones, such as abscisic acid (ABA), play a significant role in regulating plant–water relations [15,16]. Auxin in the form of indole-3-acetic acid (IAA) has been associated with the increased growth and development of photosynthetic pigments in grape and wheat [17,18]. IAA caused a wider and more circular structure of the xylem vessels in eggplant, which subsequently increased water transport [19]. In addition, cyano-fertilizer production methods can alter phytohormone concentrations, including ABA, IAA, and salicylic acid (SA) [20].

SA has been shown to increase WUE in maize and mustard [21–23]. The effects of the exogenous application of SA on growth, photosynthesis, stomatal regulation, and plant–water relations have been measured in maize and soybeans [24–26]. The WUE of broccoli plants increased following SA application due to enhanced cell division [27]. Most exogenous applications of SA to plants have been in the form of foliar application. SA may be related to the induction of the antioxidant response and to the protective role of membranes that increase the ability of plants to regulate water use [28]. Overall, the effects of SA in plants depend on its concentration and plant species [29].

Different forms of N in fertilizer can also induce various effects on plant growth and physiology. Inorganic forms of N in fertilizer have been shown to affect the WUE of French bean [30] and N assimilation in wheat [31]. Inorganic forms of N, particularly NH_4^+-N, decreased the WUE and net photosynthetic rate of tea plants [32], while a high NO_3^--N concentration in fertilizers has been shown to decrease leaf transpiration rate in maize [33]. NO_3^--N contributes to changes in stomatal aperture, increased dry matter accumulation and WUE in tomato compared to NH_4^+-N [34]. When fertilizers with high NH_4^+-N concentrations were used, the leaf area, fresh weight, and WUE of artichoke were lower [35] due to interferences with the photosynthetic production of ATP, the metabolism of carbohydrates, and amino acid synthesis [36]. Many WUE studies have been conducted on the effects of N fertilizer [30,32,33,37,38], but none of them have compared cyano-fertilizer with other organic fertilizers on sweet corn. There is also no literature describing how inorganic N forms and phytohormone concentrations in fertilizers affect the WUE of sweet corn. We hypothesized that (i) phytohormones in organic fertilizers (IAA and SA) will increase WUE and ear yield; (ii) an increasing amount of N applied as NH_4^+-N will reduce the WUE (fWUE and iWUE) and ear yield of sweet corn; (iii) an increasing amount of N applied as NO_3^--N will decrease the transpiration rate and increase the WUE (fWUE and iWUE); and (iv) micronutrients (Fe and Zn) in organic N fertilizers may influence gas exchange components in sweet corn. The overall aim of this study was to assess whether phytohormones, micronutrients, and forms of inorganic N in organic fertilizers affect the WUE (fWUE and iWUE) and leaf gas exchange components of drip-irrigated sweet corn under typical application methods and field conditions.

2. Materials and Methods

Field experiments were carried out on certified organic land at the Colorado State University (CSU) Horticulture Field Research Center (Fort Collins, CO, USA; 40°36'39.78" N, 104°59'48.25" W). Previously, the field area was planted with buckwheat followed by winter cover crops, rye (*Secale cereal*) and turnips (*Brassica rapa*). The experimental site is in a semi-arid zone with clay loam soils. The soil was classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series [39].

Soil samples were collected to a depth of 30 cm from a representative area of the field (15 m × 30 m) and analyzed by the Soil, Water, and Plant Testing Laboratory at Colorado State University. Particle size distribution was determined using the hydrometer method based on Stoke's law [40]. Chemical analyses included soil pH and electrical conductivity (EC) measured in supernatant suspension of 1:1 soil to water using a Mettler Toledo pH/EC meter (Thermo Fischer Scientific, Waltham, MA, USA). Cation exchange capacity (CEC) was determined by summation of the exchangeable bases (Ca^{2+}, K^+, Mg^{2+}, Na^+) plus hydrogen (H^+) using ammonium acetate (1 N $NH_4C_2H_3O_2$, pH 7.0) [41]. Organic matter (OM) content was determined by the loss on ignition method [42]. Soil inorganic N (NH_4^+-N and NO_3^--N) was extracted using 2 M KCl, and the filtered extract was analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX, USA). Phosphorus (P) was extracted with 0.5 M sodium bicarbonate ($NaHCO_3$) solution [43] and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA, USA). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were extracted with ammonium bicarbonate–diethylene triaminepentaacetic acid (AB-DTPA) [44] and analyzed by ICP-OES (Perkin Elmer, Waltham, MA, USA). The results of soil physical, chemical, and nutrient analyses are presented in Table 1.

Table 1. Initial soil properties (0–30 cm depth) of the sweet corn experimental site.

Soil Properties	2013	2014
pH [†]	7.5	7.7
Electrical conductivity [†] (dS m^{-1})	0.6	0.6
Cation exchange capacity, CEC [‡] (meq 100 g^{-1})	29	30
Organic matter, OM [‡‡] (%)	2.5	2.6
NH_4^+-N [‡‡‡] (ppm)	2.5	2.7
NO_3^--N [‡‡‡] (ppm)	1.9	2.1
P [§] (ppm)	30	32
K [♯] (ppm)	463	456
Ca [♯] (ppm)	4560	4650
Mg [♯] (ppm)	616	622
Fe [♯] (ppm)	6	7
Zn [♯] (ppm)	1.4	1.6

[†] pH and electrical conductivity were determined in water (1:1). [‡] CEC was determined using ammonium acetate (1 N $NH_4C_2H_3O_2$, pH 7.0) extraction. [‡‡] OM was determined by the loss on ignition method. [‡‡‡] Samples were extracted using 2 M KCl. [§] Sample was extracted with 0.5 M $NaHCO_3$. [♯] Samples were extracted using ammonium bicarbonate diethylenetriamine pentaacetate (AB-DTPA).

Treatments consisting of four different types of N fertilizer and control (no fertilizer) were arranged in a randomized complete block design with four replications. Plots were single-row plots (76 cm × 457 cm); we do not expect fertilizer movement to neighboring plots due to a calculated wetting radius of only 7 cm around each emitter and a large (>0.50 m) dry inter-row zone. The fertilizers compared in this study were liquid fertilizers (Alaska® fish emulsion (Planet Natural, Bozeman, MO, USA) and cyano-fertilizer) and solid fertilizers (blood meal and feather meal (Down To Earth Inc., Eugene, OR, USA)). For this study, N-fixing cyanobacteria (*Anabaena* sp.) were cultured from local soils and inoculated into nutrient-supplemented raceways (shallow, elliptical ponds) [45,46].

Sweet corn (*Zea mays* var. Luscious F1 se+) seeds were purchased from Johnny's Seeds (Johnny's Selected Seed, Waterville, ME, USA). Seeds were hand planted at a depth of approximately 2.5 cm on 1 July 2013 and 2 July 2014, with an in-row spacing of 12 cm in rows spaced 76 cm apart.

The amount of precipitation was obtained from a nearby CoAgMet weather station (http://www.coagmet.colostate.edu/ accessed on 5 January 2015). Water was applied daily using an automated drip irrigation system. Two lines of John Deere® drip tape (Deere & Co., Moline, IL, USA) with a flow rate of 125 L hour^{-1} 100 m^{-1} were stretched along each row. The drip system used for this study comprised of laterals (15 mm diameter), and emitters were 20 cm apart. Irrigation was applied for 15 min day^{-1} at the initial stage and was changed to 30 min day^{-1} after plants reached the V6 stage (Table 2).

Table 2. Average monthly precipitation and the amount of water applied during the 2013 and 2014 sweet corn growing seasons.

	2013	**2014**
Planting	1 July	2 July
Harvest	18 September	19 September
Precipitation (mm)		
July	39	69
August	13	26
September	119	9
Amount of water applied (mm)		
Irrigation	729	737
Fertigation	414	414
Total Precipitation + Irrigation + Fertigation ‡ (mm)	1314	1257

‡ Total amount of water applied over the growing season is the combination of precipitation, irrigation, and fertigation. All plots received the same amount of water applied via irrigation. When liquid fertilizers were applied, the control and solid fertilizer treatments were irrigated with the same amount of water as that used to fertigate the liquid fertilizer treatments.

Both the fish emulsion and cyano-fertilizer were in liquid form and were supplied in four split applications through the drip irrigation system over the growing season, while the blood meal and feather meal were in dry powdered form and incorporated into the soil prior to planting. Blood meal and feather meal were applied 6 cm from the plants and 6 cm deep through band application using a hoe. Although the fertilizers differed in application method and timing, all fertilizers were applied at 112 kg N ha^{-1} using common farmer practices for solid and liquid fertilizers, and no other nutrients were recommended based on soil testing.

Fertilizer samples were sent to the Soil, Water, and Plant Testing Laboratory at CSU and analyzed for N, C, P, K, Fe, Ca, Mg, and Zn (Tables 3 and 4). Nitrogen and C were measured with a CN analyzer (TruSpec® Micro CN Analyzer, Geleen, The Netherlands). Fertilizer samples were digested with nitric acid (HNO$_3$) and hydrogen peroxide (H$_2$O$_2$). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were analyzed using an ICP-OES (Perkin Elmer, Waltham, MA, USA). Solid fertilizers were extracted using 2 M KCl, and the filtered extracts were analyzed for NH$_4^+$-N and NO$_3^-$-N. The filtered extracts of solid and liquid fertilizers were analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX, USA) (Table 4).

Phytohormone analyses were conducted at the Proteomics and Metabolomics Facility, CSU. Fertilizer samples were adjusted to pH 7.0 with 1 N NaOH and extracted three times with water-saturated n-butanol, followed by vacuum drying [47]. The extracts obtained were filtered through membrane filters (pore size 0.45 μm). Supernatants were harvested by centrifugation at 5000× *g* for 20 min at 4 °C. Supernatants were homogenized in liquid nitrogen using a cold mortar and pestle. The resulting powder was extracted using 80% methanol containing 10 mg L^{-1} butylated hydroxytoluene at 4 °C. Samples were methylated with diazomethane and dissolved in heptane. Gas chromatography-mass

spectrometry (GC-MS) analyses were performed [48]. The concentrations and amounts of phytohormones applied over the growing season for each fertilizer treatment are presented in Table 5.

Table 3. Nutrient analysis of fertilizer samples.

Fertilizer	N ‡	C ‡	C/N Ratio	P ♯	K ♯	Fe ♯	Ca ♯	Mg ♯	Zn ♯
	%	%				mg kg^{-1}			
Cyano-fertilizer	0.041	0.038	0.9	6	0.1	6	10	18	0.1
Fish emulsion	0.045	0.066	1.5	1600	20510	131	725	921	18
Feather meal	14.4	50.5	3.5	640	1776	62	1466	575	18
Blood meal	15.5	51.4	3.3	32	366	118	904	283	14

‡ Samples were analyzed using CN analyzer and reported previously [7]. ♯ Samples were digested with HNO_3 and H_2O_2 and analyzed using ICP-OES.

Table 4. Inorganic N concentration, inorganic N ratio, and percentage of N applied as inorganic-N of fertilizers and amount of Fe and Zn applied in the fertilizers at 112 kg ha^{-1} over the course of growing season.

	NH_4^+-N *	NO_3^--N *	NH_4^+-N: NO_3^--N	N as NH_4^+-N	N as NO_3^--N	Fe	Zn
	mg kg^{-1}			%		kg ha^{-1}	
Cyano-fertilizer	4.7	0.01	470	0.24	0.0005	6.7×10^{-4}	1.1×10^{-5}
Fish emulsion	23.7	0.12	198	0.05	0.0002	1.5×10^{-2}	2.0×10^{-3}
Feather meal	1230	2.30	536	0.95	0.002	6.9×10^{-3}	2.0×10^{-3}
Blood meal	27.7	8.40	3.3	0.02	0.006	1.3×10^{-2}	1.6×10^{-3}

* Samples were extracted using 2 M KCl and were analyzed using an autoanalyzer [7].

Table 5. Phytohormone concentrations in the fertilizers and amount of phytohormones applied in the fertilizer over the growing season.

	Indole-3-Acetic Acid (IAA) †	Salicylic Acid (SA) †	Indole-3-Acetic Acid (IAA) †	Salicylic Acid (SA) †
	ng g^{-1}		kg ha^{-1}	
Cyano-fertilizer	0.06	6	4.4×10^{-5}	4.8×10^{-3}
Fish emulsion	1436	77	2.4×10^{-3}	1.2×10^{-4}
Feather meal	241	240	1.5×10^{-4}	1.5×10^{-4}
Blood meal	58	50	3.9×10^{-5}	3.4×10^{-5}

† Samples were extracted in 80% methanol containing 10 mg L^{-1} butylated hydroxytoluene at 4 °C.

Leaf gas exchange measurements of the uppermost, fully expanded leaf from three plants in each plot were carried out during tasseling in 2014 using a portable open photosynthesis system, LI- 6400XT (LI-COR Inc., Lincoln, NE, USA). Measurements were taken between 10:00 and 15:00 under ambient CO_2 (380 μL L^{-1}); the leaf temperature inside the cuvette was set to 26 °C, and the photon flux density was 2000 μmol m^{-2} s^{-1}. Relative humidity was monitored and ranged between 50 and 60% during the day. Each leaf was allowed to reach a steady state of CO_2 uptake in the LI-6400XT leaf chamber before measurements were taken.

All aboveground plant parts from each treatment were hand harvested and separated into leaves, stems, and ears at 79 days after planting. The separated plant parts were dried at 70 °C for 72 h and weighed to determine the total above-ground biomass, but marketable yields were reported as fresh weights since that is how sweet corn is generally sold. Field water use efficiency (fWUE) was calculated using fresh ear yield divided by total amount of water applied over the growing season (precipitation + irrigation + fertigation). Instantaneous water use efficiency (iWUE) was only measured in 2014 and calculated as the ratio of net photosynthetic rate to transpiration rate and expressed in μmol CO_2 mmol^{-1} H_2O.

Data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). The Univariate and Boxplot procedures were used to evaluate the normality of data distribution. Analysis of variance (ANOVA) was performed on the data by using the GLM procedure. The Tukey's Studentized Range (HSD) test of mean separation value was calculated from the obtained mean square errors to determine whether main effects or interactions were significant ($p < 0.10$). The relationships between parameters were assessed by linear correlation using the CORR procedure. PROC REG with the stepwise selection method was used to select the best combination of independent variables (gas exchange components, IAA, SA, NH_4^+-N, NO_3^--N, Fe, and Zn applied in organic N fertilizers) to predict the dependent variables (fWUE and iWUE).

3. Results

All fertilizer sources significantly increased yield as compared to the control in 2013, and fish emulsion and cyano-fertilizer treatments had significantly higher fresh yield compared to other treatments and control ($p = 0.0032$; Figure 1a). In 2014, only the cyano-fertilizer treatment had a higher yield compared with other treatments and control ($p < 0.0001$; Figure 1b). No fertilizer effect was observed in the dry weight of leaves or stems.
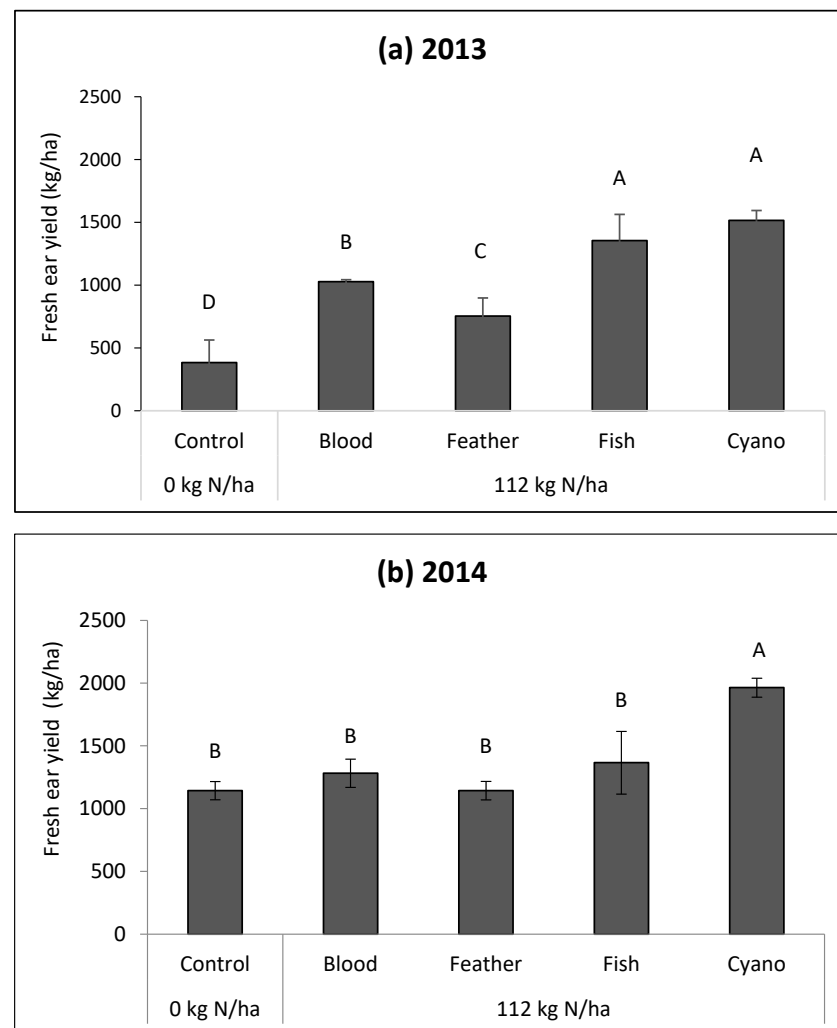
Figure 1. Effects of different organic fertilizers on fresh ear yield of sweet corn at harvest in (**a**) 2013 and (**b**) 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at $p < 0.05$ using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

The fish emulsion, cyano-fertilizer, and blood meal treatments had higher fWUEs compared to the control in 2013 (p = 0.0002; Figure 2a). The cyano-fertilizer treatment had a higher fWUE compared to blood meal and feather meal in 2013 (Figure 2a) and higher fWUE than feather meal and fish emulsion treatments in 2014 (p < 0.0001; Figure 2b). The cyano-fertilizer treatment had a higher iWUE compared to other treatments, including the control, in 2014 (p < 0.0001; Figure 3).
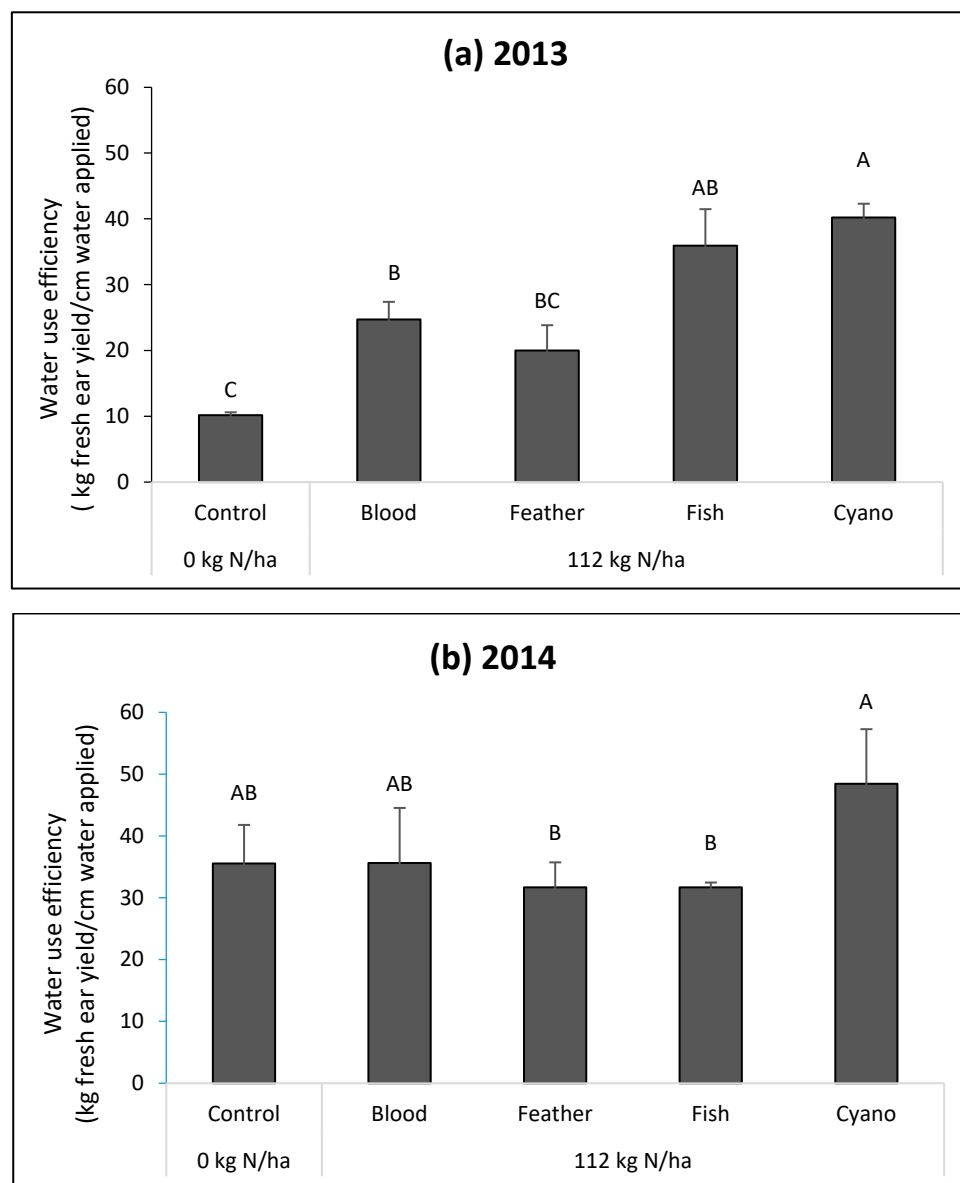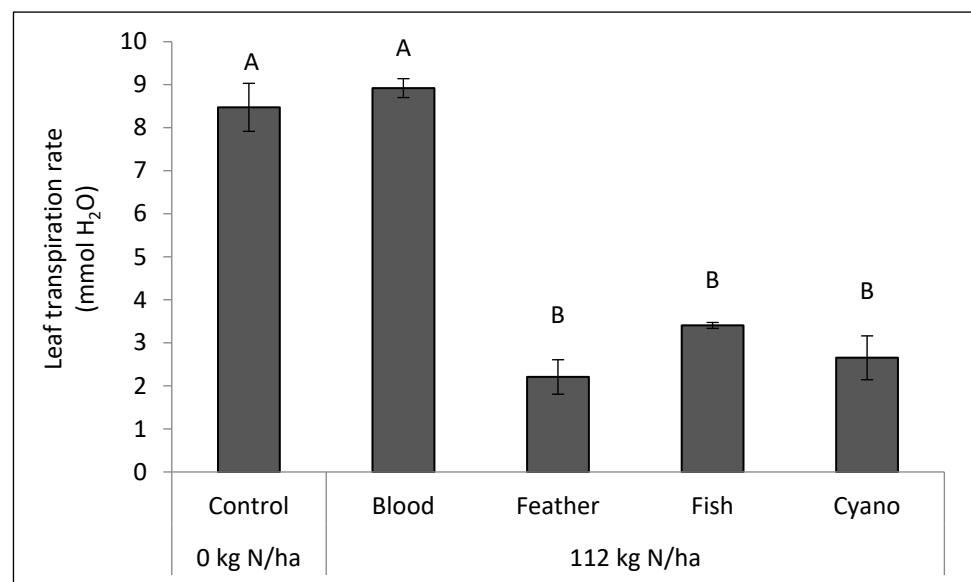

Figure 2. Effects of different organic fertilizers on field-scale water use efficiency (fWUE) at harvest in (**a**) 2013 and (**b**) 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at p < 0.05 using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

The blood meal and control treatments had a higher leaf transpiration rate (p < 0.0001; Figure 4) and leaf VPD (p < 0.0001; Figure 5) compared to the other treatments. The feather meal treatment had a lower net photosynthetic rate (p < 0.0001; Figure 6) and a higher leaf temperature compared to the other treatments, including the control (p = 0.003; Figure 7). A negative correlation was found between the amount of IAA applied in organic N fertilizers and leaf VPD (r = −0.49; p = 0.0163; Table 6), which may explain the low leaf temperature observed in the fish emulsion treatment (Figure 7). Leaf VPD (r = 0.54; p = 0.0071) and

transpiration rate (r = 0.53; *p* = 0.0057) were positively correlated with the amount of Fe applied in organic N fertilizers (Table 6). The application of SA in organic N fertilizers was negatively correlated with stomatal conductance (r = −0.79; *p* = 0.0059).
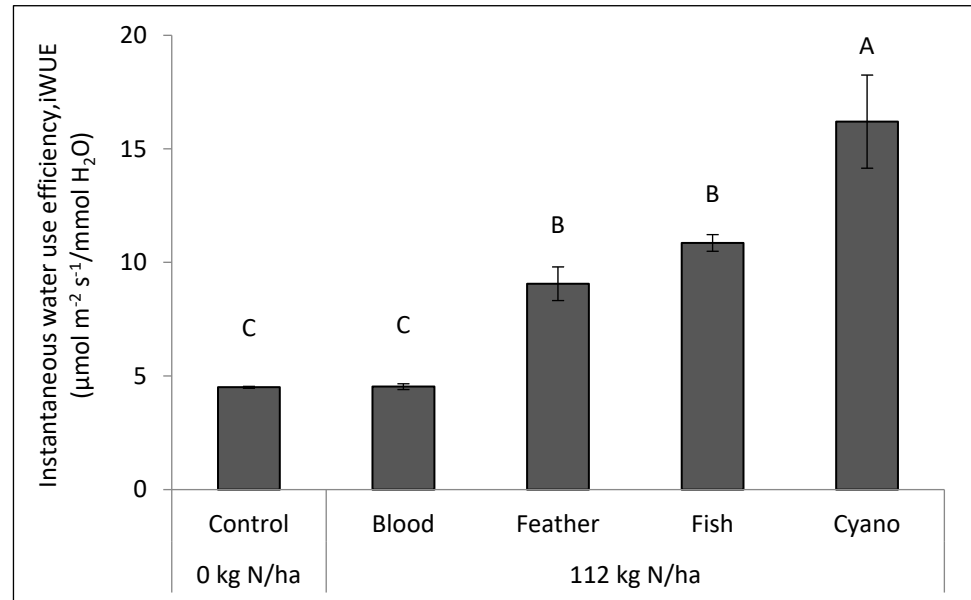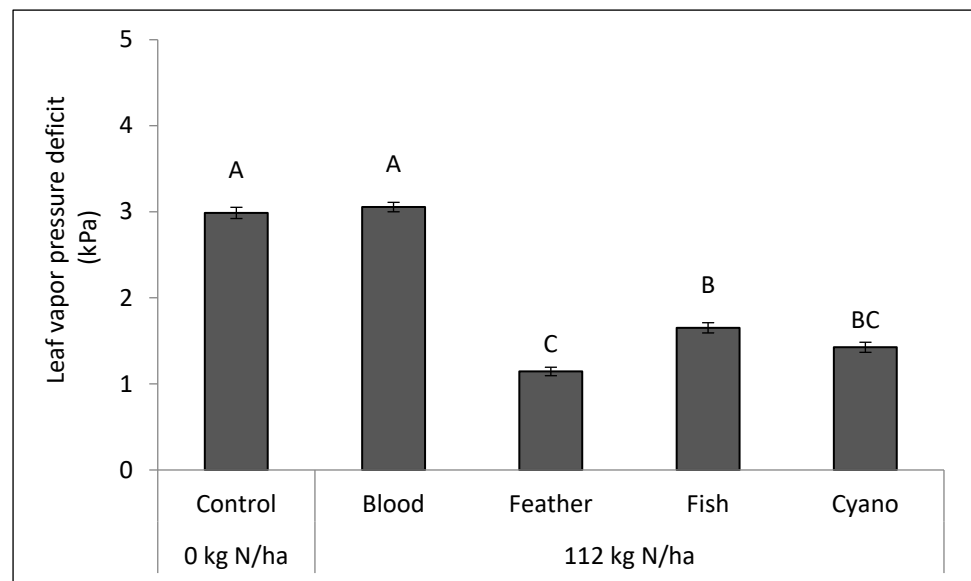

Figure 3. Effects of different organic fertilizers on instantaneous water use efficiency (iWUE) at tasseling in 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at *p* < 0.05 using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.
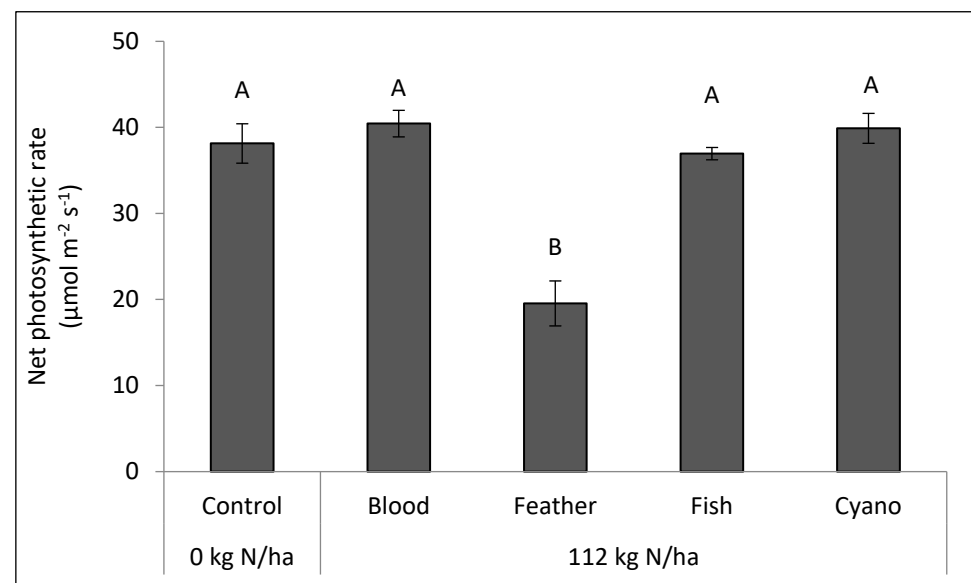

Figure 4. Effects of different organic fertilizers on leaf transpiration rate at tasseling in 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at *p* < 0.05 using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.
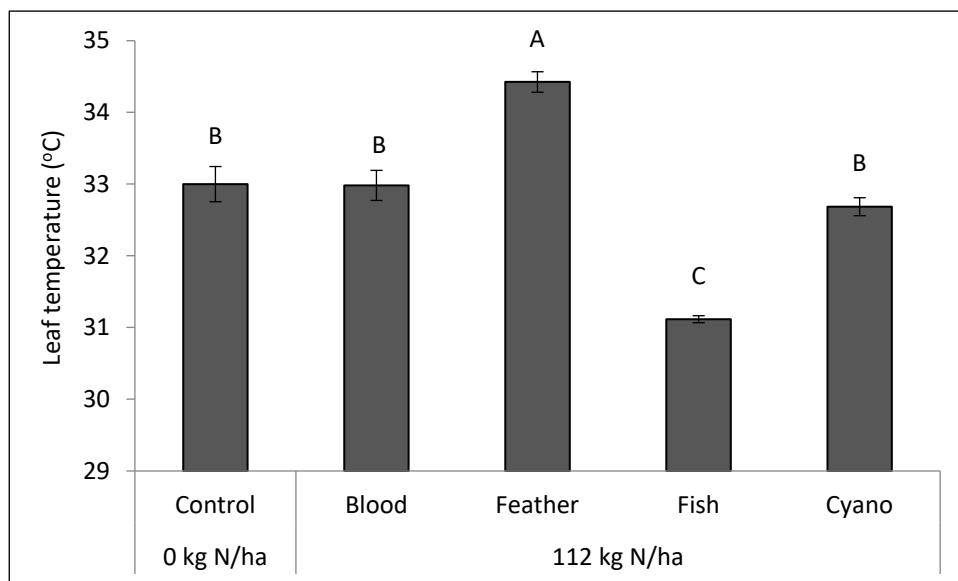
Figure 5. Effects of different organic fertilizers on leaf vapor pressure deficit (VPD) at tasseling in 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at *p* < 0.05 using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

Figure 6. Effects of different organic fertilizers on net photosynthetic rate at tasseling in 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at *p* < 0.05 using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

Figure 7. Effects of different organic fertilizers on sweet corn on leaf temperature at tasseling in 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at $p < 0.05$ using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

Table 6. Pearson correlation coefficients on amount of Fe, Zn, indole-3-acetic acid (IAA), and salicylic acid (SA) applied in the fertilizers (blood meal, feather meal, fish emulsion, and cyano-fertilizer) with gas exchange rate components, instantaneous water use efficiency (iWUE), and field-scale water use efficiency (fWUE) in 2014. p-values are shown in parentheses below the correlation coefficients.

	Lf Temp.	Lf VPD	gs	Tr	Pn	iWUE	fWUE
IAAapp	−0.14 (0.7959)	−0.49 (0.0163 *)	−0.42 (0.4080)	0.42 (0.4051)	0.70 (0.1204)	−0.32 (0.5306)	−0.32 (0.2208)
SAapp	0.078 (0.8841)	0.14 (0.7807)	−0.79 (0.0059 *)	0.090 (0.1004)	0.074 (0.8894)	0.71 (0.0126 *)	0.68 (0.0040 *)
NO3app	−0.022 (0.9668)	−0.57 (0.2328)	−0.51 (0.3063)	0.29 (0.5839)	0.74 (0.0932)	−0.37 (0.4727)	0.64 (0.0082 *)
NH4app	0.59 (0.2112)	−0.59 (0.2166)	−0.52 (0.2936)	−0.46 (0.3602)	0.41 (0.4159)	−0.49 (0.0212 *)	−0.65 (0.0066 *)
FeApp	−0.42 (0.1729)	0.54 (0.0071 *)	0.43 (0.1569)	0.53 (0.0057 *)	0.12 (0.7018)	−0.64 (0.0238 *)	−0.69 (0.0122 *)
ZnApp	0.04 (0.9012)	0.09 (0.7721)	0.19 (0.5329)	0.14 (0.6812)	−0.48 (0.2134)	−0.31 (0.2339)	−0.38 (0.3002)

* p-values are significantly different at $p < 0.05$. Lf temp. = leaf temperature; Lf VPD = leaf vapor pressure deficit; gs = stomatal conductance; Tr = leaf transpiration rate; Pn = net photosynthetic rate; iWUE = instantaneous water use efficiency; IAAapp = Indole-3-acetic acid application; SAapp = Salicylic acid application; NO3app = NO_3^--N application; NH4Napp = NH_4^+-N application, FeApp = Iron application; and ZnApp = Zinc application.

The amounts of SA, NH_4^+-N, and Fe applied in organic N fertilizers were significantly correlated with both iWUE and fWUE in 2014 (Table 6). iWUE was also positively correlated with yield (r = 0.61; $p = 0.0153$). Among other independent variables, including the IAA, SA, NH_4^+-N, NO_3^--N, Fe, and Zn applied in organic N fertilizers, only SA was selected by stepwise regression to predict both fWUE and iWUE (Table 7).

Table 7. Stepwise regression models of field water use efficiency (fWUE) and instantaneous water use efficiency (iWUE) in 2014. Variables analyzed were gas exchange rates components, amount of indole-3-acetic acid (IAA), salicylic acid (SA), NH_4^+-N, NO_3^--N, Fe, and Zn applied in the fertilizers to predict fWUE and iWUE. All variables left in the model are significant at the 0.05 level.

	Estimate	*p*-Value	Model
Field water use efficiency (fWUE)			fWUE = 32.7 + 1652SA, r^2 = 0.46
Intercept	32.7	<0.0001	
Salicylic acid application, SA	1652	0.0040	
Instantaneous water use efficiency (iWUE)			iWUE = 18.4 −54gs + 642SA, r^2 = 0.84
Intercept	18.4	<0.0001	
Stomatal conductance, gs	−54	0.0035	
Salicylic acid application, SA	642	0.0044	

4. Discussion

Although the IAA applied was not significantly correlated with WUE, greater amounts of SA were applied in the cyano-fertilizer treatment compared to other treatments (Table 5), ear yield was positively correlated with the application of SA in organic N fertilizers (r = 0.73; *p* = 0.0067), and the application of SA was positively correlated with both fWUE and iWUE (Tables 6 and 7). SA enhances the distribution of photo assimilates and has been shown to increase iWUE in grape [17]. SA induced stomatal closure, improved membrane integrity, regulated plant water use, and increased iWUE in barley [49], maize [50,51], and wheat [14]. SA has been shown to increase wheat germination and yields [52] due to enhanced cell division and ion transport [53,54].

Among the variables analyzed (amounts of IAA, SA, NH_4^+-N, NO_3^--N, Fe, Zn applied in the fertilizers), SA application and stomatal conductance were the two independent variables significantly related to iWUE, and only SA application in organic N fertilizers predicted the fWUE of sweet corn (Table 7). The stepwise regression results indicated that SA applied in organic N fertilizers was the leading fertilizer variable resulting in the high fWUE and iWUE with the cyano-fertilizer treatment. Stomatal conductance was the leading gas exchange rate component impacting iWUE. SA has been found to induce stomatal closure and regulate plant water use [22,23,52,55,56]. Although the correlation between SA application and stomatal conductance (r = −0.79; *p* = 0.0059) was significant in this study (Table 6), there was no treatment effect on stomatal conductance. Most studies conducted on SA applied to plants evaluated foliar applications. In our study, fertilizers were applied to the soil through fertigation or soil application, and results were consistent with previous maize studies [21,25,50,51].

The amount of NH_4^+-N applied in organic N fertilizers was negatively correlated with both iWUE and fWUE (Table 6). Fertilizers with high NH_4^+-N concentrations decrease fWUE and have been shown to decrease the net photosynthetic rate in maize [57,58] due to interferences with the photosynthetic production of ATP [36,59]. It may be possible that a higher percentage of N applied as NH_4^+-N in the feather meal treatment compared to other fertilizer treatments (Table 4) decreased water transport in the xylem conduit. In addition, water flow in the xylem conduit was lower in NH_4^+-N (5 mM)-treated common bean due to a reduced number of functional xylem conduits [60].

Even though it has been reported that high NO_3^--N concentration in fertilizers decreased leaf transpiration rate in corn [33], NO_3^--N applied was not significantly correlated with transpiration rate in this study (Table 6). In addition, while it had been reported that NO_3^--N application to tomato increased WUE [34], in this study, we found a significant positive correlation with fWUE but no correlation with iWUE.

Although Zn application was not correlated with any of the gas exchange components or WUE, the amounts of Fe applied in organic N fertilizers were negatively correlated with both iWUE and fWUE (Table 6). The amount of Fe applied in organic N fertilizers was positively correlated to leaf VPD (r = 0.54; *p* = 0.0071) and transpiration rate (r = 0.53; *p* = 0.0057; Table 6), thus contributing to reduced iWUE and fWUE. Among other fertilizer treatments, the blood meal treatment had the highest transpiration rate (Figure 4) and leaf

VPD (Figure 5), similar to the control. Although the amount of Fe applied in the blood meal treatment was similar to the fish emulsion treatment (Table 4), the fish emulsion had a lower transpiration rate and leaf VPD, which may have been due to the higher amount of IAA applied in the fish emulsion treatment (Table 5), since IAA application was negatively correlated with leaf VPD ($r = -0.49$; $p = 0.0163$; Table 6). Increased Fe applied in fertilizers has been shown to increase the transpiration rate of peach leaves due to physical alterations of the stomatal opening [61]. Others have found that increased Fe application in fertilizers resulted in increased transpiration rate and Fe translocation in plants, which affected photosynthetic pigments and gas exchange rates of French bean [62].

5. Conclusions

In conclusion, we found that SA applied in organic N fertilizers was the leading fertilizer variable resulting in the consistently high fWUE and iWUE with the cyano-fertilizer treatment. We also found that the amounts of SA, NH_4^+-N, and Fe applied in organic N were correlated with the fWUE, iWUE, and leaf gas exchange components of sweet corn. We conclude that organic growers could increase WUE by fertigating their sweet corn with cyano-fertilizer, which had the consistently highest yield and water use efficiency of the tested fertilizers. Fish emulsion had a comparable yield and fWUE to cyano-fertilizer in 2013, but a significantly lower yield, fWUE, and iWUE in 2014. A positive relationship was observed between the amount of SA applied and both iWUE and fWUE; apparently, cyano-fertilizer may have had a higher WUE due to the high amount of SA applied as part of the cyano-fertilizer. Cyanobacteria can produce cyanotoxins, especially when they are grown in water with high N and P concentrations [63]; therefore, prior to their use, cyano-fertilizers should be tested for the presence of cyanotoxins [20]. Further research needs to be conducted to better understand the mechanisms through which phytohormones, NH_4^+-N, and Fe in organic N fertilizers regulate water transport and utilization in plants. In addition, the practicality and optimization of cyano-fertilizers require further assessment prior to their widespread adoption.

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