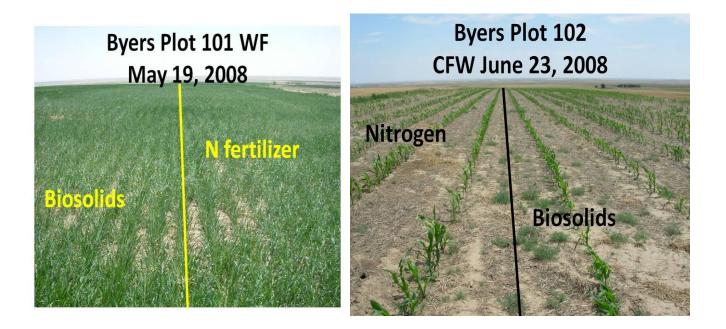


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# Biosolids Application to No-Till Dryland Rotations: 2007 Results



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# The Cities of Littleton and Englewood, Colorado and the Colorado Agricultural Experiment Station (project number 15-2924) funded this project.

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### INTRODUCTION

Biosolids recycling on dryland winter wheat (*Triticum aestivum*, L.) can supply a reliable, slow-release source of nitrogen (N) and organic carbon (Barbarick et al., 1992). Barbarick and Ippolito (2000, 2007) found that continuous application of biosolids from the Littleton/Englewood, CO wastewater treatment facility to dryland winter wheat-fallow rotation provides 16 to 18 lbs N per dry ton. This research involved tilling the biosolids into the top 8 inches of soil. A new question related to soil management in a biosolids beneficial-use program is: How much N would be available if the biosolids were surface-applied in a no-till dryland agroecosystem with winter wheat-fallow (WF) and winter wheat-corn (*Zea mays*, L.)-fallow (WCF) crop rotations?

Our objective was to compare agronomic rates of commercial N fertilizer to an equivalent rate of biosolids in combination with WF and WCF crop rotations. Our hypotheses were that biosolids addition, compared to N fertilizer, will:

- 1. Produce similar crop yields;
- Not differ in grain P, Zn, and Cu levels (Ippolito and Barbarick, 2000) or soil P, Zn, and Cu AB-DTPA extractable concentrations, a measure of plant availability (Barbarick and Workman, 1987); and
- Not affect soil salinity (electrical conductivity of saturated soil-paste extract, EC) or soil accumulation of nitrate-N (NO<sub>3</sub>-N).

#### **MATERIALS AND METHODS**

In 1999, we established our research on land owned by the Cities of Littleton and Englewood (L/E) in eastern Adams County, approximately 28 miles east of Byers, CO. The Linnebur family manages the farming operations for L/E. Soils belong to the Adena-Colby association where the Adena soil is classified as an Ustollic Paleargid and Colby is classified as an Ustic Torriorthent. No-till management is used in conjunction with crop rotations of WF and WCF. We originally also used a wheat-wheat-corn-sunflower (*Helianthus annuus*, L.)-fallow rotation. After the 2004 growing season, we abandoned this rotation because of persistent droughty conditions that restricted sunflower production.

We installed a Campbell Scientific weather station at the site in April 2000; Tables 1 and 2 present mean temperature and precipitation data, and growing season precipitation, respectively.

With biosolids application in August 1999, we initiated the study. Planting sequences are given in Table 3. We used two replications of each rotation (20 plots total) and we completely randomized each replicated block. Each phase of each rotation was present every year. Each plot was 100 feet wide by approximately 0.5 mile

(2640 feet) long. The width was split so that one 50-foot wide section received commercial N fertilizer applied with the seed and sidedressed after plant establishment (Table 3), and the second 50-foot wide section received biosolids applied by L/E with a manure spreader. We randomly selected which strip in each rotation received N fertilizer or biosolids. Characteristics of the L/E biosolids are provided in Table 4. We based the N fertilizer and biosolids applications on soil test recommendations determined on each plot before planting each crop. The Cities of L/E completed biosolids application for the summer crops in March 2000, 2001, 2002, 2003, 2004, and 2005. We planted the first corn crop in May 2000. We also established wheat rotations in September 2000, 2001, 2002, and 2003, corn rotations in May 2001, 2002, 2003, and 2004, and sunflower plantings in June 2001, 2002, and 2003. Soil moisture was inadequate in June 2004 to plant sunflowers (see Table 1).

We completed wheat harvests in July 2000, 2001, 2002, 2003, 2004, 2005, and 2007 and corn and sunflowers in October 2000 and 2001, sunflowers in December 2003, and corn in 2004, 2006, and 2007. We experienced corn and sunflower crop failures in 2002, a corn crop failure in 2003 and 2005, and a wheat-crop failure in 2006 due to lack and proper timing of precipitation (Table 1). For each harvest, we cut grain from four areas of 5 feet by approximately 100 feet within each subplot. We determined the yield for each area and then took a subsample from each cutting for subsequent grain protein or N, P, Zn, and Cu analyses (Huang and Schulte, 1985).

Following each harvest, we collected soil samples using a Giddings hydraulic probe. For AB-DTPA extractable Cu, P, and Zn (Barbarick and Workman, 1987) and EC (Rhoades, 1996) and pH (Thomas, 1996), we sampled to one foot and separated the samples into 0-2, 2-4, 4-8, and 8-12 inch depth increments. For soil NO<sub>3</sub>-N (Mulvaney, 1996) analyses, we sampled to 6 feet and separated the samples into 0-2, 2-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-60, and 60-72 inch depth increments.

For the wheat rotations, the experimental design was a split-plot design where type of rotation was the main plot and type of nutrient addition (commercial N fertilizer versus L/E biosolids) was the subplot. For crop yields and soil-sample analyses, main plot effects, subplot effects, and interactions were tested for significance using least significant difference (LSD) at the 0.10 probability level. Since we only had one corn rotation, we could only compare the commercial N versus L/E biosolids using a "t" test at the 0.10 probability level.

#### **RESULTS AND DISCUSSION**

#### Precipitation Data

Table 1 presents the monthly precipitation records from the time we established the weather station at the Byers research site. The plots received more than 11 inches of total annual rainfall in 2000, 2001, and 2007, only 5 inches in 2002, about 12 inches in 2003, 10 inches in 2004 and 2005, 9 inches in 2006. The critical precipitation months for corn are July and August (Nielsen et al., 1996). The Byers site received 6.0, 3.8, 1.3, 2.6, 2.5, 3.5, 4.5, and 5.4 inches of precipitation in July and August 2000, 2001, 2002, 2003, 2004, 2005, 2006, and 2007, respectively.

#### 2007 Crop Grain Data

As shown in Figure 1, the WF rotation produced significantly larger wheat yields than the WCF rotation. Neither rotation nor nutrient source affected the grain protein, P, or Cu concentrations (Figures 2, 3, and 5). The biosolids treatment in the WCF rotation produced the highest wheat-grain Zn (Figure 4).

Biosolids significantly increased the corn-grain P concentration relative to the N fertilizer treatment but did not affect the grain yields or Cu or Zn levels (Table 5).

#### 2007 Soil Data

The AB-DTPA-extractable P concentration (Figure 6) in the 0-2-inch depth is considered medium or high according to the Colorado P Index Risk Assessment (Sharkoff et al., 2003). Overall, this site would most likely have a "medium" risk assessment in terms of the potential for off-site P movement. Interpreted, biosolids land application can still follow crop N requirements.

The AB-DTPA-extractable P, Zn, and Cu concentrations in the top 2 inches of soil were significantly affected by rotation, treatment, and the interaction between rotation and treatment. For all three nutrients, the higher concentrations were found in the WCF rotation that received biosolids.

The salinity level (EC; Figure 9) showed inconsistent trends with rotation and nutrient source. Soil pH (Figure 10) was higher in the WCF rotation in the top 8 inches of soil.

Only the 2-4-inch soil depth showed an increase in  $NO_3$ -N in the biosolids plots (Figure 11). The residual  $NO_3$ -N in the top 36 inches also indicates that future biosolids

and fertilizer applications to both wheat and corn should be ceased until the soil  $NO_3$ -N levels are reduced to below 15 mg kg<sup>-1</sup> (ppm). Nitrogen additions to winter wheat are needed when soil  $NO_3$ -N concentrations are less than 15 mg kg<sup>-1</sup> (ppm) in the top foot (Davis et al., 2005). Nitrogen additions to dryland corn are needed when soil  $NO_3$ -N concentrations are less than 12 mg kg<sup>-1</sup> (ppm) in the top foot (Mortvedt et al., 1996).

## CONCLUSIONS

Relative to our hypotheses listed on page 3, we have found the following trends:

- In the wheat plots, we observed similar concentrations of P, Zn, and Cu in wheat grain and surface-soil levels following biosolids or N fertilizer application. We found no differences in soil NO<sub>3</sub>-N concentrations (except at 2-4 inches) at depths to 6 feet.
- 2. We found that biosolids application did not provide consistent effects on soil salinity (EC) in the wheat plots as compared to N fertilizer applications.
- Previous biosolids and N fertilizer applications, based on soil test N and crop N requirements, have caused an accumulation of NO<sub>3</sub>-N in the soil profile. Therefore, near-future biosolids and N fertilizer applications will be ceased until soil NO<sub>3</sub>-N is reduced by wheat and corn removal.

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	(Weat	her station	ı was insta	<u>lled in A</u> pi	ril, 2000).										
Month		2000			2001			2002			2003			2004	
	Max	Min <sup>°</sup> F	Precip	Max	Min <sup>°</sup> F	Precip	Max	Min <sup>°</sup> F	Precip	Max	Min⁰F	Precip	Max	Min <sup>°</sup> F	Precip
	°F		inches	°F		inches	°F		inches	°F		inches	°F		inche
January	+	+	+	41.0	20.7	0.2	44.1	17.0	0.1	50.4	23.3	0.0	44.9	20.2	0.0
February	+	+	+	42.1	19.0	0.1	48.2	19.7	0.2	39.9	17.1	0.1	42.6	20.4	0.1
March	+	+	+	49.9	27.5	0.2	46.5	17.7	0.2	55.0	29.6	1.0	61.2	31.3	0.1
April	68.9	38.4	0.6	64.2	36.4	1.5	65.8	35.2	0.3	65.0	37.5	1.5	61.9	35.6	0.9
May	78.4	47.0	0.9	70.0	43.7	2.4	73.5	41.8	0.7	71.3	45.3	1.8	75.8	44.8	1.4
June	80.4	49.3	0.9	85.9	53.5	2.4	89.0	56.9	1.2	76.8	51.1	4.7	78.3	51.1	4.1
July	91.9	61.0	2.5	92.2	61.1	1.9	93.3	62.2	0.2	97.4	62.1	0.2	86.9	57.6	1.0
August	90.8	60.2	3.5	88.8	59.0	1.9	88.2	57.0	1.1	91.0	60.5	2.4	85.2	54.6	1.5
September	80.6	49.8	0.8	82.0	51.6	0.8	78.1	50.5	0.7	76.2	45.6	0.1	80.8	50.7	0.6
October	65.9	38.7	1.6	68.0	37.2	0.2	58.6	33.0	0.2	72.3	41.2	0.1	67.3	38.6	0.4
November	40.8	20.0	0.3	56.2	28.9	0.8	50.2	27.1	0.1	51.3	24.3	0.0	48.0	26.6	0.3
December	41.7	17.0	0.3	45.4	21.4	0.0	47.1	22.8	0.0	47.2	20.8	0.0	46.4	22.4	0.1
Total			11.4			12.4			5.0			11.9			10.5
Month		2005			2006			2007							
	Max	Min <sup>°</sup> F	Precip	Max <sup>°</sup> F	Min <sup>°</sup> F	Precip	Max	Min ⁰F	Precip						
	°F		inches			inches	°F		inches						
January	43.9	21.5	0.1	52.2	24.6	0.0	30.9	11.1	0.1						
February	49.4	24.5	0.0	41.2	15.3	0.0	34.7	16.3	0.1						
March	53.0	27.2	0.2	52.9	25.5	0.6	59.1	33.5	0.7						
April	59.0	34.0	1.1	65.0	34.5	0.4	57.8	32.8	1.8						
May	72.0	44.6	0.8	76.5	44.6	0.7	73.2	45.3	1.5						
June	80.1	50.4	2.4	86.5	54.2	0.2	81.3	52.0	0.4						
July	94.2	61.1	1.3	90.6	61.8	1.9	91.5	61.6	2.8						
August	84.6	56.7	2.2	86.1	59.0	2.6	89.3	61.5	2.6						
September	83.3	51.9	0.1	69.5	43.3	1.4	80.8	51.3	0.6						
October	65.1	39.1	1.3	62.5	35.9	1.1	68.7	38.8	0.3						
November	56.5	29.7	0.5	53.3	26.9	0.0	56.9	27.9	0.1						
December	41.6	17.5	0.0	42.2	21.1	0.1	38.5	15.8	0.2						
Total			10.0			9.0			11.2						
<sup>+</sup> We in	stalled th	e weather	station in	mid-April.	2000.		•								

Table 1. Monthly mean maximum (Max) and minimum (Min) temperatures and precipitation (Precip) in inches at the Byers research site, 2000-2007. (Weather station was installed in April, 2000).

We installed the weather station in mid-April, 2000.

## Table 2.Growing season precipitation.

Stage	Dates	Precipitation, inches
Wheat vegetative	September 2000 - March 2001	3.3
Wheat reproductive	April 2001 - June 2001	6.3
Corn/Sunflowers preplant	July 2000 – April 2001	9.5
Corn/Sunflowers growing season	May 2001 – October 2001	9.6
Wheat vegetative	September 2001 - March 2002	2.1
Wheat reproductive	April 2002 - June 2002	2.2
Corn/Sunflowers preplant	July 2001 – April 2002	6.1
Corn/Sunflowers growing season	May 2002 – October 2002	3.9
Wheat vegetative	September 2002 - March 2003	1.1
Wheat reproductive	April 2003 - June 2003	3.3
Corn/Sunflowers preplant	July 2002 – April 2003	3.4
Corn/Sunflowers growing season	May 2003 – October 2003	9.2
Wheat vegetative	September 2003 - March 2004	0.3
Wheat reproductive	April 2004 - June 2004	2.3
Corn/Sunflowers preplant	July 2003 – April 2004	3.0
Corn/Sunflowers growing season	May 2004 – October 2004	8.6
Wheat vegetative	September 2004 - March 2005	1.7
Wheat reproductive	April 2005 - June 2005	4.3
Corn preplant	July 2004 – April 2005	5.3
Corn growing season	May 2005 – October 2005	8.6
Wheat vegetative	September 2005 - March 2006	2.5
Wheat reproductive	April 2006 - June 2006	1.3
Corn preplant	July 2005 – April 2006	6.4
Corn growing season	May 2006 – October 2006	7.9
Wheat vegetative	September 2006 - March 2007	3.5
Wheat reproductive	April 2007 - June 2007	3.7
Corn preplant	July 2006 – April 2007	8.8
Corn growing season	May 2007 – October 2007	8.2

Year Planted	Date Planted	Crop	Variety	Biosolids Biosolids tons/acre	Treatment Bio/N equiv. lbs	Nitrogen N Ibs/acre with seed	Fertilizer N Ibs/acre after planting	Treatment Total N Ibs/acre	P₂O₅ lbs/acre	Zn Ibs/acre
1999	Early Oct.	Wheat	Halt	2.4	38.4	5	40	45	20	0
2000	May	Corn	Pioneer 3752	4	64	5	40	45	15	5
2000	June	Sunflowers	Triumph 765, 766 (confection type)	2	32	5	40	45	15	5
2000	9/25/00	Wheat	Prairie Red	0	0	4	0	4	20	0
2001	5/11/01	Corn	DK493 Round Ready	5.5	88	5	40	45	15	5
2001	6/20/01	Sunflowers	Triumph 765C	2	32	5	40	45	15	5
2001	09/17/01	Wheat	Prairie Red	Variable	Variable	5	Variable	Variable	20	0
2002		Corn	Pioneer 37M81	Variable	Variable	5	Variable	Variable	15	5
2002		Sunflowers	Triumph 545A	0	0	5	0	0	15	5
2002		Wheat	Stanton	Variable	Variable	5	Variable	Variable	20	0
2003	05/21/03	Corn	Pioneer K06							
2003	06/28/03	Sunflowers	Unknown							
2003		Wheat	Stanton	Variable	Variable	5	Variable	Variable	20	0
2004		Corn	Triumph 9066 Roundup Ready	Variable	Variable	5	Variable	Variable	15	5
2004		Sunflowers	Triumph 765 (confection type)	0	0	5	0	0	15	5
2004	09/17/04	Wheat	Yumar	3	54	0	50	50	15	5
2005	05/10/05	Corn	Pioneer J99	4	72	0	75	75	15	5
2005	Sept.	Wheat	Yumar	0	0	0	0	0	0	0
2006	May	Corn	Pioneer J99	0	0	0	0	0	0	0
2006	Sept.	Wheat	Yumar	0	0	0	0	0	0	0
2007	May	Corn	Pioneer J99	0	0	0	0	0	0	0

Table 3.Biosolids and fertilizer applications and crop varieties used at the Byers research site, 1999-2007.

Parameter	1999	2000 Corn,	2001 Corn,	2001	2003 Corn,	2003	2004	2005	Avg.	Range
1	Wheat	Sunflowers	Sunflowers	Wheat	sunflowers	Wheat	Wheat	Corn		
Solids, g kg⁻¹	217		210	220	254	192	197	211	214	192-254
рН	7.6	7.8	8.4	8.1	8.5	8.2	8.8	8.2	8.2	7.6-8.8
EC, dS m <sup>-1</sup>	6.2	11.2	10.6	8.7	7.6	7.4	4.5	5.1	7.7	4.5-11.2
Org. N, g kg⁻¹	50	47	58	39	54	46	43	38	47	38-58
NH <sub>4</sub> -N, g kg <sup>-1</sup>	12	7	14	16	9	13	14	14	12	7-16
NO₃-N, g kg <sup>-1</sup>	0.023	0.068	0.020	0.021	0.027	0.016	0.010	0	0.023	0-0.068
K, g kg⁻¹	5.1	2.6	1.6	1.9	2.2	2.6	2.1	1.7	2.5	1.6-5.1
P, g kg⁻¹	29	18	34	32	26	28	29	13	26	13-34
Al, g kg <sup>-1</sup>	28	18	15	18	14	15	17	10	17	10-28
Fe, g kg⁻¹	31	22	34	33	23	24	20	20	26	20-34
Cu, mg kg⁻¹	560	820	650	750	596	689	696	611	672	560-82
Zn, mg kg <sup>-1</sup>	410	543	710	770	506	629	676	716	620	410-77
Ni, mg kg⁻¹	22	6	11	9	11	12	16	4	11	4-22
Mo, mg kg⁻¹	19	22	36	17	21	34	21	13	23	13-36
Cd, mg kg <sup>-1</sup>	6.2	2.6	1.6	1.5	1.5	2.2	4.2	2.0	2.7	1.5-6.2
Cr, mg kg⁻¹	44	17	17	13	9	14	18	14	18	9-44
Pb, mg kg⁻¹	43	17	16	18	15	21	26	16	22	15-43
As, mg kg <sup>-1</sup>	5.5	2.6	1.4	3.8	1.4	1.6	0.5	0.05	2.1	0.05-5.
Se, mg kg <sup>-1</sup>	20	16	7	6	17	1	3	0.07	8.8	0.07-2
Hg, mg kg⁻¹	3.4	0.5	2.6	2.0	1.1	0.4	0.9	0.1	1.4	0.1-3.4
Ag, mg kg⁻¹					15	7	0.5	1.2	5.9	0.5-15
Ba, mg kg⁻¹							533	7	270	7-533
Be, mg kg <sup>-1</sup>							0.05	<0.001	0.05	<0.001 0.05
Mn, mg kg⁻¹							239	199	219	0.05 199-23

Table 4.Littleton/Englewood biosolids composition used at the Byers Research site, 1999-2005.

Table 5.Corn grain characteristics for the corn rotation (CFW) at the Byers research site for<br/>2007. *Highlighted parameters* are significant at the 0.10 probability level.

Parameter, units	Biosolids	Nitrogen	Probability level
Yield, bushels/acre	54	56	0.376
Cu, mg/kg	1.3	1.2	0.176
P, g/kg	2.9	2.6	0.061
Zn, mg/kg	9.6	9.2	0.494

Table 6.Soil characteristics for the corn rotation (CFW) at the Byers research site for<br/>2007. *Highlighted parameters* are significant at the 10% probability level.

Parameter, units	Depth, inches	Biosolids	Nitrogen	Probability level
AB-DTPA P, mg kg <sup>-1</sup>	0-2	43.6	15.4	0.017
	2-4	13.3	5.7	0.058
	4-8	3.6	3.3	0.554
	8-12	2.4	2.2	0.695
AB-DTPA Zn, mg kg <sup>-1</sup>	0-2	5.2	1.3	0.038
	2-4	1.4	0.4	0.057
	4-8	0.2	0.2	0.454
	8-12	0.2	0.1	0.194
AB-DTPA Cu, mg kg <sup>-1</sup>	0-2	9.1	2.0	0.028
	2-4	3.9	2.2	0.046
	4-8	3.4	3.1	0.660
	8-12	2.8	3.0	0.702
ECe, dS m⁻¹	0-2	0.80	0.48	0.002
	2-4	0.63	0.39	0.084
	4-8	0.47	0.30	0.011
	8-12	0.51	0.32	0.122
NO <sub>3</sub> -N, mg kg <sup>-1</sup>	0-2	38.6	9.5	0.006
	2-4	22.4	4.5	0.024
	4-8	9.6	3.0	0.058
	8-12	9.8	2.0	0.135
	12-24	12.0	4.8	0.308
	24-36	29.4	11.9	0.134
	36-48	33.8	14.3	0.064
	48-60	13.4	16.7	0.598
	60-72	1.8	14.7	0.181

Figure 1. Wheat grain yields for 2007 dryland-wheat-rotation harvests comparing Littleton/Englewood biosolids to commercial fertilizer. In the statistical summary, LSD<sub>0.10</sub> represents the least significant difference at the 10% probability level and NS indicates non-significant differences. (WF = wheat-fallow and WCF = wheat-corn-fallow rotations).

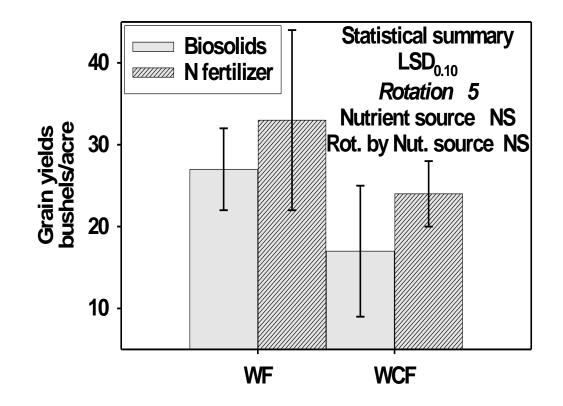


Figure 2. Wheat grain protein concentrations for 2007 dryland-wheat-rotation harvests comparing Littleton/Englewood biosolids to commercial fertilizer. In the statistical summary, LSD<sub>0.10</sub> represents the least significant difference at the 10% probability level and NS indicates non-significant differences. (WF = wheat-fallow and WCF = wheat-corn-fallow rotations).

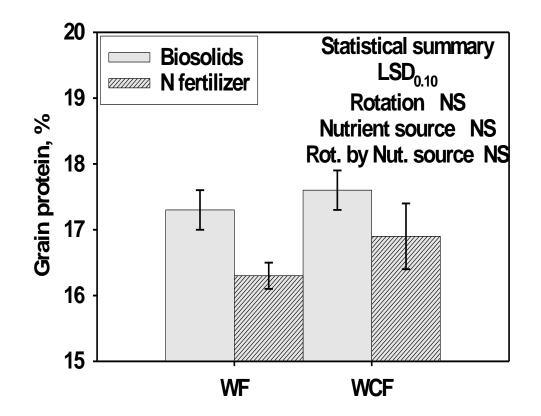


Figure 3. Wheat grain P concentrations for 2007 dryland-wheat-rotation harvests comparing Littleton/Englewood biosolids to commercial fertilizer. In the statistical summary, LSD<sub>0.10</sub> represents the least significant difference at the 10% probability level and NS indicates non-significant differences. (WF = wheat-fallow and WCF = wheat-corn-fallow rotations).

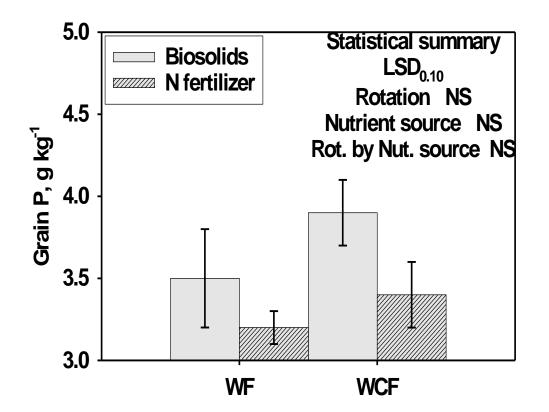


Figure 4. Wheat grain Zn concentrations for 2007 dryland-wheat-rotation harvests comparing Littleton/Englewood biosolids to commercial fertilizer. In the statistical summary, LSD<sub>0.10</sub> represents the least significant difference at the 10% probability level and NS indicates non-significant differences. (WF = wheat-fallow and WCF = wheat-corn-fallow rotations).

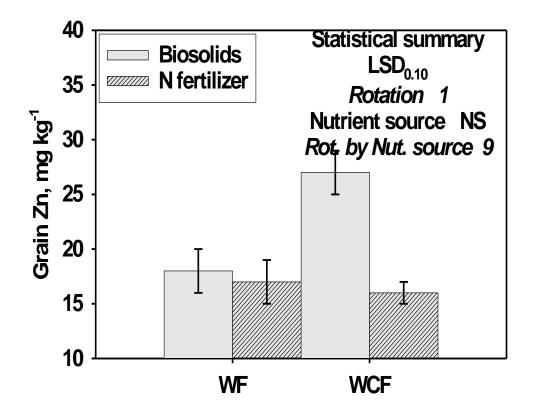


Figure 5. Wheat grain Cu concentrations for 2007 dryland-wheat-rotation harvests comparing Littleton/Englewood biosolids to commercial fertilizer. In the statistical summary, LSD<sub>0.10</sub> represents the least significant difference at the 10% probability level and NS indicates non-significant differences. (WF = wheat-fallow and WCF = wheat-corn-fallow rotations).

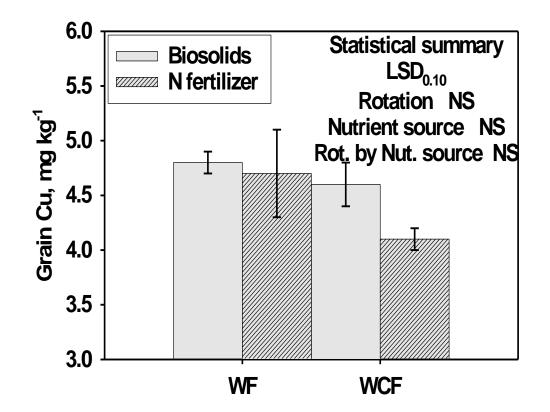


Figure 6. Soil AB-DTPA-extractable P concentration following 2007 dryland-wheat-rotation harvests comparing Littleton/Englewood biosolids to commercial N fertilizer. In the statistical summary, LSD<sub>0.10</sub> represents the least significant difference at the 0.10 probability level and NS indicates non-significant differences.

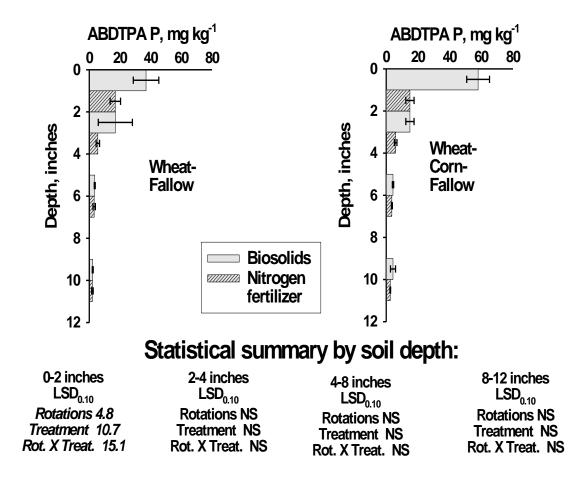


Figure 7. Soil AB-DTPA-extractable Zn concentration following 2007 dryland-wheat-rotation harvests comparing Littleton/Englewood biosolids to commercial N fertilizer. In the statistical summary, LSD<sub>0.10</sub> represents the least significant difference at the 0.10 probability level and NS indicates non-significant differences.

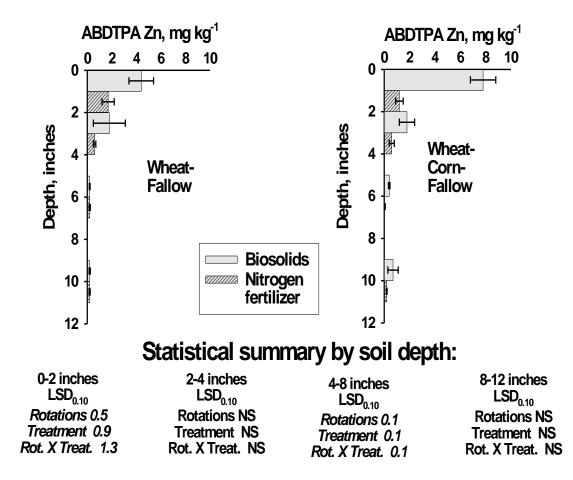


Figure 8. Soil AB-DTPA-extractable Cu concentration following 2007 dryland-wheat-rotation harvests comparing Littleton/Englewood biosolids to commercial N fertilizer. In the statistical summary, LSD<sub>0.10</sub> represents the least significant difference at the 0.10 probability level and NS indicates non-significant differences.

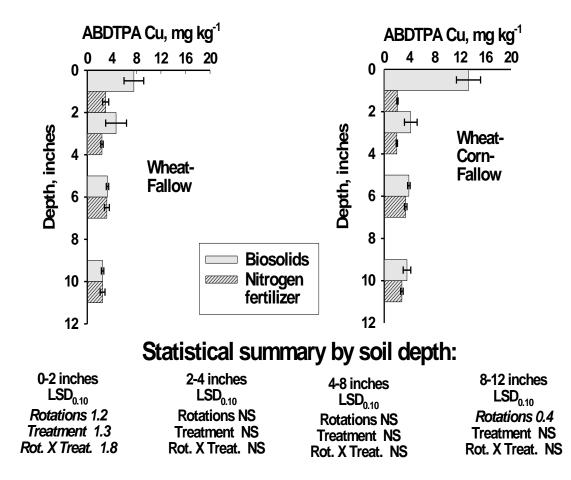


Figure 9. Soil saturated-paste electrical conductivity (EC) following 2007 dryland-wheat-rotation harvests comparing Littleton/Englewood biosolids to commercial N fertilizer. In the statistical summary, LSD<sub>0.10</sub> represents the least significant difference at the 0.10 probability level and NS indicates non-significant differences.

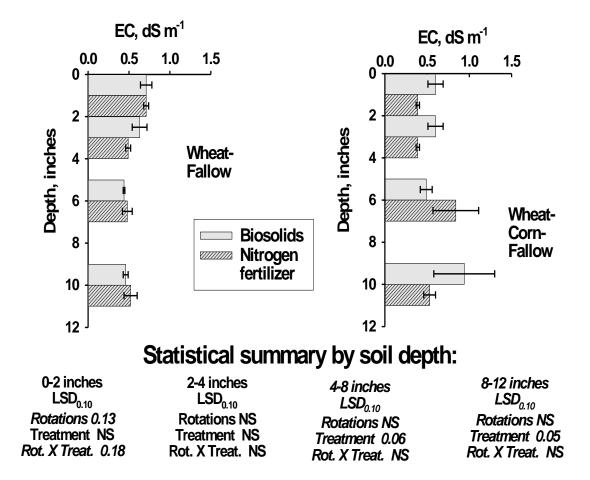


Figure 10. Soil saturated-paste pH following 2007 dryland-wheat-rotation harvests comparing Littleton/Englewood biosolids to commercial N fertilizer. In the statistical summary, LSD<sub>0.10</sub> represents the least significant difference at the 0.10 probability level and NS indicates non-significant differences.

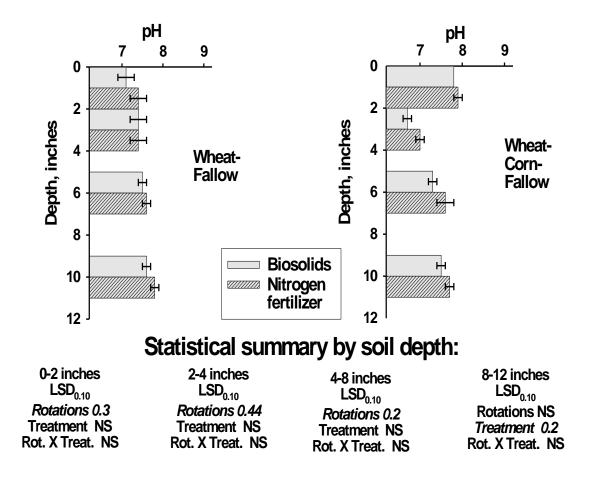


Figure 11. Soil NO<sub>3</sub>-N concentrations following 2007 dryland-wheat-rotation harvests comparing Littleton/Englewood biosolids to commercial N fertilizer. In the statistical summary, LSD<sub>0.10</sub> represents the least significant difference at the 0.10 probability level and NS indicates non-significant differences.

