Journal of Environmental Quality

TECHNICAL REPORTS

WASTE MANAGEMENT

Long-Term Crop and Soil Response to Biosolids Applications in Dryland Wheat

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Biosolids have the potential to improve degraded soils in grain–fallow rotations. Our objectives were to determine if repeated biosolids applications in wheat (*Triticum aestivum* L.)–fallow could supply adequate but not excessive N for grain production and increase soil C without creating a high risk of P loss. A replicated on-farm experiment was established in 1994 in central Washington, comparing anaerobically digested biosolids with anhydrous NH_3 and a zero-N control. Biosolids were applied at 5, 7, and 9 Mg ha^{-1} every fourth year through 2010 and incorporated 10 cm deep, while anhydrous NH_3 plots received 56 kg ha^{-1} N every second year. Grain yield and protein were determined. Soil chemical, biological, and bulk density analyses were made in 2012. Medium and high biosolids rates significantly increased grain yield (3.63 vs. 3.13 Mg ha^{-1}) and protein (103 vs. 85 g kg^{-1}) compared with anhydrous NH_3 averaged across all crops. The medium biosolids rate had significantly lower bulk density (1.05 vs. 1.22 g kg^{-1}) and greater total C (0–10-cm depth) (16.9 vs. 9.4 g kg^{-1}), mineralizable N (156 vs. 52 mg kg^{-1}), and extractable P (114 vs. 16 mg kg^{-1}) than anhydrous NH_3. The P index site vulnerability increased from low for anhydrous NH_3 to medium for the biosolids treatments. Soil NO_3–N was nearly always <10 mg N kg^{-1} soil (0–30-cm depth). Medium and high biosolids rates significantly increased bacteria/fungi ratios, Gram-negative bacteria, and anaerobic bacteria markers compared with anhydrous NH_3. Biosolids can be an agronomically and environmentally sound management practice in wheat–fallow systems.

J. Environ. Qual. 42:1872–1880 (2013)
doi:10.2134/jeq2013.05.0109
Received 28 Mar. 2013.
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A 2-yr wheat–fallow rotation is practiced in semi-arid regions of the Pacific Northwest and Great Plains of the United States as a way to capture fallow-year moisture for the next crop of wheat. Wheat–fallow rotation accelerates the decline in soil organic C compared with annual crops (Collins et al., 1992) because of decreased biomass inputs and increased susceptibility to erosion during the fallow (Rasmussen and Parton, 1994). Researchers and farmers have sought means of improving the soil C status in wheat–fallow rotations, such as conservation tillage (Brown and Huggins, 2012; Gollany et al., 2013).

Organic amendments are another means for restoring organic matter in depleted soils (Brown et al., 2011). Powlson et al. (2012) reported that amendments such as biosolids and animal manures were more effective than reduced tillage at storing C. In a long-term (>60-yr) experiment in Pendleton, OR, Rasmussen and Parton (1994) found that the addition of beef feedlot manure (22 Mg ha^{-1}) every 2 yr maintained soil C in a wheat–fallow rotation. Reeve et al. (2012) observed significantly greater levels of soil C in samples collected from the 0- to 5-cm depth 16 yr following the application of 50 Mg ha^{-1} dry weight of dairy manure compost to a wheat–fallow rotation compared with unamended plots (143 g C kg^{-1} in amended soil vs. 89 g C kg^{-1} in unamended soil).

Biosolids produced by municipal wastewater treatment plants are an affordable organic amendment for farmers and have been shown to increase soil C across a range of agroecosystems (Powlson et al., 2012; Ippolito et al., 2010; Jin et al., 2011, Mantovi et al., 2005). We lack information on biosolids' effects on soil C in wheat–fallow rotations, where application rates are light (typically 5–8 Mg ha^{-1} dry weight biosolids every 2 to 4 yr) and at least half of the rotation period is in bare fallow.

Biosolids are well documented as an N and P source, and N availability has been evaluated and agronomic rate recommendations developed in short-term studies on wheat–fallow rotations in the Pacific Northwest (Sullivan et al., 2009; Cogger et al., 1998) and in long-term studies on the Great Plains (Barbarick et al., 2010; Lagae et al., 2009). Agronomic rates of biosolids can produce equivalent or better grain yields than typical applications of inorganic N (Sullivan et al., 2009; Koenig et al., 2011), but higher rates can lead to yield loss through

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Assigned to Associate Editor Tiequan Zhang.

lodging (Mantovi et al., 2005) or moisture stress (Cogger et al., 1998). Biosolids N can also increase grain protein (Sullivan et al., 2009), which is a benefit for hard wheat but a detriment for the soft white wheat typically grown in the Pacific Northwest. Biosolids provide P in excess of crop needs when applied at agronomic rates based on N (Ippolito et al., 2007). The risk of P loss is not simply related to the P supplied but depends also on soil, site, and biosolids factors that can be assessed through a P index approach (Elliott and O'Connor, 2007).

Past research has suggested that biosolids containing high concentrations of heavy metals (Brown et al., 1998) could reduce plant growth, harm the soil microbial community, or alter soil function (Ibekwe et al., 1997). This is unlikely to be a concern for current low-metal biosolids (Kelly et al., 2007) or for organic contaminants in biosolids, such as pharmaceuticals and personal care products (Smith, 2009). Biosolids nonetheless can affect soil microbial communities and function, and microbial indicators can assess changes in soil health (Kennedy and Papendick, 1995). Sullivan et al. (2006) observed changes in microbial community structure in a semiarid grassland 12 yr following surface biosolids applications (2.5–30 Mg ha^{-1}). The changes appeared to be related to C, N, and P supplied by the biosolids and to changes in plant community structure. Jin et al. (2011) reported increased N mineralization following 8 yr of annual biosolids applications to perennial forage, but only at annual application rates of 45 Mg ha^{-1} or higher. Information on soil microbial changes with amendment additions or management choices is critical to fully understanding if a practice builds and improves soil or degrades the soil and the soil microbial community (Petersen et al., 2002). There is little information on the impact of biosolids on soil microbial communities in tilled wheat–fallow rotations.

This experiment was conducted to determine the long-term response of crop and soil to biosolids applications in a dryland wheat–fallow rotation. The objectives of our study were to determine if repeated use of biosolids in wheat–fallow rotations could (i) supply adequate but not excessive N for grain production and (ii) maintain or increase soil C and soil health without creating a high risk of loss of accumulated P.

Materials and Methods

Site Description

Plots were established in 1994 on a commercial dryland wheat farm on the Waterville Plateau in Douglas County in north-central Washington (47°55′ N, 119°48′ W, elevation 750 m). The Waterville Plateau has a semiarid temperate climate. The mean annual temperature is 8°C, with a January mean of −4°C and a July mean of 20°C. Mean annual precipitation is 270 mm, with an average of 60% of the total falling between November and March. Precipitation during the study ranged from 354 mm for the 2-yr crop cycle in 2000 to 2002 to 735 mm in 1996 to 1998, measured at Chief Joseph dam, 15 km northwest of the study site.

The soil is classified as a Timentwa ashy fine sandy loam (an ashy over loamy, glassy over mixed, superactive, mesic Vitrandic Haploxeroll), developed in glacial till mixed with ash and loess in the upper layer. The surface soil in the plot area contains 68% sand, 27% silt, and 5% clay. The soil (0–30-cm depth) had a pH of 6.7 and bicarbonate-extractable P of 11 mg kg^{-1} at the start of the experiment. The site has been in a wheat–fallow rotation for nearly 100 yr.

Field Experiment

The experiment was a randomized complete block design with five treatments, each replicated three times. The treatments included three rates of biosolids (mean rates of 4.8, 6.9, and 9.0 Mg ha^{-1}) applied to every second crop (Table 1), an inorganic N treatment (56 kg N ha^{-1} as anhydrous NH_3) applied to every crop, and a zero-N control. The plot size was 15 by 215 m, and the plots were located within a commercial production field.

Table 1. Biosolids dry matter and N application rates, 1994 to 2010.

Year	Biosolids treatment	Biosolids application	Total N application	Estimated available N†	
				One crop	Two crops
		Mg ha^{-1} dry wt.		kg ha^{-1}	
	low	4.3	221	85	94
1994	medium	6.9	361	139	153
	high	9.9	513	198	217
	low	4.9	270	103	114
1998	medium	6.3	344	131	145
	high	8.5	466	178	196
	low	4.9	287	111	122
2002	medium	6.9	405	157	172
	high	8.1	470	182	200
	low	4.9	290	115	125
2006	medium	6.9	409	161	176
	high	8.7	515	203	221
	low	4.9	318	125	136
2010	medium	7.4	478	187	204
	high	9.6	622	243	266

† Estimated available N includes NH_4^+–N retained and organic N mineralized; NH_4^+–N retained was estimated at 0.50 NH_4^+–N applied and incorporated within 24 h (Cogger and Sullivan, 2007); organic N mineralized for one crop was estimated at 0.35 organic N applied for the fallow and crop year, and organic N mineralized for two crops included estimated N release from Years 3 and 4 (0.03 and 0.02 organic N applied) (Sullivan et al., 2009; Cogger and Sullivan, 2007).

Anaerobically digested, dewatered biosolids were obtained from the King County wastewater treatment plant at Renton, WA (Table 2). The biosolids met USEPA limits for trace element concentrations and Class B requirements for pathogen reduction (USEPA, 1993). Biosolids were applied in the fall of the fallow year in 1994 and again in 1998, 2002, 2006, and 2010 (Table 1). The biosolids were stockpiled adjacent to the application site for 1 to 2 wk and applied using a spreader with a 15-Mg capacity. Each spreader load was weighed to determine the actual rate of biosolids applied.

The recommended N application rate for the experimental site was 95 to 108 kg N ha^{-1} for a yield goal of 3700 to 4000 kg ha^{-1} under the conditions at the start of the experiment (Koenig, 2005; Sullivan et al., 2009). The low rate of biosolids application supplied a similar amount of available N for the first crop as the recommendation, while the medium and high rates provided excess N (Table 1), based on biosolids N availability estimates (Cogger and Sullivan, 2007). The high rate of biosolids application provided similar available N to the recommended rate for two crops (Table 1), although most of the N would be released for the first crop. The standard farmer rate applied to each crop for the anhydrous NH_3 plots (56 kg N ha^{-1}) was less than the recommended rate.

Biosolids were incorporated to a depth of 10 cm within 24 h of application. Anhydrous NH_3 was applied via injection to the inorganic N treatment in the spring of every fallow year, 2 to 3 mo before planting. The anhydrous NH_3 plots also received 11 kg ha^{-1} of S at the time of N application, based on typical practice. No supplemental P was applied, even though the soil test indicated a possible response (Koenig, 2005), because farmers in the area seldom apply P at similar soil test levels.

The collaborating farmer did all farming activities during the fallow and crop seasons. Tillage typically included plowing, disking, and two cultivations. The herbicide 2,4-D LV6 (2-ethylhexyl 2,4-dichlorophenoxyacetate) was applied by the farmer to manage weeds.

Eltan winter wheat was planted at a rate of 40 kg ha^{-1} by the collaborating farmer in late August of each crop season at the same time the entire production field was planted. Wheat was harvested in early to mid-August of the following year by taking a 7.6-m-wide combine swath from the center of each plot across the entire 215-m length of the plot. The harvested grain from each swath was weighed by transferring it from the combine to a grain truck placed on truck scales. A representative 10-L grain sample was collected as the grain was discharged from the combine to determine the grain N concentration. Wheat harvest occurred every second year beginning in 1996, a cycle of two wheat harvests following each biosolids application.

Soil and Biosolids Sampling

We collected soil samples for NO_3^-–N analysis after harvesting each crop from 1996 to 2006 using a hydraulic hollow-core probe with an inside diameter of 4 cm or a hammer probe with an inside diameter of 2.5 cm. Ten cores (0–30-cm depth) were collected and combined for each plot. Samples were also collected from the 30- to 60-cm depth through 2002, but it was difficult to sample the entire depth in the glacial till subsoil because of rocks.

Soil samples were collected in 2012 for determination of bicarbonate-extractable P, exchangeable K, total C, total N, pH, electrical conductivity, and microbiological markers. These samples were collected from the 0- to 10-, 10- to 20-, and 20- to 30-cm depths using 2.5-cm-diameter hammer probes, combining 12 cores per plot. Soil samples for chemical analyses were air dried at 30°C, ground, and sieved (2 mm). Samples for microbiological analyses were placed on ice, delivered to the laboratory, and refrigerated at 4°C. Microbiological analyses were done only on samples from the 0- to 10-cm depth. Soil bulk density was determined in 2012 using a hammer-driven core sampler, collecting three samples per plot (Grossman and Reinsch, 2002).

We collected six composite subsamples of the dewatered biosolids at each application date. Three subsamples were dried at 60°C for determination of total solids, and three subsamples were acidified to pH 4 to 5 using 1 mol L^{-1} H_2SO_4 and dried at 60°C before N analysis (Sullivan et al., 1997).

Chemical Analyses

Total C and N were determined for biosolids and soil samples using a combustion analyzer (Gavlak et al., 2005). Soil and biosolids NO_3–N and biosolids NH_4–N were extracted with 1 mol L^{-1} KCl (Gavlak et al., 2005). Nitrate was determined by an automated Cd reduction method and NH_4 by colorimetric analysis on a flow-injection analyzer (Gavlak et al., 2005). Grain N was determined using a combustion analyzer and converted to protein by multiplying grain N (g kg^{-1}) by 5.75.

Soil test P (bicarbonate), soil pH, electrical conductivity (1:1 soil/water suspension), and total C and N (combustion analyzer) were determined using standard procedures (Gavlak et al., 2005). Trace elements and K in the biosolids were determined at the King County wastewater treatment plant laboratories using acid wet digestion by USEPA Method 3050B, followed by inductively couple plasma atomic emission spectroscopy (USEPA, 1996). Phosphorus was determined at King County using a vanadomolybdate colorimetric analysis following acid digestion (American Public Health Association, 1999).

Table 2. Selected nutrients, trace elements, C, and solids in King County biosolids, 1994 to 2010. All concentrations except solids are on a dry-weight basis.

Year	Total N	NH_4^+–N	P	K	S	Cu	Cd	Zn	Total C	Solids
			g kg^{-1}				mg kg^{-1}		g kg^{-1}	
1994	52	13	27	2.0	12	890	5	890	–	220
1998	54	11	29	2.4	11	800	6	700	310	187
2002	58	14	31	2.9	12	600	4	750	310	183
2006	59	17	24	2.9	11	510	5	870	350	205
2010	65	18	26	2.0	10	460	2	920	360	221

Laboratory Nitrogen Mineralization Incubation

The long-term effect of biosolids applications on the release of plant-available inorganic N (NH_4^+–N + NO_3^-–N) was estimated by conducting a 150-d laboratory incubation with soils collected in 2011. Soil samples (0–15-cm depth, composite of six cores per plot) were collected from all replicates of the medium-rate biosolids plots (cumulative biosolids application of 34 Mg ha^{-1} and cumulative total N application of 2000 kg ha^{-1}) and the anhydrous NH_3 plots (cumulative N application of 448 kg ha^{-1}). The incubation was conducted under conditions similar to those of Fortuna et al. (2003). Soil was passed through a 2-mm sieve, weighed (50-g oven-dry equivalent) into 120-mL specimen vials (diameter 53 mm, height 60 mm), packed to a bulk density of 1.2 g cm^{-3}, and preincubated for 7 d. Soils were incubated at 25°C and maintained at 40% water-filled pore space, 18% gravimetric water content. Vials were weighed weekly, and distilled water was added to the soil surface with a pipette to maintain the water-filled pore space. Gaseous exchange was fostered by loosely placing lids on the incubation vessels. Specimen vials were destructively sampled at 0, 7, 21, 56, 70, 90, 120, and 150 d of the incubation. Inorganic N was extracted from 10-g soil samples with 100 mL of 1 mol L^{-1} KCl. Aliquots were run on an automated, continuous-flow QuikChem 8000 injection flow analysis system (Hach Instruments). Nitrate-N was determined using the Cd reduction method. Ammonium N was determined using a salicylate–nitroprusside method (Mulvaney, 1996).

Carbon and Nitrogen Storage

Carbon storage was estimated as the difference in soil C (Mg ha^{-1}) in the 0- to 10-cm depth between the biosolids treatments and the anhydrous NH_3 treatment. Soil C (Mg ha^{-1}) was calculated by multiplying the measured soil C (g kg^{-1}) by measured bulk density (0–10-cm depth). The proportion of applied C stored was determined from quadratic regression of soil C vs. the C application rate. The proportion of applied N stored was determined using a similar regression of soil total N vs. N application rate.

Microbiological Analyses

Soil samples (2 g) were extracted using the modified method of Bligh and Dyer as described by Petersen and Klug (1994). The total lipid extract was fractionated into glyco-, neutral, and polar lipids (Ibekwe and Kennedy, 1998). Reagents used in the procedure were high performance liquid chromatography grade supplied by Sigma unless otherwise stated. The polar lipid fraction was transesterified with mild alkali to recover the phospholipid fatty acids as methyl esters in hexane. Solid-phase extraction was used to separate the samples for phospholipid analysis with 100-mg silica columns (Varian) as described by Pritchett et al. (2011). A gas chromatograph (Agilent Technologies GC 6890) equipped with a fused silica column, flame ionizer detector, and integrator was used to analyze fatty acid methyl esters. Integration and analysis of samples were operated with ChemStation software (Agilent Technologies). Microbial Identification Systems software provided parameters that were used for peak identification and integration of areas. Peak chromatographic responses were converted to mole responses by using internal standards, and recalculation of responses was done as needed. Various peaks were used as markers for bacteria as described by Pritchett et al. (2011).

Statistical Analyses

Statistics for grain yield, grain protein, grain N uptake, soil NO_3^-–N, P, K, pH, electrical conductivity, bulk density, and microbial markers were computed using PROC GLM (SAS Institute, 2008). Biosolids and fertilizer treatments were fixed effects. Years were tested as repeated measures for analyses of yield, grain protein, and soil NO_3^-–N. There was a significant year × treatment interaction, so years were analyzed individually. Least significant differences were compared after a protected ($P < 0.05$) F test. Data for grain protein, grain N uptake, soil NO_3^-–N, and bicarbonate-extractable P were transformed to a $\log_{10}$ distribution before doing statistical analyses because the untransformed data deviated from normality. Data reported in the text and tables are untransformed.

Net inorganic N mineralized at each time interval of the 150-d incubation was fitted to an exponential model that estimated a single pool of potentially mineralizable N, mineralizing at a rate proportional to its concentration:

$$N_m = N_o\left[1-\exp\left(-kt\right)\right] \quad [1]$$

where N_m is the amount of N mineralized at time t, t is the incubation time, N_o is the potentially mineralizable pool of N, and the constant k is the mineralization rate.

Curve fitting for the N mineralization data was approximated by least-squares iteration using the SAS NLIN procedure (SAS Version 9.2; SAS Institute, 2008). Reduced and full models were tested to determine whether the source of N affected the fit of the exponential model. The confidence intervals for each parameter were used to test for significant differences among parameters for each treatment.

Results and Discussion

Grain Yield, Protein, Soil Nitrate, and Agronomic Rate

The medium and high biosolids rates increased grain yields compared with anhydrous NH_3 when averaged across all harvests, while the low biosolids rate was not significantly different from anhydrous NH_3 (Table 3). In 2000 and 2008 (both first crop harvests after biosolids applications), the low rate biosolids treatment had greater grain yield than the high rate treatment (Fig. 1). The high rate biosolids treatment had noticeably more vigorous vegetative growth, which probably led to more rapid soil moisture depletion, depressing grain yields. In 2004 (another first crop harvest year), dry conditions depressed all yields. In three of the second crops following biosolids applications (1998, 2006, and 2010 harvest years) the high biosolids rate treatment had greater grain yield than the low rate, indicating greater residual N supply from the previous biosolids application. No difference was seen among treatments in 2002, the most moisture-stressed crop cycle of the study, when crop cycle (2-yr) precipitation was 354 mm, <70% of normal.

Figure 1 shows a trend with time of increasing yield in the second crop following biosolids application relative to the first crop. The trend suggests increased soil N supply with repeated

Table 3. Mean winter wheat grain yield, protein, and N uptake.

Treatment†	Grain yield			Grain protein			Grain N uptake		
	All crops	First crop‡	Second crop	All crops	First crop	Second crop	All crops	First crop	Second crop
	kg ha^{-1}			g kg^{-1}			kg ha^{-1}		
Zero N	2450 c§	2350 b	2550 c	74 d	70 c	79 c	32 c	28 d	35 d
Anhydrous NH_3	3130 b	3420 a	2850 c	85 c	83 b	87 ab	46 b	49 c	43 bc
Biosolids low	3480 ab	3860 a	3090 bc	84 c	90 b	78 c	50 b	59 b	42 cd
Biosolids medium	3600 a	3680 a	3530 ab	97 b	112 a	82 bc	61 a	72 a	50 b
Biosolids high	3640 a	3450 a	3830 a	109 a	129 a	89 a	67 a	76 a	58 a

† Biosolids low, 24 Mg ha^{-1} cumulative application; biosolids medium, 34 Mg ha^{-1} cumulative application; biosolids high, 45 Mg ha^{-1} cumulative application; anhydrous NH_3, 56 kg N ha^{-1} per crop.

‡ First and second crop refer to first and second crops grown following biosolids applications.

§ Means within a column followed by different letters are significantly different ($P < 0.05$) by protected LSD.

biosolids applications. This is consistent with the observations of Barbarick et al. (2010), who used planar regressions to describe the effects of application rate and number of applications on grain N uptake in dryland wheat.

Biosolids P may also have affected wheat yields. Although farmers in the wheat–fallow climate zone in the Pacific Northwest typically do not apply P, extension guidance would have recommended 20 to 40 kg ha^{-1} P based on the initial soil test results at the site (Koenig, 2005).

Average grain protein was greater in the medium and high biosolids treatments compared with anhydrous NH_3, with all of the difference occurring in the first crop following biosolids application (Table 3). The soft white wheat grown in the Pacific Northwest is used for cookies and pastries, and its baking quality declines with increased protein. Mean first crop protein for the medium and high biosolids rates were both greater than desirable (100 g kg^{-1} or less). No increase in protein was observed in the second crop following biosolids application compared with anhydrous NH_3 (Table 3).

Grain N uptake increased with biosolids rate. All biosolids treatments had greater N uptake than the anhydrous NH_3 treatment averaged across the first crop harvests following application, while the two higher biosolids rates had greater N uptake than anhydrous NH_3 averaged across all harvests (Table 3). Cumulative grain apparent N recovery (treatment grain N uptake minus zero-N control grain N uptake) was equivalent to 15% of total biosolids N applied through 2008, compared with 29% for anhydrous NH_3–N.

The elevated protein levels for the medium and high biosolids rates for the first crop following biosolids application indicate that the agronomic rate was exceeded. The low rate would be closest to the agronomic rate for the first crop following biosolids application based on N recommendations and protein data. The low biosolids rate was less than the agronomic rate for the second crop following biosolids application but still equivalent to the anhydrous NH_3 treatment (Table 3).

Biosolids applications had a small effect on post-harvest soil NO_3–N, with significant differences among treatments occurring after three out of the six harvests that were sampled (Table 4). Soil NO_3–N levels were low (<10 mg N kg^{-1} soil) in all samples except the medium and high biosolids rates in 2004. Although our data are limited with depth and time, they suggest that even the highest biosolids rate did not greatly increase the risk of NO_3 loss compared with the zero-N control.

Soil Carbon, Nitrogen, and Bulk Density

Repeated biosolids applications had a large positive effect on total soil C and N in the 0- to 10-cm depth (Table 5), with nearly double the concentration of soil C in the medium and high biosolids rate treatments compared with the zero-N and anhydrous NH_3 treatments. Significant differences were confined to the 0- to 10-cm sampling depth, indicating little change below the shallow tillage zone used in these farming systems.

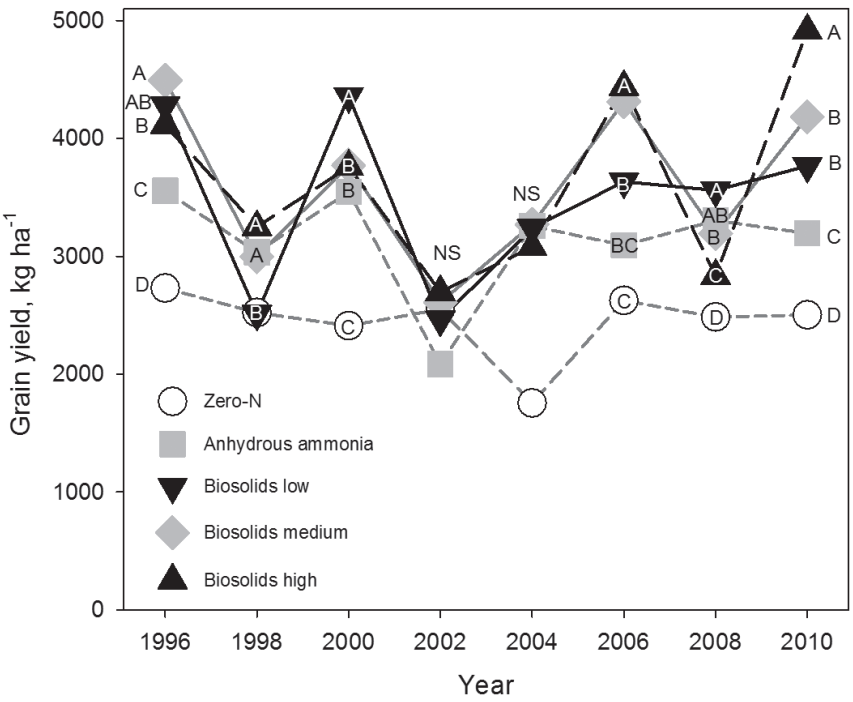

Fig. 1. Winter wheat grain yield by harvest, 1996 to 2010. The first crops following biosolids applications were in 1996, 2000, 2004, and 2008. The second crops following biosolids applications were in 1998, 2002, 2006, and 2010.

Table 4. Post-harvest soil NO_3–N, 1996 to 2006.

Treatment†	Soil NO_3–N									
	1996		1998		2000		2002		2004	2006
	0–30 cm	30–60 cm	0–30 cm	30–60 cm	0–30 cm	30–60 cm	0–30 cm	30–60 cm	0–30 cm	0–30 cm
	mg kg^{-1}									
Zero N	5.3	2.3	4.3 b‡	5.0	2.9	3.2	2.6	2.6	3.8 c	1.1 c
Anhydrous NH_3	5.7	4.0	4.2 b	5.2	2.7	3.1	2.8	3.0	4.2 c	0.8 c
Biosolids low	5.7	2.7	5.4 ab	5.5	3.8	3.4	2.4	2.2	6.9 b	1.2 bc
Biosolids medium	5.7	3.3	6.4 a	5.9	3.5	3.3	3.4	3.4	10.8 a	1.9 ab
Biosolids high	7.7	3.7	6.6 a	6.2	3.7	4.5	3.9	3.7	13.4 a	3.1 a

† Biosolids low, 24 Mg ha^{-1} cumulative application; biosolids medium, 34 Mg ha^{-1} cumulative application; biosolids high, 45 Mg ha^{-1} cumulative application; anhydrous NH_3, 56 kg N ha^{-1} per crop.

‡ Means within a column followed by different letters are significantly different ($P < 0.05$) by protected LSD.

Bulk density is expected to decrease with increasing soil C, but some long-term biosolids studies have shown little or no change in bulk density (Jin et al., 2011). We observed a significant decrease in bulk density at all rates of biosolids application (Table 5), with bulk density ranging from 1.26 g cm^{-3} in the zero-N treatment to 1.02 g cm^{-3} at the high rate of biosolids. This probably reflects the large proportional increase in soil C concentration observed.

Soil C (Mg ha^{-1}) in the 0- to 10- cm depth vs. biosolids C applied fit a quadratic regression ($y = -0.03x^2 + 0.92x + 10.45$, $r^2 = 0.78$, $P < 0.001$). At the medium biosolids rate (34 Mg ha^{-1} cumulative biosolids applied), the difference in soil C compared with the zero-N treatment was equivalent to 57% of the biosolids C added. This is considerable C storage in a farming system that is not designed to preserve organic matter (tilled fallow every other year). The increase in C was probably a combination of recalcitrant biosolids C and plant-based C resulting from increased biomass production in the biosolids treatments. These results indicate that biosolids applications at agronomic rates can sequester a substantial amount of C in depleted soils. Little additional C accumulated in the soil at the high biosolids rate, suggesting that C storage was approaching a plateau. This apparent plateau may be because of saturation of one or more pools of organic matter (Six et al., 2002) from the higher rates of amendment application.

Total soil N in the 0- to 10-cm depth (Mg ha^{-1}) vs. N applied also fit a quadratic regression ($y = -0.13x^2 + 0.53x + 1.01$, $r^2 = 0.77$, $P < 0.001$). The increase in soil total N for the medium biosolids rate compared with the zero-N control was equivalent to 33% of the biosolids N applied, as calculated from the regression equation. Anhydrous NH_3 had a negligible effect on soil total N.

Laboratory Incubation Nitrogen Mineralization

Laboratory N incubation results indicated a significantly ($P < 0.01$) larger pool of mineralizable N in the field soil after repeated biosolids applications. Potentially mineralizable N (N_o) calculated from the exponential N mineralization model was

Table 5. Total soil C and N, bulk density, pH, electrical conductivity (EC), and Olsen P, 2012.

Treatment†	Total C	Total C	Total N	Bulk density	pH	EC	Olsen P
	g kg^{-1}	Mg ha^{-1}	g kg^{-1}	g cm^{-3}		dS m^{-1}	mg kg^{-1}
	0–10-cm depth						
Zero N	8.4 c‡	10.5 c	0.8 c	1.26 a	6.1 a	0.08 c	15 c
Anhydrous NH_3	9.4 c	11.4 c	0.9 c	1.22 a	5.7 b	0.08 bc	16 c
Biosolids low	13.9 b	15.2 b	1.3 b	1.09 b	5.5 bc	0.13 ab	74 b
Biosolids medium	16.9 a	17.7 a	1.5 a	1.05 bc	5.4 c	0.18 a	114 a
Biosolids high	16.4 a	16.8 ab	1.4 a	1.02 c	5.4 c	0.16 a	128 a
	10–20-cm depth						
Zero N	6.0	–	0.7	–§	6.7 a	0.10	8 c
Anhydrous NH_3	6.0	–	0.7	–	6.5 ab	0.11	7 c
Biosolids low	6.8	–	0.7	–	6.4 abc	0.10	18 b
Biosolids medium	6.8	–	0.8	–	6.3 bc	0.15	29 a
Biosolids high	6.5	–	0.7	–	6.1 c	0.12	35 a
	20–30-cm depth						
Zero N	5.7	–	0.7	–	7.2	0.09 c	7 c
Anhydrous NH_3	6.0	–	0.8	–	7.2	0.11 bc	7 c
Biosolids low	6.0	–	0.7	–	7.0	0.13 ab	9 bc
Biosolids medium	6.3	–	0.8	–	7.0	0.14 a	11 ab
Biosolids high	6.2	–	0.7	–	7.1	0.11 abc	12 a

† Biosolids low, 24 Mg ha^{-1} cumulative application; biosolids medium, 34 Mg ha^{-1} cumulative application; biosolids high, 45 Mg ha^{-1} cumulative application; anhydrous NH_3, 56 kg N ha^{-1} per crop.

‡ Means within a column and depth followed by different letters are significantly different ($P < 0.05$) by protected LSD.

§ Bulk density data were not collected below the surface layer.

156 mg kg^{-1} for the medium rate biosolids treatment (34 Mg ha^{-1} cumulative biosolids applied), compared with 52 mg kg^{-1} for the anhydrous NH_3 treatment, using soil samples collected in 2011. Decay constants were 0.014 d^{-1} for the biosolids treatment, compared with 0.021 d^{-1} for anhydrous NH_3.

The increased pool of mineralizable N may be a factor in the observed changes in relative yield between the biosolids and anhydrous NH_3 treatments with time. Yields became greater in the biosolids treatments relative to anhydrous NH_3 in the second crop following biosolids application in the later years of the study (Fig. 1). Because the second crop would rely more on N mineralized from soil organic matter, this result is consistent with a larger mineralizable N pool following repeated biosolids applications. Jin et al. (2011) also observed increased N mineralization at a long-term biosolids site, but only at biosolids application rates of 45 Mg ha^{-1} yr^{-1} or greater, much higher rates than applied in this study.

Soil Chemical Properties

Soil pH ranged from 5.4 to 6.1 at the 0- to 10-cm depth, decreasing with increasing organic N rate (Table 5). The medium and high biosolids treatments had slightly but significantly ($P < 0.05$) lower pH than anhydrous NH_3. The soil pH was higher in the 10- to 20-cm depth, but a similar trend with N rate was observed. The soil pH was 7.0 to 7.2 in the 20- to 30-cm depth, with no significant treatment differences. Electrical conductivity was low (<0.2 dS m^{-1}), with statistically significant but biologically unimportant increases in the biosolids treatments.

Biosolids had a large effect on bicarbonate-extractable P, increasing from 15 mg kg^{-1} at the 0- to 10-cm depth in the zero-N treatment to 128 mg kg^{-1} at the high rate of biosolids (Table 5). Smaller but statistically significant increases in bicarbonate-extractable P were observed at the 10- to 20- and 20- to 30-cm depths, indicating limited downward movement of P. Ippolito et al. (2007) also reported evidence of downward movement of P following biosolids applications in a dryland wheat–fallow system.

Water quality risks from excess biosolids P depend on soil and transport factors, as well as the P binding capacity of the biosolids itself (Elliott and O'Connor, 2007). Washington and Oregon use P indexes to estimate water quality risks based on source factors (soil test P, P application rate, and application method) and transport factors (distance to a water body, runoff class, and soil erosion rate) (Sullivan and Stevens, 2003). Using the eastern Washington and Oregon P index calculations, the three biosolids treatments had scores ranging from 51 to 92, placing them in the medium site vulnerability class (scores of 31–130). The anhydrous NH_3 treatment was in the low site vulnerability class. This indicates that biosolids applications increased the site P vulnerability, but in the near term, biosolids applications to the site can continue on a N basis.

Soil exchangeable K was high across all treatments and depths, ranging from 305 to 425 mg kg^{-1} of soil, with no significant treatment effects at any depth (data not shown). Biosolids are low in K, and repeated applications can lead to K depletion if supplemental K is not added (Cogger et al., 2013). In this experiment, no differences were observed, even after eight crop harvests.

Soil Microbiological Properties

Soils were sampled 2 yr after the last biosolids application, so the samples represent long-term rather than transient changes in the microbial community. Microbial biomass ranged from 386 (zero N) to 619 μg C g^{-1} soil (medium biosolids), but values were not significantly different among treatments, indicating that the biosolids did not add or detract from overall microbial growth (Table 6). Gram-positive bacteria markers were similar between biosolids treatments and anhydrous NH_3. All other bacterial markers (total, aerobic, Gram negative, and anaerobic) were significantly greater in the high biosolids treatment than anhydrous NH_3. The medium biosolids treatment had significantly greater Gram-negative and anaerobic bacteria, while the low biosolids treatments had no significant differences compared with anhydrous NH_3. Total bacteria and aerobic bacteria markers were lowest in the zero-N compared with the other treatments. Fungal markers were significantly lower in the medium biosolids rate compared with anhydrous NH_3, but they were not different at the high rate (Table 6). Bacteria/fungi ratios ranged from 2.3 to 4.2 and were highest in soil treated with the medium and high biosolids rates. This indicates an increase in bacterial growth relative to fungi, probably due to the addition of biosolids nutrients and C. While the ratios were statistically different, they were within an acceptable range (Landesman and Dighton, 2011) and did not indicate any negative impact or imbalance due to the biosolids.

These results are broadly consistent with those observed by others across a range of agroecosystems. Sullivan et al. (2006) observed increased Gram-positive biomarkers in a long-term study following biosolids applications at rates of 10 Mg ha^{-1} or greater in semiarid grassland. Gram-negative markers significantly increased only at the highest biosolids rate (30

Table 6. Microbial markers from soil at the study site, 2012.

Treatment†	Microbial biomass	Bacteria	Aerobic bacteria	Gram-positive bacteria	Gram-negative bacteria	Anaerobic bacteria	Fungi	Bacteria/fungi ratio
	μg C g^{-1} soil	mol %						
Zero N	386	0.199 c‡	0.199 c	0.147 b	0.053 b	0.048 c	0.115 ab	2.3 c
Anhydrous NH_3	423	0.242 b	0.240 b	0.179 ab	0.061 b	0.057 bc	0.133 a	2.3 c
Biosolids low	464	0.273 ab	0.272 ab	0.205 a	0.067 ab	0.065 ab	0.128 ab	2.7 bc
Biosolids medium	626	0.268 ab	0.265 ab	0.184 ab	0.081 a	0.074 a	0.086 b	4.2 a
Biosolids high	619	0.302 a	0.297 a	0.214 a	0.083 a	0.068 a	0.100 ab	3.8 ab

† Biosolids low, 24 Mg ha^{-1} cumulative application; biosolids medium, 34 Mg ha^{-1} cumulative application; biosolids high, 45 Mg ha^{-1} cumulative application; anhydrous NH_3, 56 kg N ha^{-1} per crop.

‡ Means within a column followed by different letters are significantly different ($P < 0.05$) by protected LSD.

Mg ha^{-1}), while arbuscular mycorrhizal fungi were lower in the biosolids treatments than the unfertilized control. They concluded that increases in bacterial biomarkers and decreased arbuscular mycorrhizae coincided with long-term nutrient enrichment of the soil from the biosolids applications. In a short-term study on river sediments amended with high rates of biosolids in a humid environment, Kelly et al. (2007) also observed increased Gram-negative bacteria following biosolids applications, while Gram-positive bacteria and fungi decreased. They attributed these effects to the ability of Gram-negative bacteria to take advantage of the high inputs of C following biosolids applications. They also showed that biosolids applications increased the microbial biomass and activity but did not affect arbuscular mycorrhizae. Zerzghi et al. (2009, 2010) observed increased microbial activity and density but a similar bacterial community structure following 20 yr of biosolids applications to irrigated cotton (*Gossypium hirsutum* L.). These results suggest that nutrients and C from biosolids affect microbial communities, and in our study we were able to observe changes at relatively low biosolids rates.

Long-Term Implications for Biosolids Management in Dryland Wheat

Agronomic rates of biosolids reliably produced equivalent or greater grain yields than the standard anhydrous NH_3 treatment and increased soil organic matter. Biosolids also have the potential to increase grain protein in the first crop after application, although this is not a benefit when growing soft white wheat. Repeated applications of biosolids increase the soil N supply, suggesting that rates need to be reduced in the long term to avoid potential N loss and yield loss associated with excess N in a semiarid environment.

The increase in soil C observed was substantial, given the modest rates of biosolids applied (24–45 Mg ha^{-1} cumulative from 1994–2012) and the tillage- and fallow-dependent cropping system that does not conserve soil C. This indicates that agronomic biosolids applications are an effective and low-cost tool to increase soil C and improve soil quality in soils depleted of organic matter after years of grain–fallow rotation.

Repeated biosolids applications to meet plant N needs will increase soil P in excess of plant requirements. Excess P may become a future water quality risk, depending on site-specific P transport factors. The use of environmental P indices to identify P loss risks, adoption of conservation tillage to reduce erosion, rotation of biosolids applications to sites with low soil test P levels, and development of wastewater treatment processes that reduce biosolids P will enhance the sustainability of biosolids applications to grain–fallow rotations and allow continued C and N benefits associated with biosolids use.

Acknowledgments

The Northwest Biosolids Management Association, the King County Dep. of Natural Resources, and the Agricultural Research Center at Washington State Univ. (Hatch Projects 0711 and 0722) funded this project. We thank Elizabeth Myhre and Jeremy Hansen for their technical support.

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