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Effects of Biosolids on Root Growth and Nitrogen Metabolism in Kentucky Bluegrass under Drought Stress

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Abstract. **Biosolids are valued as a source of plant nutrients, soil organic matter, and biologically active substances. This greenhouse study was designed to examine if application of biosolids can improve plant drought tolerance by affecting nitrogen (N) and hormone metabolism as well as root growth in kentucky bluegrass (*Poa pratensis* L.; KBG). The three treatments, which provided N rates equivalent to 75 mg plant-available N/kg soil, included: 1) biosolids at 1× agronomic (Ag) N rate (75 mg N/kg soil completely provided with biosolids); 2) biosolids at 0.5× Ag N rate (37.5 mg N/kg soil provided with biosolids and 37.5 mg N/kg soil provided with NH_4NO_3); and 3) control (75 mg N/kg soil completely provided with NH_4NO_3). The treated KBG was grown under either well-watered (90% container capacity) or drought stress (≈25% container capacity) conditions. Biosolids application improved turf quality and delayed leaf wilting under drought stress. The grass treated with biosolids at 1× N rate had higher leaf proline and amino acid content and greater nitrate reductase activity than the control under drought stress. Biosolids treatments also increased leaf and soil indole-3-acetic acid (IAA) content. Moreover, biosolids at 1× N rate increased root length density by 23% compared with the control under drought stress. The results of this study suggest that biosolids may enhance plant drought tolerance by improving N and hormone metabolism and root growth in KBG.**

Biosolids are treated sewage sludge that have strict requirements for pollutant concentrations as well as reduction of pathogens and vector attractions (USEPA, 1993; Virginia Department of Health, 1997). Biosolids are valued as a source of plant nutrients and soil organic matter and, in the case of alkaline-stabilized materials, liming agents (David et al., 2010; Zhang et al., 2007). Proper application of biosolids cannot only improve soil physical properties (Cogger, 2005), but also enhance the biological and chemical properties of the soil (De Andres et al., 2012; Seran et al., 2010; Singh and Agrawal, 2008, 2010). In recent years, research has provided evidence that biosolids may contain several groups of biologically active substances (e.g., humic substances, amino acids, vitamins, and phytohormones) (Zhang et al., 2005, 2009, 2012). It has been documented that proper application of biosolids can enhance plant growth (Antolín et al., 2005; Pascual et al., 2004) including turfgrass (Zhang et al., 2005, 2007, 2009) and improve plant tolerance to abiotic stresses (David et al., 2010).

Zhang et al. (2009) pointed out that the positive effects of biosolids may result from biologically active substances that provide hormones (particularly IAA) directly or stimulate the activity of microbes that supply substrates and hormones, especially under drought stress. They also noted that biosolids treatment may affect N metabolism of tall fescue. However, little information was reported on responses of root characteristics to biosolids in this study

The capacity of root systems in absorbing water and nutrients has long been recognized as an important factor in plant resistance to drought stress (Huang et al., 1997). Root traits (root length, root length density) are associated with drought resistance in field crops (Asch et al., 2005) and turfgrass (Huang and Fry, 1998; Huang and Gao, 2000). Plants with greater root length density may have greater access to available water (Githinji et al., 2008) and nutrients (Bowman et al., 2002). Zhang et al. (2009) noted that addition of biosolids to the soil increased auxin levels and promoted plant root biomass. However, no studies investigated the effects of biosolids on root length density and root surface area of turfgrass under moisture stress conditions.

It has been reported that N metabolites (such as proline) are closely related to drought tolerance (Caravaca et al., 2004). Nitrate reductase is one of the key enzymes in N metabolism and nitrate reductase activity is associated with efficiency of nitrate reduction and N assimilation (Subramanian and Charest, 1999). Antolín et al. (2010) reported that sludge treatment increased levels of N compounds (such as proline) of nodulated alfalfa (*Medicago sativa* L.) plants under drought stress.

Kentucky bluegrass, a cool-season turfgrass, is widely used for home lawns and sport fields in many parts of the world. This grass has relatively poor to moderate drought resistance. The objective of this study was to examine if application of biosolids can improve plant drought tolerance by affecting N and hormone metabolism as well as root growth in KBG.

Materials and Methods

Biosolids characterization. The biosolids used in this experiment were obtained from the Beijing Wastewater Treatment Plant, Beijing, China. The biosolids were anaerobically digested and dewatered by centrifuge. The fresh biosolids (80% moisture content) were stored in sealed plastic bags at 4 °C until use. Moisture content and N concentrations of the biosolids were determined at the Institute of Plant Nutrition and Natural Resource, Beijing Academy of Agriculture and Forestry Sciences (Beijing, China). Total Kjeldahl N (TKN) was determined using USEPA Method 351.3 (USEPA, 1979) and mineral nutrients and heavy metals were determined using the USEPA method SW 846-6010B (USEPA, 2003). Plant hormone IAA was analyzed using the procedure by Zhang et al. (2012). The biosolids contained 1.62 $\mu g \cdot g^{-1}$ IAA, 68.4 $mg \cdot g^{-1}$ TKN, 26.1 mg NH_4^+-N, 39.5 mg organic N, 2.8 mg NO_3^--N, 19.3 $mg \cdot g^{-1}$ total phosphorus (P), 7.1 $mg \cdot g^{-1}$ total potassium (K), 7.8 $mg \cdot g^{-1}$ calcium, 3.5 $mg \cdot g^{-1}$ iron, 2.4 $mg \cdot g^{-1}$ magnesium, and 52 $mg \cdot kg^{-1}$ manganese, 952 $mg \cdot kg^{-1}$ zinc, 153 $mg \cdot kg^{-1}$ cadmium (Cd), 456 $mg \cdot kg^{-1}$ copper, 56 $mg \cdot kg^{-1}$ lead. All of these elements are within the most stringent EPA standards, except for Cd which is higher than EPA ceiling limits.

Preliminary experiment on actual plant-available N in the biosolids. This preliminary experiment was designed to determine the actual plant-available N in the biosolids. There were five treatments, including four inorganic N rates (0, 25, 50, and 75 mg N/kg soil) supplied as NH_4NO_3 and one biosolids treatment applied at 3 $g \cdot kg^{-1}$ soil. The biosolids rate was calculated to supply 51.08 mg plant-available N/kg soil using the following assumptions: 1) 100% plant availability of inorganic N and 50% plant availability of the organic N in the biosolids as estimated from field and modeling studies (Gilmour et al., 2003); and 2) 35% of the calculated N from Assumption 1 would become available during the course of the greenhouse study (Zhang et al., 2005, 2009). All treatments (N and biosolids) were added to pots (15-cm diameter × 11-cm depth) filled with 700 g

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calcined clay (Profile Products, Chicago, IL). Calcined clay is an inorganic amendment with a uniform texture and good water-holding capacity. It does not contain any organic matter and mineral nutrients so that fertilization rate can be accurately controlled. 'Midnight' KBG seeds were planted in each pot at 15 $g \cdot m^{-2}$. The pots were irrigated to 90% container capacity with a full-strength N-free Hoagland's solution before seeding and maintained at this moisture content throughout the course of the experiment. "Container capacity" was the amount of water held by initially saturated calcined clay after 24 h free drainage as defined previously (Zhang et al., 2009). A completely randomized block design was used with four replications.

The experiment lasted for 8 weeks. The grass was clipped at 9 cm twice each week. The clippings were collected and dried at 65 °C for 72 h and subjected to total N concentration analysis using the Kjeldahl method. Kentucky bluegrass N uptake (in mg N/pot) was determined by multiplying accumulative above-ground biomass/pot by KBG N concentration. The N provided by the biosolids was determined using a linear regression calibration equation: $Y = 0.353X + 5.268$ ($R^2 = 0.959$; $P < 0.001$), where Y represents N uptake (mg/pot) and X represents N applied ($mg \cdot kg^{-1}$). The biosolids were calculated to provide plant-available N at 38.02 $mg \cdot kg^{-1}$ soil. Based on this value, a rate of 1.97 g biosolids/kg soil was used to supply 75 mg N/kg soil in the following experiment.

Plant culture, biosolids application, and drought stress treatment. Plastic-lined pots (23-cm diameter × 15-cm depth) were filled with 2.5 kg of calcined clay, and full-strength N-free Hoagland's solution (Hoagland and Arnon, 1950) was added to all pots to supply adequate P, K, and other macro- and micronutrients at one time. All pots were seeded with 'Midnight' KBG at 15 $g \cdot m^{-2}$ on 20 Apr. 2011 and grown in a greenhouse with photosynthetic active radiation at 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (at 1400 HR) and temperatures at 24/18 °C (day/night). The pots were watered to 90% container capacity by hand twice a week before seeding and maintained at this moisture content for 7 weeks until the drought stress treatments were initiated.

Nitrogen in the form of biosolids and/or NH_4NO_3 was applied to all treatments at a plant-available N rate equivalent to 75 mg N/kg soil. The treatments included: 1) biosolids at 1× Ag N rate (75 mg N/kg soil completely provided with biosolids); 2) biosolids at 0.5× Ag N rate (37.5 mg N/kg soil provided with biosolids and 37.5 mg N/kg soil provided with NH_4NO_3); 3) control (75 mg N/kg soil completely provided with NH_4NO_3). The biosolids and NH_4NO_3 solution (25 mg N/kg soil) were applied to top 5 cm of the pot before planting. The rest of NH_4NO_3 solution was applied at 14 d (5 May 2011; 25 mg N/kg soil) and 28 d (19 May 2011; 25 mg N/kg soil) after planting. All pots received 75 mg N/kg soil during grass growth.

Four weeks after the final N was applied, the KBG was subjected to two soil moisture regimes: well watered (Expt. 1) and drought stress (Expt. 2). The "well-watered" pots were maintained at 90% container capacity (49.4% soil water content) throughout the experiment. For drought stress treatment, the KBG was dried down by allowing soil moisture to drop gradually to ≈25% of container capacity (8% soil water content, near wilting point) and then irrigated so that the grass could recover. The container capacity was determined based on the procedure by Zhang et al. (2005, 2009). Briefly, each of six pots was weighed and holes on the bottom of the pots were covered with two layers of paper towel. Then the pots were filled with 2.5 kg of calcined clay each. The pots were weighed, saturated with water, and then covered with plastic film to prevent evaporation. After 24 h, each pot was weighed to determine the water content. The total amount of water at 90%, 50%, 30%, and 25% container capacity was calculated based on the initial water content calculated previously. Soil moisture content was also measured with a ThetaProbe soil moisture sensor (ML2; Delta-T Devices, Cambridge, U.K.).

Sample collection. Morphological, physiological, and biochemical parameters were determined during dry-down and recovery periods. Turfgrass was mowed to ≈9 cm once a week. Leaf samples were collected for biochemical constituents. The samples were taken from the top of the canopy on 19 June (normal moisture, 90% container capacity), 4 July (50% container capacity), 10 July (30% container capacity), 16 July (25% container capacity), and 23 July (90% container capacity, recover for 7 d). The samples were wrapped with aluminum foil, frozen with liquid N_2, and stored at –80 °C until use. The calcined clay growth medium was sampled by taking two cores (1.25-cm diameter × 6.5 cm deep) from each pot at the beginning and the end of experiment. The samples were stored at –80 °C until use. After sampling, the holes from which the cores were taken were refilled with calcined clay from extra pots receiving the same treatments as those for the experiment.

Turfgrass quality, leaf wilting, and relative water content. Turfgrass quality was rated on color, density, texture, and uniformity of the turfgrass surface. Turfgrass quality ratings were based on a numerical scale from 1 to 9: 1 = brown, dead turfgrass, 6 = minimal acceptable turfgrass, 9 = ideal green, healthy turfgrass (Waddington et al., 1992). Leaf wilting was rated based on a visual scale of 0% to 100% with 100% indicating complete, permanent wilting of the canopy. Leaf relative water content was determined according to Man et al. (2011).

Leaf amino acid and proline content. Leaf amino acid was determined according to Zhang et al. (2011) with slight modifications. Frozen leaf samples (100 mg) were cut into 5-mm sections and then placed into tubes containing 4 mL of 3% 5-sulfosalicylic acid. The mixture was boiled at 100 °C in a water bath for 10 min. The resultant 2 mL supernatant was mixed with 2 mL acetic acid and 2 mL acidic ninhydrin solution, then the mixed liquid was boiled at 100 °C for 30 min, and 3 mL methylbenzene was added to the mixed liquid. After vortexing for 1 min, the supernatant was used for the analysis at 580 nm with a spectrophotometer (ultraviolet-2802S; UNICO, Spain). The amino acid content was calculated based on a standard curve with glycine as the standard. Proline content was determined spectrophotometrically at 520 nm with a spectrophotometer (ultraviolet-2802S; UNICO) (Bates, 1973).

Leaf and root nitrate reductase activity. The leaf and root nitrate reductase activity was assayed in vivo according to Caravaca et al. (2004) with some modifications. Leaf and root samples were collected in the morning between 0830 and 1100 HR solar time and cut into 5-mm sections. Approximately 200 mg of leaf punches and 200 mg of roots from each pot were placed into tubes containing 2 mL of an incubation medium consisting of 0.05 M tris-HCl pH 7.8 and 0.25 M KNO_3. The tubes were sealed and kept in the dark for 1 h at 30 °C. The nitrite released into the medium was determined after incubation by treating 1 mL of the aliquots with 1 mL of 1% sulphanilamide in 1 M HCl and 1 mL of 0.01% n-1-napthyl-ethylenediamine hydrochloride. After 15 min, the optical density was measured at 540 nm with a spectrophotometer (ultraviolet-2802S; UNICO). For each run, blanks and four nitrite standards (1, 5, 10, and 25 μM KNO_2) were included. An extract from frozen spinach (*Spinacia oleracea* L.) leaf was used as a positive control each time.

Leaf indole-3-acetic acid and abscisic acid content, root and soil indole-3-acetic acid content. Leaf or root tissue (1 g) was ground with a mortar and pestle in liquid N_2 and homogenized in 10 mL Na-phosphate buffer (0.05 M, pH 7.0) containing 0.02% (w/v) sodium diethyldithiocarbamate as an antioxidant. The samples were extracted by continuous shaking for 24 h at 4 °C, transferred into 10-mL microcentrifuge tubes after extraction, and adjusted pH to ≈2.6 with 2.0 M HCl. The sample was slurried with 1 g of Amberlite XAD-7 (Sigma Aldrich, St. Louis, MO) for 1 h. After removal of the buffer, the XAD-7 was washed with 2 × 3 mL of 1% acetic acid before being slurried with 2 × 3 mL of dichloromethane for 2 × 1 h (Edlund et al., 1995). The combined dichloromethane fractions were reduced to dryness with N gas. The samples were redissolved with 200 μL methanol and 200 μL 0.075% (v/v) acetic acid. The samples were stored at –20 °C until analysis. IAA and abcissic acid (ABA) contents were assayed using Agilent Technologies 1260 Infinity HPLC (Agilent Technologies Inc., Santa Clara, CA). An Agilent Zorbax XDB-C18 column (4.6 × 250 mm; inner diameter: 5-μm particle size) (Agilent Technologies Inc.) was used and eluted with a mixture of methanol:water containing 0.075% (v/v) acetic acid at a flow rate of 1 $mL \cdot min^{-1}$ and a temperature of 30 °C. The sample was injected at a volume of 10 μL on

an autosampler (Agilent Technologies Inc.). The gradient elution conditions and settings were as follows: 10% methanol and 90% high-performance liquid chromatography (HPLC) water with 0.075% (v/v) acetic acid from 0 to 10 min, 90% methanol and 10% HPLC water with 0.075% acetic acid from 10 to 15 min, and 10% methanol and 90% HPLC water with 0.075% acetic acid from 15 to 22 min. The liquid chromatography ultraviolet detector was set at 280 nm for IAA and 262 nm for ABA. The retention time for IAA was ≈10.8 min and ≈11.4 min for ABA. The IAA and ABA concentrations were calculated based on the standard curve and expressed as $ng \cdot g^{-1}$ dry leaf weight. Soil and biosolid IAA were assayed using a similar procedure, except that the sample (2 g) was extracted in 10 mL sodium phosphate buffer (0.05 M, pH 7.0) containing 0.02% sodium diethyldithiocarbamate as an antioxidant overnight and centrifuged at 10,000 g for 10 min.

Root length, root length density, root surface area, and biomass. Root samples were collected according to Baldwin and Liu (2008) with some modifications. Root samples were collected at the end of the trial using a cylinder root sampler (4-cm diameter, 15-cm depth). Two soil cores were taken per pot and stored in commercial freezers (–20 °C) to stop microbial activities before root washing. Roots were washed free of soil using a 1-mm sieve. Once all soil was completely removed using tap water, roots were clipped from the shoot tissue base. Before quantifying root biomass, root growth traits (root length, root surface area) were analyzed using WinRHIZO Pro (Regent Instruments Inc., Quebec, Canada). WinRHIZO provides a computerized method of measuring root length density and total root length (cm) per volume of soil (cm^3) as described by Tennant (1975). Root mass in each pot was determined at the end of the experiment. The roots were removed from the pots and washed free of soil and dried at 65 °C for 48 h and weighed.

Root viability. Fresh root tissue (0.4 g) was collected for root viability measurement at the end of experiment. The root viability was determined using a modified 2,3,5-triphenyltetrazolium chloride reduction technique (Knievel, 1973). Roots were incubated in the dark for 24 h in 0.6% 2,3,5-triphenyltetrazolium chloride at 37 °C, rinsed with deionized water, and ground in a mortar and pestle with ethyl acetate. The absorbance of the incubation solution was measured at 490 nm with a spectrophotometer (ultraviolet-2802S; UNICO). Five independent samples were determined for each treatment. Root activity was expressed as the reduction intensity of red tetrazoline.

Experimental design and statistical analysis. The experimental design was similar to the previous study by Zhang et al. (2009). Two experiments were conducted at the same time, one in which no soil moisture stress was imposed (well-watered regime, 90% container capacity, Expt. 1) and one in which soil moisture was limiting and one wilt and recovery cycle was imposed (moisture stress regime, ≈25% container capacity, Expt. 2). Each experiment was arranged on the greenhouse bench as a randomized complete block design with four replications. The data from each experiment were subjected to

Table 1. Biosolids impact on turfgrass quality, leaf relative water content, and leaf wilting of kentucky bluegrass subjected to well-watered (Expt. 1) and drought stress (Expt. 2) regimes.

		19 June	4 July	10 July	16 July	23 July
		Turfgrass quality				
Moisture	Treatment	1–9; 9 = ideal green; healthy turfgrass				
Water+[z]	Biosolids at 1× N rate	8.26 aB[y]	8.42 aA	8.4 aAB	8.5 aA	8.48 aA
	Biosolids at 0.5× N rate	8.18 aB	8.22 aB	8.5 aA	8.62 aA	8.44 aA
Control		8.06 aA	7.82 bAB	8.1 bA	7.5 bC	7.6 bBC
Water–	Biosolids at 1× N rate	8.22 aA	7.82 aB	6.7 aD	5.54 bE	7.46 aC
	Biosolids at 0.5× N rate	8.22 aA	7.7 aB	6.68 aC	5.84 aD	7.62 aB
	Control	7.84 bA	7.28 bB	6.44 aC	5.16 cD	7.08 bB
		Leaf relative water content				
Water+	Biosolids at 1× N rate	92.3 aA	90.11 aA	92.03 aA	91.08 aA	90.46 aA
	Biosolids at 0.5× N rate	91.66 aA	89.26 aA	91.98 aA	90.46 aA	90.04 aA
	Control	91.48 aA	88.5 aAB	87.61 bAB	86.85 bB	89.01 aAB
Water–	Biosolids at 1× N rate	92.1 aA	79.78 aB	65.81 aC	46.26 aD	88.68 aA
	Biosolids at 0.5× N rate	92.06 aA	78.55 abC	61.48 bD	44.44 aE	86.85 aB
Control		91.47 aA	75.65 bC	58.6 bD	39.35 bE	86.54 aB
		Leaf Wilting				
		0–100%; 100% = complete, permanent wilting				
Water+	Biosolids at 1× N rate	4.2 aA	2.8 aB	3 bB	3 bB	4.2 bA
	Biosolids at 0.5× N rate	4.2 aA	2.6 aB	2 cBC	2.4 cBC	1.4 cC
	Control	5.4 aB	3.6 aC	4 aBC	5 aBC	7.2 aA
Water–	Biosolids at 1× N rate	4 bD	11.8 aBC	16.6 bB	40 bA	10.6 bC
	Biosolids at 0.5× N rate	4.6 bC	8.4 bBC	17 bB	39bA	10.2 bBC
	Control	6.4 aD	7.2 bD	23 aB	48 aA	15.8 aC

[z]Water+ = well-watered regime (maintained at 90% container capacity throughout the course of the experiment). Water– = drought and rewater regime (soil moisture was gradually decreased from 90% to ≈25% container capacity from 19 June to 16 July and then 90% container capacity from 17 to 23 July.
[y]Means with same lowercase letters within column at each moisture regime (water+ or water–) or uppercase letters within each row of each data set are not significantly different at $P \leq 0.05$.
N = nitrogen.

Table 2. Biosolids impact on proline, amino content and nitrate reductase activity of kentucky bluegrass subjected to well-watered (Expt. 1) and drought stress (Expt. 2) regimes.

		19 June	4 July	10 July	16 July	23 July
		Leaf proline content				
Moisture	Treatment	$\mu g \cdot g^{-1}$ FW				
Water+[z]	Biosolids at 1× N rate	320 aA[y]	385 aA	421 aA	299 abA	288 aA
	Biosolids at 0.5× N rate	373 aB	474 aA	470 aA	375 aB	200 aC
	Control	361 aAB	430 aA	431 aA	256 bAB	227 aB
Water–	Biosolids at 1× N rate	413 aC	764 aB	1123 aA	1312 aA	342 aC
	Biosolids at 0.5× N rate	378 aD	746 aC	1105 aB	1324 aA	315 aD
	Control	352 aC	727 aB	979 aA	1066 bA	273 aC
		Leaf amino acid content				
		$mg \cdot g^{-1}$ FW				
Water+	Biosolids at 1× N rate	2.20 aA	2.53 bA	1.90 aA	2.18 aA	2.18 aA
	Biosolids at 0.5× N rate	2.71 aAB	3.29 aA	2.39 aB	2.04 abB	1.93 aB
	Control	2.35 aA	2.35 bA	1.82 aAB	1.25 bBC	1.00 bC
Water–	Biosolids at 1× N rate	2.50 aC	3.14 aBC	3.89 aA	3.72 abAB	2.57 aC
	Biosolids at 0.5× N rate	2.49 aC	3.20 aB	3.69 aAB	4.14 aA	2.44 aC
	Control	2.46 aB	2.57 aB	2.67 bB	3.44 bA	2.66 aB
		Nitrate Reductase Activity				
		$\mu molNO_2/g/FW/h$				
Water+	Biosolids at 1× N rate	1.24 aA	0.93 aA	0.92 aA	1.02 aA	1.05 aA
	Biosolids at 0.5× N rate	1.02 bA	1.04 aA	0.97 aA	1.29 aA	1.01 aA
	Control	0.94 bA	0.86 aA	0.75 aA	0.94 aA	0.77 aA
Water–	Biosolids at 1× N rate	1.19 aA	0.73 aB	0.46 aBC	0.22 bC	0.77 abB
	Biosolids at 0.5× N rate	1.09 aA	0.66 aB	0.50 aBC	0.31 aC	0.98 aA
	Control	1.07 aA	0.64 aB	0.35 bBC	0.13 cC	0.57 bB

[z]Water+ = well-watered regime (maintained at 90% container capacity throughout the course of the experiment). Water– = drought and rewater regime (soil moisture was gradually decreased from 90% to ≈25% container capacity from 19 June to 16 July and then 90% container capacity from 17 to 23 July.
[y]Means with same lowercase letters within column at each moisture regime (water+ or water–) or uppercase letters within each row of each data set are not significantly different at $P \leq 0.05$.
FW = fresh weight; N = nitrogen.

a repeated-measures general linear model using a multivariate approach. In this model, between-subject variable (biosolid treatments) main effect, within-subject variable (sampling dates) main effect, and the interaction were analyzed. In addition, one-way analysis of variance was used for analysis of sampling date effect. Mean separations were performed using a protected Fisher's least significant difference at a 5% probability level (Zhang et al., 2009).

Results

Turfgrass quality, leaf RWC, and leaf wilting. Drought stress reduced turfgrass quality (Table 1). After rewatering, turfgrass recovered gradually to the acceptable level (rating 6 or greater). The biosolids at 1× N rate and biosolids at 0.5× N rate improved turfgrass quality when compared with the control under both moisture regimes. However, biosolids at 0.5× N rate increased turfgrass quality ratings by 5% and 13%, respectively, when compared with biosolids at 1× N rate and the control at the end of drought stress (16 July).

The leaf relative water content (RWC) declined during drought stress and recovered after rewatering (Table 1). Biosolids application at 1× N rate improved RWC compared with the control as measured during drought stress (4, 10, and 16 July). Biosolids application at 1× N rate and 0.5× N rate also reduced leaf wilting relative to the control under drought stress (10 and 16 July), although the reduction was not large compared with the overall drought stress effect.

Leaf proline and amino acid content, nitrate reductase activity. Leaf proline accumulated in response to drought stress and declined after rewatering (Table 2). Biosolids application increased proline content relative to the control at the time of greatest drought stress (16 July).

Drought stress induced accumulation of amino acids in leaf tissues (Table 2). The biosolids at 0.5× N rate increased amino acid content during drought stress (10 and 16 July). Biosolids treatments also had increased leaf amino acid content under well-watered conditions.

Leaf nitrate reductase activity gradually declined during drought stress and recovered after rewatering in the three treatments (Table 2). Biosolids application alleviated declines in leaf nitrate reductase activity under moisture stress (10 and 17 July) and the recovery period (23 July). Leaf nitrate reductase activity of the grass treated with biosolids at 0.5× N rate was 41% and 138% higher than that of biosolids at 1× N rate and control, respectively, as measured at the time of greatest drought stress (16 July). Biosolids at 0.5× N rate increased root nitrate reductase activity by 51% under drought stress and by 42% under the well-watered condition when compared with the control at the end of the experiment (Fig. 1).

Leaf IAA and ABA content. Drought stress reduced leaf IAA content at the end of the drought stress (16 July; Table 3). Biosolids at 1× N rate increased leaf IAA content by 107% relative to the control under drought stress as measured on 16 July. Biosolids at 1× N rate and 0.5× N rate also increased leaf IAA content under well-watered conditions at two sampling dates (10 and 16 July).

Drought stress increased leaf ABA content (Table 3). The grass amended with biosolids had lower ABA content relative to control at the end of drought stress.

Root and soil IAA content. There was no difference in root IAA content between treatments under drought stress (Fig. 2). Under well-watered conditions, the biosolids at 1× N rate and 0.5× N rate increased root IAA content by 96% and 83%, respectively, when compared with the control.

No difference was found in soil IAA content between the treatments at the beginning of soil moisture treatment (19 June). Biosolids application at 1× N rate had greater soil IAA content relative to the control regardless of soil moisture treatment at the end of experiment (Table 4).

Root characteristics, biomass, and viability. Drought stress reduced root length density, root surface area, and root biomass (Figs. 3 to 5). Biosolid applications at 1× N rate improved root length density and surface area when compared with the control under both soil moisture regimes and also increased

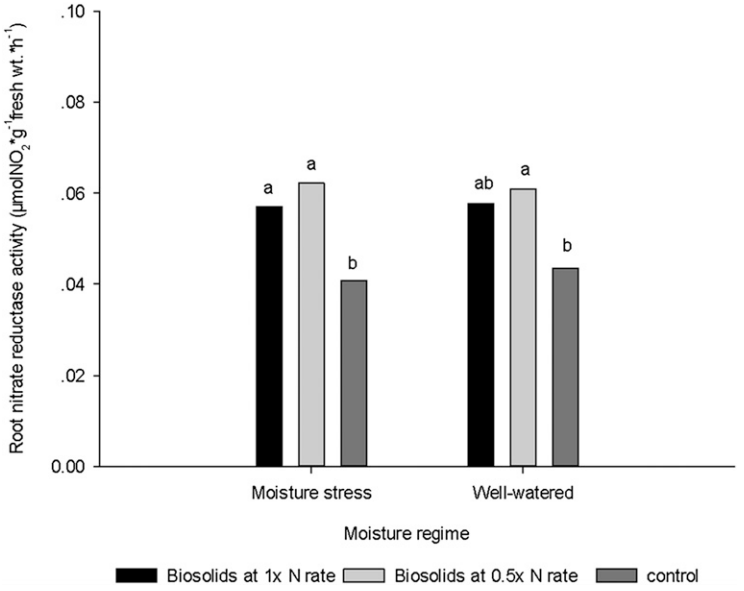

Fig. 1. Root nitrate reductase activity responses to biosolids application in kentucky bluegrass subjected to well-watered (Expt. 1) and moisture stress (Expt. 2) regimes. Root samples were collected at the end of the experiment. Bars marked with the same letter for each sampling dates are not significantly different at $P \leq 0.05$.

Table 3. Biosolids impact on leaf indole-3-acetic acid (IAA) and abscisic acid (ABA) contents of kentucky bluegrass subjected to well-watered (Expt. 1) and drought stress (Expt. 2) regimes.

		19 June	4 July	10 July	16 July	23 July
		Leaf IAA				
Moisture	Treatment	ng·g^{-1} dry wt				
Water+[z]	Biosolids at 1× N rate	586 bB[y]	569 aB	878 abA	649 aB	239 cC
	Biosolids at 0.5× N rate	805 aB	833 aB	1111 aA	724 aB	536 aC
	Control	573 bB	597 aB	844 bA	275 bC	389 bBC
Water–	Biosolids at 1× N rate	807 abB	1162 aA	1053 abAB	511 aC	282 aC
	Biosolids at 0.5× N rate	1011 aA	1134 aA	1159 aA	287 abB	115 bB
	Control	554 bB	790 bA	808 bA	247 bC	193 abC
		Leaf ABA				
		ng·g^{-1}dry wt				
Water+	Biosolids at 1× N rate	288 aB	138 bC	584 bA	129 aC	74 aC
	Biosolids at 0.5× N rate	448 aB	405 aB	779 aA	156 aC	28 aC
	Control	309 aB	239 bBC	754 aA	196 aC	43 aD
Water–	Biosolids at 1× N rate	504 aC	644 aC	852 aB	1051 bA	76 bD
	Biosolids at 0.5× N rate	344 aD	572 aC	797 aB	1120 bA	63 bE
	Control	341 aC	689 aB	741 aB	1309 aA	128 aD

[z]Water+ = well-watered regime (maintained at 90% container capacity throughout the course of the experiment). Water– = drought and rewater regime (soil moisture was gradually decreased from 90% to ≈25% container capacity from 19 June to 16 July and then 90% container capacity from 17 to 23 July.
[y]Means with same lowercase letters within each column at each moisture regime (water+ or water–) or uppercase letters within each row of each data set are not significantly different at $P \leq 0.05$.
N = nitrogen.

root biomass under well-watered conditions. Under moisture stress, biosolids at 1× N rate increased root length density and surface area by 23% and 31%, respectively, when compared with the control. There was no significant difference in root viability between the treatments under both soil moisture regimes (data were not shown).

Discussion

The results of this study indicated that biosolids application alleviated turfgrass quality decline and leaf wilting and increased leaf proline and amino acid content and nitrate reductase activity, particularly under drought stress. This is in agreement with previous studies (Garling and Boehm, 2001; Zhang et al., 2005, 2007, 2009, 2012). Because each treatment received similar N from biosolids and/or chemical fertilizer, the beneficial effects of biosolids likely resulted from factors other than mineral nutrients. Biosolids contain several groups of biologically active substances including humic substances and IAA (Zhang et al., 2005). It was also reported that humic acids contain IAA and cytokinins (Zhang et al., 2005; Zhang and Ervin, 2004). The IAA contents of the humic acid fractions of variously processed biosolids range from 0.5 to 2.4 $\mu g \cdot g^{-1}$ (Zhang et al., 2005). Previous studies showed that exogenous cytokinin increased nitrate reductase activity in creeping bentgrass (Wang et al., 2012) and rice plants (Hemalatha, 2002). Lower molecular-weight humic acids were found to increase nitrate uptake (Qaussoin et al., 2013). It was also reported that microbial activity in root zones is positively related to nitrate reductase activity in lettuce plants subjected to drought stress (Ruíz-Lozano and Azcón, 1996). It is likely that the plants with a higher nitrate reductase activity may assimilate nitrate and synthesize amino acids more efficiency than those with a lower nitrate reductase activity. Accumulation of amino acids, particularly proline, in leaf tissues may increase RWC, reduce leaf wilting, and improve tolerance to drought stress. This suggests that biosolids application may increase nitrate reductase activity and N metabolism by providing humic acids and hormones (IAA and cytokinins) and promoting microbial activity in the root zones, particularly under drought stress.

The results of this study indicated that biosolids increased leaf IAA content under both drought stress and well-watered conditions. In addition, leaf ABA level was lower in the grass treated with biosolids relative to the control under drought stress. This is in agreement with the previous study by Zhang et al. (2009) who reported that biosolids and auxin (indole-3-butyric acid) application increased leaf IAA content in tall fescue subjected to drought stress. IAA can increase root growth and delay leaf senescence, especially under drought stress (Zhang et al., 2009, 2012). Man et al. (2011) found that a drought-tolerant tall fescue (*Festuca arundinacea* Schreb) cultivar contained higher

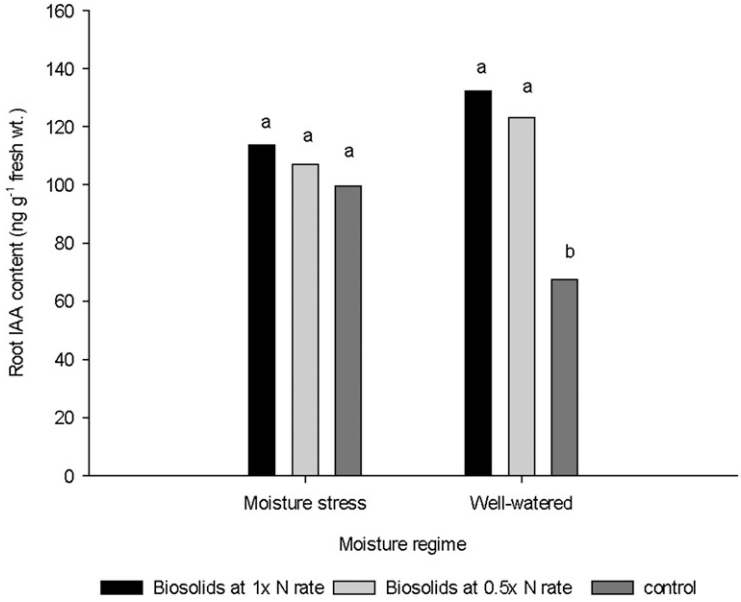

Fig. 2. Root indole-3-acetic acid (IAA) content response to biosolids application in kentucky bluegrass subjected to well-watered (Expt. 1) and moisture stress (Expt. 2) regimes. Root samples were collected at the end of the experiment. Bars marked with the same letter for each sampling dates are not significantly different at $P \leq 0.05$.

Table 4. Soil indole-3-acetic acid (IAA) content of kentucky bluegrass subjected to well-watered (Expt. 1) and drought stress (Expt. 2) regimes.

		19 June	23 July
		Soil IAA	
Moisture	Treatment	---------- $ng \cdot g^{-1}$ FW ----------	
Water+[z]	Biosolids at 1× N rate	15.69 a[y]	6.85 a
	Biosolids at 0.5× N rate	10.88 a	5.12 b
	Control	8.73 a	4.65 b
Water–	Biosolids at 1× N rate	10.06 a	6.29 a
	Biosolids at 0.5× N rate	11.24 a	5.11 ab
	Control	7.13 a	4.33 b

[z]Water+ = well-watered regime (maintained at 90% container capacity throughout the course of the experiment). Water– = drought and rewater regime (soil moisture was gradually decreased from 90% to ≈25% container capacity from 19 June to 16 July and then 90% container capacity from 17 to 23 July.
[y]Means with same letters within each column at each moisture regime (water+ or water–) are not significantly different at $P \leq 0.05$.
FW = fresh weight; N = nitrogen.

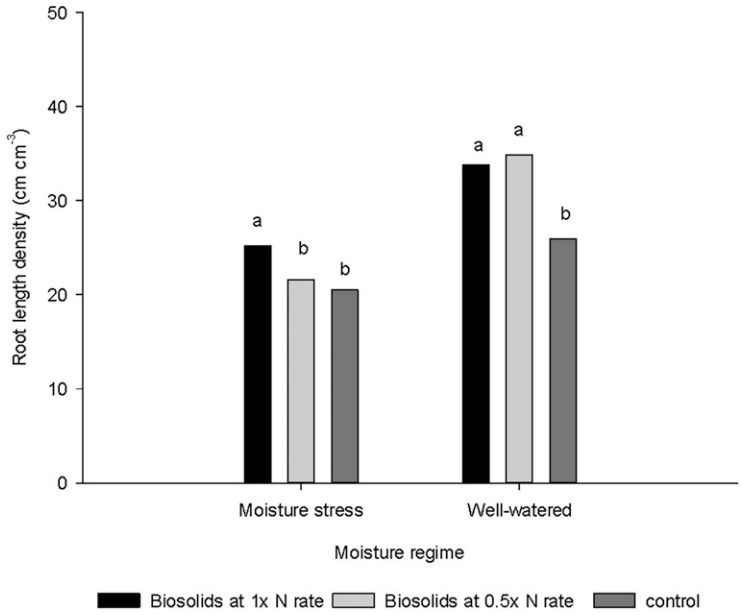

Fig. 3. Root length density response to biosolids application in kentucky bluegrass subjected to well-watered (Expt. 1) and moisture stress (Expt. 2) regimes. Root samples were collected at the end of the experiment. Bars marked with the same letter for each sampling dates are not significantly different at $P \leq 0.05$.

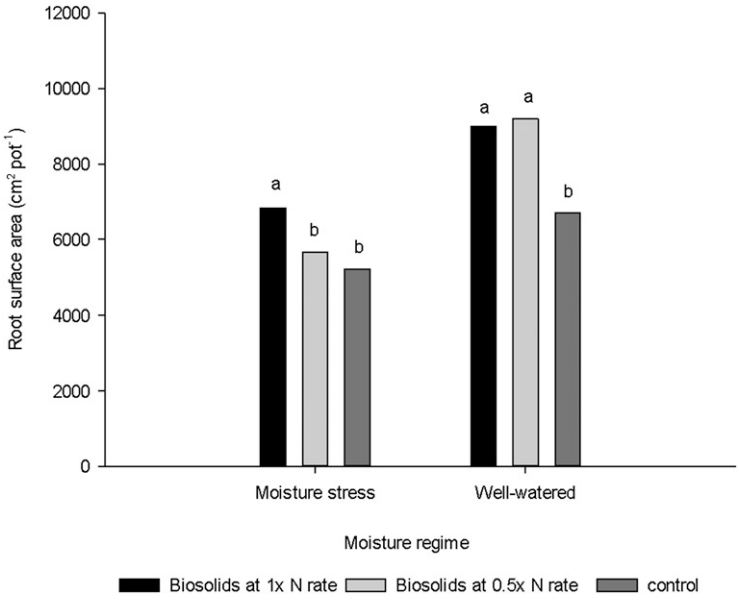

Fig. 4. Root surface area response to biosolids application in kentucky bluegrass subjected to well-watered (Expt. 1) and moisture stress (Expt. 2) regimes. Root samples were collected at the end of the experiment. Bars marked with the same letter for each sampling dates are not significantly different at $P \leq 0.05$.

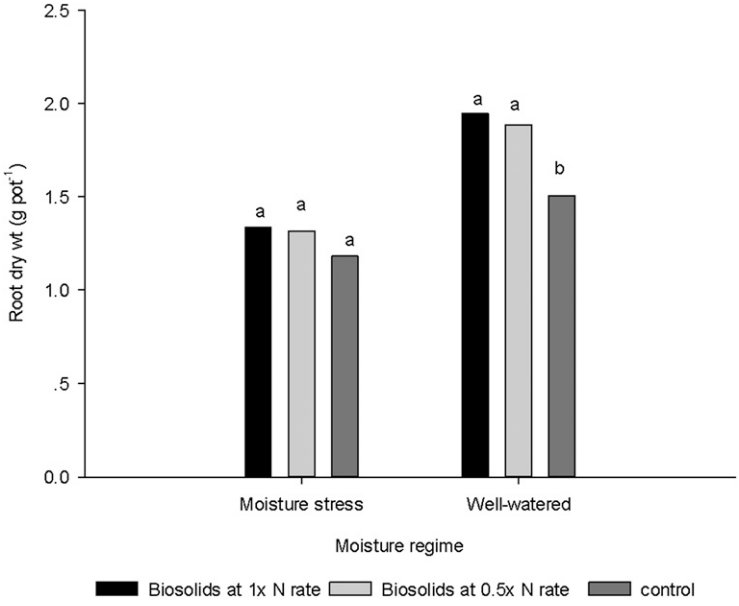

Fig. 5. Root mass response to biosolids application in kentucky bluegrass subjected to well-watered (Expt. 1) and moisture stress (Expt. 2) regimes. Root samples were collected at the end of the experiment. Bars marked with the same letter for each sampling dates are not significantly different at $P \leq 0.05$.

levels of IAA than a drought-sensitive cultivar under drought stress. In the present study, drought stress reduced leaf and root IAA. Application of biosolids alleviated IAA decline in leaf tissues. Man et al. (2011) pointed out that the grass with higher IAA may have a greater root system and water use efficiency under drought stress. Biosolids may enhance root growth by providing IAA directly to the growth media and/or by stimulating microbial production of IAA by supplying substrates. The results of the study suggest biosolids application may improve plant drought tolerance by enhancing hormones and N metabolism.

The results of this study showed that biosolids at 1× N rate increased soil IAA, root length density, and root surface area under both drought stress and well-watered conditions. The biosolids treatment also increased root biomass under well-watered conditions. This is consistent with the results in tall fescue by Zhang et al. (2007, 2009, 2012). The greater root length density and root surface area in the grass treated with biosolids may result from biologically active substances in biosolids, which provided hormones directly or stimulated the activity of microbes that supply substrates and hormones (Zhang et al., 2009). Biosolids may enhance root growth and function allowing the plants to use water and nutrients more efficiently under drought stress conditions. It has been documented that soil organic matter may affect soil physical properties and moisture retention (Qaussoin et al., 2013). In this study, however, biosolids application rate (1.97 g·kg^{-1} soil) was low. Therefore, the biosolids treatments may not significantly affect soil physical property. Biosolids application may improve turfgrass drought tolerance by promoting N metabolism, increasing hormones (particularly IAA) and root growth.

In summary, the results of this study indicated that application of biosolids to the soil improved turf quality, delayed leaf wilting, and increased proline and total amino acids and IAA content, especially under drought stress conditions. The biosolids treatments also increased root length density and root surface area under both well-watered and drought stress conditions. The results of this study suggested that biosolids may improve plant drought tolerance by promoting N and hormone metabolism and root growth. Proper application of biosolids may be a practical approach to improve plant fitness under drought stress environments. This growth chamber study used calcined clay as the growth media. The microbial activity in the calcined clay growth media may be much lower than in the native soil conditions. Future research is needed to investigate the mechanisms of biosolids' impact on crop plant drought tolerance in field conditions.

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