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Research Article

Establishment of Native Grasses with Biosolids on Abandoned Croplands in Chihuahua, Mexico

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The objective of the work was to evaluate establishment and forage production of native grasses with application of biosolids, a byproduct of waste-water treatment, at an abandoned field, in Ejido Nuevo Delicias, Chihuahua, Mexico. Four biosolids rates from 0 (control) to 30 dry Mg ha^{-1} and two methods of application, surface applied (BioSur) and soil incorporated (BioInc), were evaluated. Seedbed preparation included plowing and harrowing before rainfall. Field plots of 5 × 5 m were manually sown with a mix of blue grama (*Bouteloua gracilis*) (50%) and green sprangletop (*Leptochloa dubia*) (50%) in early August 2005. Experimental design was a randomized block with a split plot arrangement. Grass density, height, and forage production were estimated for three years. Data were analyzed with mixed linear models and repeated measures. Green sprangletop density increased under all biosolids rates regardless of method of application, while blue grama density slightly decreased. Biosolids were more beneficial for green sprangletop height than for blue grama height. Blue grama forage production slightly increased, while green sprangletop forage production increased the most at 10 Mg ha^{-1} biosolids rate under BioSur method. It was concluded that BioSur application at 10 and 20 Mg ha^{-1} rates had positive effects on the establishment and forage production of native grasses, especially green sprangletop.

1. Introduction

Native grasslands in Mexico comprehend about 9.9 million ha within central and north regions of the country [1]. Approximately 6.5 million ha shows different degradation types, including soil, wind, and water erosion and chemical and physical degradation [2]. Chihuahua grasslands, at northern Mexico, comprise about 4.5 million ha, supplying forage to livestock, habitat for wildlife, as well as ecosystem services [3, 4], and recreational activities. A study performed in 2002 [5] found that most of Chihuahua grasslands were affected by overgrazing, that caused grassland degradation. Also, recent studies showed that most of Chihuahua grasslands were in moderate-extreme to extreme rangeland health [6] and severely invaded by native shrubs and exotic grasses [7].

There are several techniques to rehabilitate degraded rangelands, including grazing management, brush management, prescribed fire, and range seeding. Each technique is recommended according to the severity of the grassland degradation and climate conditions. Range seeding is an alternative for rehabilitation of severely degraded rangelands and abandoned croplands. Some authors [8, 9] recommended native grasses such as sideoats grama (*Bouteloua curtipendula* (Michx.) Torr.), green sprangletop (*Leptochloa dubia* (Kunth) Nees), and Arizona cottontop (*Digitaria californica* (Benth.) Henr.) for restoration of degraded grasslands in Mexico. Blue grama (*Bouteloua gracilis* (Willd. ex Kunth.) Lag. ex Griffiths) is a native grass that needs high soil humidity and moderate temperatures for germination, emergence, and establishment [10, 11]. Therefore, techniques that increase soil water may

have a beneficial effect on blue grama seedling establishment. Other successful strategies such as the use of biofertilizers on blue grama and sideoats grama [12, 13] and straw amendments under greenhouse conditions [14] have also been used to increase grass establishment. Green sprangletop is a grass species showing adequate germination rates and good establishment under dry conditions in desert grasslands and is recommended for rangeland revegetation [15, 16]. Use of grass mixes for rangeland seeding is recommended to increase the success of seeding rangelands [17]. Blue grama and green sprangletop are two of the most important grasses in the semiarid region of Chihuahua and are decreasing because of overgrazing and drought [18, 19].

Application of biosolids, produced from wastewater treatment plants, may be a sustainable approach for promoting grass establishment as shown by several studies and biosolids disposal practice. In fact, surface application of biosolids can increase soil water infiltration [20, 21] and supply nutrients for plant growth [22, 23] in grasslands of arid and semiarid regions. However, research on the effects of biosolids on grass emergence under controlled conditions has shown variable results. A study conducted in Texas (USA) found no effects of surface-applied biosolids on blue grama or green sprangletop emergence under greenhouse conditions [24], whereas biosolids have increased seedling biomass of blue grama with surface-applied and soil-incorporated biosolids [25]. In a greenhouse study, beneficial effects of biosolids applied both at the surface or incorporated into soils on sideoats grama seedling growth were reported [26].

However, little research has been done on the use of biosolids for seeding native grasses under field conditions in arid or semiarid conditions. The only one field study found an increase in grass emergence with biosolids applied at the surface with moderate irrigation in desert grassland [24]. No research has been done either on the effects of biosolids on native grasses emergence and growth in abandoned croplands. Therefore, this study was designed to evaluate the effect of biosolids on grass density and forage production on an abandoned cropland in Chihuahua, Mexico.

2. Materials and Methods

The study was conducted in an abandoned field for 3 years at the Ejido Nuevo Delicias, Chihuahua, 75 km north of Chihuahua, Mexico. The study site is located within the Central Valleys region of Chihuahua, with a dry temperate climate, mean annual precipitation of 350 mm, mean annual temperature of 16°C, and an altitude of 1,640 m [27]. Soils have alluvial-colluvial origin with a sandy-loam texture. Original vegetation before crop cultivation was a shortgrass prairie with *Bouteloua-Aristida* grasses [28].

Thirty-two field plots of 5 × 5 m, with 10 m distance from each other, were established in June 2005 and marked with metal bars. Seedbed preparation included disk plowing and harrowing in June before rainy season (July to September). At the beginning of the rainy season, another disk harrowing was done to eliminate weeds and prepare seedbed. Seeding was broadcasted manually with a mix of blue grama var. Hachita and green sprangletop on August 3, 2005, according to recommended seeding rates [17]. Blue grama was 64.3% pure live seed and 66% germination rate, and seeding rate at 50% was 1.5 kg ha^{-1}, with 8 gr plot^{-1}; Green sprangletop was 95% pure live seed, and 60% germination rate, and seeding rate at 50% was 1 kg ha^{-1}, with 4 gr plot^{-1}. After seeding, seeds were raked down to approximately 1 cm soil depth.

Table 1: Soil chemical properties before biosolids application and biosolids composition from the North Wastewater Treatment Plant at Chihuahua city, Mexico.

Constituent[a]	Soil	Biosolids	Element	Soil	Biosolids
pH	6.5	6.7	Ca (mg kg^{-1})	850	3,412
OM (%)	0.55	12	Mg (mg kg^{-1})	125	1,037
TCC (%)	Free	Free	Na (mg kg^{-1})	237	1,312
EC (dS m^{-1})	1.4	3	Cu (mg kg^{-1})	1	9
N-NO_3^- (mg kg^{-1})	60	66	Fe (mg kg^{-1})	4	30
P (mg kg^{-1})	8	7	Mn (mg kg^{-1})	14	15
K (mg kg^{-1})	362	962	Zn (mg kg^{-1})	3	111

[a]OM: organic matter; TCC: total calcium carbonate; EC: electrical conductivity; N-NO_3 and the rest of the elements are extractable and expressed on a dry weight basis.

Anaerobic-stabilized biosolids from the North Wastewater Treatment Plant at Chihuahua city, receiving domestic sewage and treated with aerobic process, were applied to field plots, according to water content of biosolids (determined gravimetrically), plot size, and biosolids rate. Six biosolids samples of about 1 kg each were taken from the biosolids pile (approximately three tons) and a composite sample with about 200 gr of each 1 kg sample was taken and used for the following chemical analysis (Table 1): pH value (in $CaCl_2$), organic matter (OM) content by the $K_2Cr_2O_7$ wet oxidation method (Walkley and Black) [29], electrical conductivity (EC) by the saturated paste method, extractable nitrate-nitrogen (NO_3-N) [30], and plant available P (Bray-1 fraction) [31]. Concentrations of plant available K, Ca, Mg, and Na were determined by extractions with ammonium acetate, whereas the plant available Cu, Mn, Fe, and Zn were determined by extractions with DTPA [32]. The elemental analysis was carried out by Atomic Absorption Spectroscopy.

Biosolids were applied at 0 (control), 10, 20, or 30 ton DM ha^{-1} rates using two application methods, either surface-applied (BioSur) or soil incorporated (BioInc), at the same date of seeding. For the BioSur method, seeding was done first and then biosolids were surface-applied and distributed within the plot through raking, while for the BioInc treatments, biosolids were surface-applied, distributed within the plot through raking, and incorporated into the soil through harrowing at approximately 20 cm soil depth before seeding. Study area was protected from grazing through fencing with barbed wire and poultry netting. Precipitation was measured during the growing seasons (Figure 1) and was low (84 mm) in the seeding year (2005) and during 2007 (180 mm) and above normal during 2006 (450 mm) and 2008 (285 mm).

Soil sampling at 0–15 cm soil depth was done with soil auger before biosolids application. Biosolids were removed from the soil surface (in biosolids-applied plots), and approximately 250 gr soil samples were taken in each plot, placed

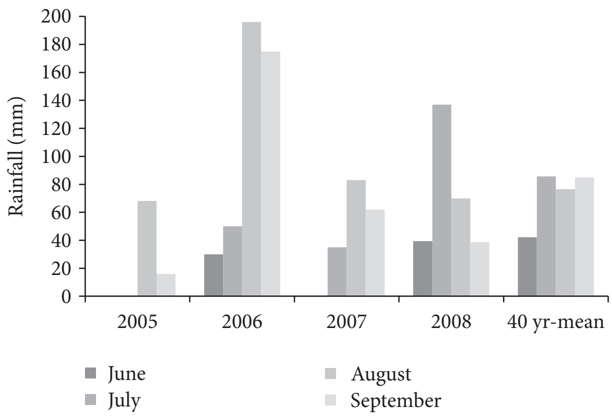

Figure 1: Monthly precipitation (June to September) at the study site from 2005 to 2008. Ejido Nuevo Delicias, Chihuahua.

in plastic bags in a portable cooler, and dried at ambient conditions for 48 hr. Then, soil samples were stored in a refrigerator for a week until chemical analysis. Results of the analysis are shown in Table 1. Soil sampling was repeated in early September, approximately one month after the biosolids application, for the measurement of electrical conductivity, organic matter content, and NO_3-N, P, and K availability with the same above mentioned methods.

Grass density was estimated by counting plants from both grasses within two 0.25 m^2 random quadrats $plot^{-1}$ at the end of each growing season (September) from 2006 to 2008. Grass height in four random plants $species^{-1}$ $plot^{-1}$ and aboveground grass forage production by clipping in a one m^2 random quadrat $plot^{-1}$ were estimated annually at the end of each growing season (end of September) during 2006 to 2008. Forage samples were oven-dried at 60°C for 48 hr, and forage production at kg ha^{-1} was estimated on a dry weight basis.

Experimental design was a randomized block with a split plot arrangement [33] with method of application as the main plot and biosolids rate as the subplot, with four replications. Linear models were used for statistical analysis on soil properties with Proc GLM [34]. Linear mixed models and year as repeated measures were used for grass height and density and forage production statistical analysis with Proc Mixed [35]. Several covariance structures were used to fit the best model including unstructured variance, compound symmetry, heterogeneous compound symmetry, and autoregressive. The best model for all variables was that with heterogeneous compound symmetry as a covariance structure, showing the smaller Akaike Information Criterion (AIC). A $P = 0.05$ probability was used to indicate significant effects. Mean separation was done using LSD test when significant effects were observed. Trend analyses were performed on some variables [34], and significant effects were declared at $P = 0.05$.

3. Results and Discussion

Soil organic matter and concentration of available P and K were not affected by biosolids rate or application method

Table 2: Chemical soil properties at 0–15 cm depth (mean ± SE) one month after biosolids application at an abandoned field in Chihuahua, Mexico.

Biosolids rate (Mg ha^{-1})	EC[a] (dS m^{-1})	NO_3-N (mg kg^{-1})
0	0.76 ± 0.2^{b}	6.8 ± 1.3^{b}
10	0.97 ± 0.1^{b}	11.0 ± 1.6^{ab}
20	1.01 ± 0.1^{b}	15.3 ± 3.3^{a}
30	1.65 ± 0.2^{a}	14.7 ± 2.8^{a}

[a]EC: electrical conductivity. Biosolids rate mean values within each soil property followed by different letters are significantly different ($P < 0.05$; $n = 8$).

Table 3: Grass density (plants m^{-2}; mean ± SE) under different biosolids rates and two methods of biosolids application at a seeded abandoned field in Chihuahua, Mexico.

Biosolids rate (Mg ha^{-1})	BioInc[a]	BioSur
0	5.6 ± 0.7^{bcA}	6.0 ± 0.7^{abA}
10	8.0 ± 0.7^{aA}	7.6 ± 0.7^{aA}
20	4.9 ± 0.7^{cB}	7.3 ± 0.7^{aA}
30	7.2 ± 0.7^{abA}	4.5 ± 0.7^{bB}

[a]BioInc: soil-incorporated method; BioSur: surface-applied method. Biosolids rate mean values followed by different lowercase letters within each method of application are significantly different ($P < 0.05$; $n = 24$). Method of application mean values followed by different uppercase letters within each biosolids rate are significantly different ($P < 0.05$; $n = 24$).

with an overall mean of 0.59 ± 0.02%, 51.3 ± 5.4 mg kg^{-1}, and 278 ± 8 mg kg^{-1}, respectively, whereas the soil electrical conductivity (EC) was significantly increased ($P = 0.0023$) by the highest biosolids amendment rates (Table 2). The soil nitrate-nitrogen was significantly increased ($P = 0.0239$) by the 20 and 30 Mg ha^{-1} biosolids amendment rates (Table 2).

Beneficial effects of biosolids application related to the increase in soil water infiltration and soil NO_3-N have been observed on arid grasslands [20–22]. Increase in water availability and plant available nitrogen were also observed with biosolids application on semiarid grasslands in central Mexico [23] and at greenhouse research with surface-applied and soil-incorporated biosolids [25, 26].

Grass density was influenced ($P = 0.0089$) by a rate × method interaction, where grass density increased at 10 Mg ha^{-1} rate regardless of method of application (Table 3). At 20 Mg ha^{-1} biosolids rate, grass density was higher with BioSur method, whereas at 30 Mg ha^{-1} rate the grass density was higher at the BioInc method. A significant rate × species interaction ($P = 0.0001$) was also found for grass density (Figure 2). In the control plots, green sprangletop density was low and was increased by all biosolids rates, whereas blue grama density was high in the control plots and decreased with biosolids application.

Effects of surface-applied biosolids on blue grama and green sprangletop emergence were evaluated under field conditions in west Texas rangelands [24], observing an increase in plant density with surface-applied biosolids and moderate irrigation in both grasses. Decreasing the blue grama density could be partially attributed to two factors: the possible

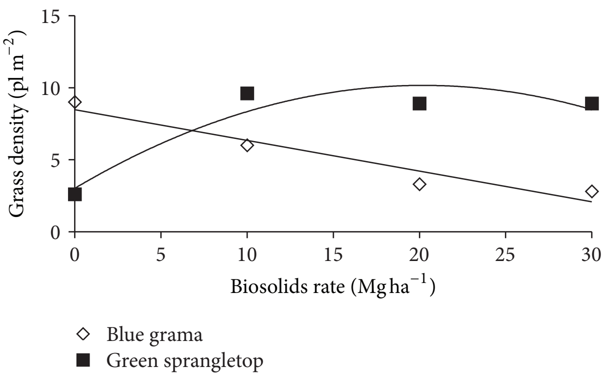

Figure 2: Grass density of blue grama (*Bouteloua gracilis*) and green sprangletop (*Leptochloa dubia*) under different biosolids rates during four years at a seeded abandoned field in Chihuahua, Mexico. $Y_{\text{Green-sprangletop}} = 3.02 + 0.707x - 0.017x^2$, $P = 0.001$, $r^2 = 0.89$, $n = 24$; $Y_{\text{Blue-grama}} = 8.47 - 0.213x$, $P = 0.0001$, $r^2 = 0.92$, $n = 24$.

Table 4: Grass species height (mean ± SE) of blue grama (*Bouteloua gracilis*) and green sprangletop (*Leptochloa dubia*) under different biosolids rates at a seeded abandoned field in Chihuahua, Mexico.

Biosolids rate (Mg ha^{-1})	Blue grama (cm)	Green sprangletop (cm)
0	27.2 ± 4.8^{cA}	19.6 ± 4.8^{bA}
10	71.6 ± 4.8^{aA}	84.4 ± 4.8^{aA}
20	52.2 ± 4.8^{bB}	81.1 ± 4.8^{aA}
30	39.1 ± 4.8^{bcB}	83.3 ± 4.8^{aA}

Biosolids rate mean values followed by different lowercase letters within each grass species are significantly different ($P < 0.05$; $n = 24$). Grass species mean values followed by different uppercase letters within each biosolids rate are significantly different ($P < 0.05$; $n = 24$).

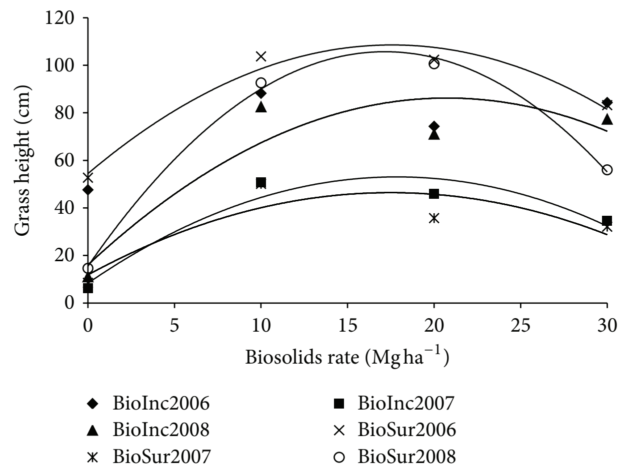

Figure 3: Grass height under different biosolids rates, methods of application (BioInc: soil-incorporated method; BioSur: surface-applied method), and dates (year 2006, 2007, or 2008) at a seeded abandoned field in Chihuahua, Mexico. $Y_{\text{BioInc2006}}$ = no linear ($P = 0.1320$) or quadratic effect ($P = 0.2348$), $n = 8$; $Y_{\text{BioInc2007}} = 8.33 + 5.00x - 0.14x^2$, $P = 0.0001$, $r^2 = 0.92$, $n = 8$; $Y_{\text{BioInc2008}} = 16.04 + 6.76x - 0.163x^2$, $P = 0.0139$, $r^2 = 0.84$, $n = 8$. $Y_{\text{BioSur2006}} = 54.43 + 6.15x - 0.17x^2$, $P = 0.96$, $r^2 = 0.96$, $n = 8$; $Y_{\text{BioSur2007}} = 11.84 + 3.95x - 0.11x^2$, $P = 0.0003$, $r^2 = 0.75$, $n = 8$; $Y_{\text{BioSur2008}} = 15.47 + 10.51x - 0.30x^2$, $P = 0.0001$, $r^2 = 0.93$, $n = 8$.

physical obstruction effect of biosolids that interfered with blue grama emergence and/or a plant competition between blue grama, which is a slow growth species [36], and green sprangletop, which is a rapid growth grass [37]. Greenhouse studies have also shown a decrease in blue grama [25] and sideoats grama [26] emergence under both biosolids surface applied and soil incorporated.

The observed significant ($P = 0.0001$) rate × species interaction showed that grass height increased with increasing biosolids rate for both grass species (Table 4). However, grass height was higher for green sprangletop than that of blue grama grass. A significant ($P = 0.0138$) three-way interaction (rate × method × sampling time) was also found for grass height (Figure 3). In the control plots, the grass height was low and increased with biosolids rate for both methods of application. However, BioSur showed a stronger effect on plant height compared to BioInc, especially at the intermediate biosolids rates, except in 2007 where BioSur and BioInc resulted in similar plant heights. Increases in grass height with surface application of biosolids have also been reported in tobosagrass (*Pleuraphis mutica* Buckl.), alkali sacaton (*Sporobolus airoides* (Torr.) Torr.), and blue grama [38–40] under arid and semiarid conditions, attributed to the improvement of plant growing conditions with biosolids application.

A significant ($P = 0.0031$) three way interaction (rate × method × species) was found for forage production (Figure 4). Blue grama forage production slightly increased at the 10 Mg ha^{-1} rate regardless of the application method, whereas it was similar between control and 20 and 30 Mg ha^{-1} rates. Differently, green sprangletop forage production was much higher than blue grama at all biosolids rates regardless of application method. Also, BioSur applications benefited green sprangletop forage production more than BioInc applications. Species × date interaction was also significant ($P = 0.0001$) in forage production (Table 5). Blue grama forage production was lower than that of green sprangletop and also varied among sampling times. However, correlation between forage production and precipitation was very low ($r = 0.05$), which is possibly attributed to the different morphology and slow growth rate of blue grama [36]. Green sprangletop forage production was high during 2006, a wet year, and decreased the following two years that were drier than 2006, showing a low correlation to precipitation ($r = 0.31$).

Forage production was highly benefited by biosolids application in some rate-method combinations, which is attributed to the improvement of plant growing conditions as mentioned earlier. However, these benefits were species specific. Green sprangletop is characterized as a rapid seed spread rate, high seedling vigor, and a higher moisture user [37]; therefore, this grass may have taken advantage of the improved soil conditions under biosolids application. Conversely, blue grama is a slow seed spread rate, low seedling vigor, and a low moisture user grass [36] which could have negatively

Table 5: Forage dry matter production (mean ± SE) of blue grama (*Bouteloua gracilis*) and green sprangletop (*Leptochloa dubia*) under different dates at a seeded abandoned field in Chihuahua, Mexico.

Date	Blue grama (kg ha^{-1})	Green sprangletop (kg ha^{-1})
Growing season 2006	240 ± 48^{abB}	2,297 ± 360^{aA}
Growing season 2007	166 ± 37^{bB}	1,117 ± 159^{bA}
Growing season 2008	394 ± 89^{aB}	1,211 ± 250^{bA}

Date mean values followed by different lowercase letters within each grass species are significantly different ($P < 0.05$; $n = 32$). Grass species mean values followed by different uppercase letters within each date are significantly different ($P < 0.05$; $n = 32$).

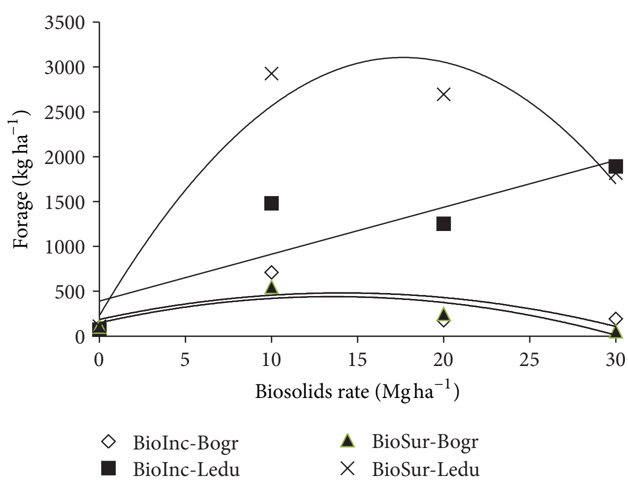

Figure 4: Forage production under different biosolids rates, methods of application, and grass species at a seeded abandoned field in Chihuahua, Mexico. BioInc: soil-incorporated biosolids; BioSur: surface-applied biosolids; Bogr: *Bouteloua gracilis*; Ledu: *Leptochloa dubia*; $Y_{\text{BioInc-Ledu}} = 389.4 + 52.29x$, $P = 0.0003$, $r^2 = 0.74$, $n = 12$; $Y_{\text{BioSur-Ledu}} = 229.95 + 325.99x - 9.23x^2$, $P = 0.0001$, $r^2 = 0.94$, $n = 12$; $Y_{\text{BioInc-Bogr}} = 185.65 + 41.86x - 1.48x^2$, $P = 0.0039$, $r^2 = 0.39$, $n = 12$; $Y_{\text{BioSur-Bogr}} = 147.77 + 43.12x - 1.59x^2$, $P = 0.0017$, $r^2 = 0.75$, $n = 12$.

affected blue grama growth when sown along with green sprangletop. Indications of plant competition such as reduced survival, height, and forage, production have been observed in blue grama when sown in western wheatgrass (*Agropyron smithii* Rydb.) sod [41]. Also, forage production of blue grama decreased when seeded in mixtures with western wheatgrass or green needlegrass (*Stipa viridula* Trin.) [42].

In general, BioSur method showed better results than those of BioInc method on both grass height and forage production, especially at the 10 and 20 Mg ha^{-1} rates. Research supports that surface-applied biosolids increase water infiltration in grassland sites [21]. Also, studies on biosolids application to grasslands have shown that soil volumetric water increases with BioSur compared to BioInc method at 10 and 20 Mg ha^{-1} rates [25]. Surface applications could have reduced soil water evaporation underneath biosolids aggregates, while BioInc applications apparently did not affect surface soil water since biosolids are mixed into the soil. Also, method of amendment application matters, since soil-incorporated wood chip amendments decreased grass growth compared to surface-applied amendment [43].

Although only small benefits were observed on blue grama height and forage production, biosolids application exerted an impressive beneficial effect on green sprangletop. This opens the possibility of using biosolids or other organic amendments to increase establishment and forage production of native grasses in degraded grasslands or abandoned fields in arid and semiarid regions. Biosolids generation will increase in the future along with human population and wastewater treatment, and their beneficial use on land application must be preferred as an option for biosolids disposal over incineration or land filling.

4. Conclusions

This field study provided evidence that biosolids application was beneficial for blue grama and green sprangletop establishment and forage production, especially at 10 Mg ha^{-1} rate. Surface-applied biosolids showed more beneficial effects on grass growth than soil-incorporated biosolids and may represent a strategy for the sustainable management of biosolids produced by wastewater treatment plants. However, these preliminary data need further confirmation by more field experiments involving more plant and soil types and longer sampling periods.

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