ASSESSMENT OF THE AGRONOMIC EFFECTS AND POTENTIAL CARBON SEQUESTRATION ASSOCIATED WITH BIOSOLID APPLICATIONS ON RANGELANDS IN SOLANO COUNTY

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Section 1: **EXECUTIVE SUMMARY**

Soil and plant tissue samples were collected and analyzed to assess the agronomic effects, potential carbon sequestration, and determine the fate or movement, if any, of trace metals in soil associated with biosolids applications on rangelands in Solano County. Forage quantity and quality data was used to create a crop agronomic growth model to estimate potential yield expected from the application of biosolids and to estimate weight gain of cows grazed on pastures receiving biosolids applications. Forage and soil data was used to estimate the potential for enhanced carbon sequestration from biosolids applications.

Forage and soil samples were collected and analyzed from areas that receive biosolids – "Biosolids Amended Soil" (BAS) – and compared to samples from areas where biosolids are not applied – "Non-Biosolids Amended Soil" (NBAS). Soil samples were collected from BAS and NBAS sites after biosolids were applied in 2015 to establish baseline concentrations in the soil profile of plant essential nutrients, trace metals, and salts prior to significant winter rains and the forage growing season ("pre-forage"). "Post-forage" soil sampling was conducted after the growing season, and after water movement through the soil profile may have moved analytes and plants may have incorporated nutrients into tissue.

The scope of this study was to assess differences in metals and nutrient concentrations in soil and forage, as well as physical soil characteristics including soil texture, moisture retention, bulk density, and salinity between BAS areas and NBAS areas. Previous studies have evaluated pesticides, pharmaceuticals, personal care products, or other constituents of concern. This study was conducted over the course of 8 months, on two adjacent rangeland fields in Solano County. Eight sampling sites were selected; four were located within BAS areas and four were located within NBAS areas. Results of this study are representative of conditions occurring in these fields, at the particular sites investigated during the study period.

Soil sampling indicates that fields amended with biosolids have more nitrate-nitrogen (NO₃) in the upper soil profile (0-1 feet). BAS sites have a higher percentage of organic matter in the same layer than NBAS sites. The concentration of metals and salts (as measured by EC) at BAS and NBAS sites was not significantly different, and no movement of these constituents through the soil profile was noted.

During the growing season, exclosures were constructed at sampling sites in each field to allow forage plants to grow without grazing pressure. Plant tissue was sampled three times during the growing season to assess yield and forage quality differences between fields receiving biosolids application and those not receiving biosolids application. Average forage yield at BAS sites was approximately 55% higher than NBAS sites on a dry matter basis. Analysis of selenium (Se), copper (Cu), and other compounds that could adversely affect grazing animal health were below levels of concern, in regard to animal health. Forage nutrition and quality was not significantly different between BAS and NBAS sites; as such, forage productivity (yield) is the primary difference between the two treatments.

The benefit of increased forage yields from applying biosolids was estimated using an agronomic growth model. The model inputs include pre-growing season soil nitrate-nitrogen, estimated tissue nitrogen, and amount of nitrate-nitrogen to be applied to give an estimate of the potential yields if biosolids are applied.

The benefit of increased yields on biosolids amended fields to grazing animals and ranchers is higher grazing density or number of animals per acre of pasture. Cow/calf pairs and sheep grazed on areas that receive biosolids are able to meet energy demands of lactation, pregnancy, reserves and body condition maintenance. A daily weight gain of 1.6 pounds was estimated for a mature cow given forage quality and quantities available.

Carbon sequestration estimates were made using data collected from soil and forage sampling. Soil organic carbon and estimates of carbon additions through increases in root biomass were investigated. Only long term forms of soil carbon storage were assessed. Preliminary estimates indicate that organic carbon concentrations were generally greater in BAS. A sequestration rate of 0.656 tons C/acre per year is achieved on biosolids amended fields compared to 0.424 tons C/acre per year on non-biosolids amended fields. This initial data suggests that there is a potential environmental benefit from biosolids application. However, due to the preliminary nature of this data and the limited scope of the investigation, further investigation is warranted.

The application of biosolids allows for the recycling of material that would otherwise be landfilled while providing a benefit to rangeland application areas. Increased available plant nutrients in soil grows more forage, allows for improved animal yield per acre grazed, and adds carbon to soils in a form that suggests a potential for long-term carbon sequestration.

Section 2: PROJECT APPROACH

Biosolids are treated and processed domestic sewage. Once biosolids are processed to meet regulatory requirements for odor, vector, and pathogen reduction, the nutrient-rich material may be applied as a soil supplement to approved parcels. Solano County allows for the application of Class B biosolids to rangelands where forage is grown. Application of biosolids to land as a soil supplement may be more environmentally beneficial than disposing of it at landfills or other disposal sites. The majority of biosolids applied in Solano County come from wastewater treatment plants located in the San Francisco Bay area, and from other generators within and outside of the County.

The land application of biosolids is conducted under Solano County code, Chapter 25. The Solano County Environmental Health Services Division ("Environmental Health") is responsible for the enforcement of Solano County Code regulating land application of biosolids. Solano County's biosolids regulations stem from 40 CFR Part 503 – Standards for the Use or Disposal of Sewage Sludge, which contains regulatory requirements for pathogen reduction, vector attraction reduction, and pollutant thresholds. The County supplements these regulations by implementing policies that address application timing windows, nuisance odor prevention, tracking of material onto public roads, and establish a complaint and formal protest system.

Since 2000, regulated applications of Class B biosolids have been applied to approved rangelands in Solano County. Prior to application of biosolids, a parcel must also be enrolled in the State Water Resources Control Board General Order No. 2004-0012-DWQ - General Waste Discharge Requirements for the Discharge of Biosolids to Land for Use as a Soil Amendment in Agricultural, Silvicultural, Horticultural, And Land Reclamation Activities or an individual Waste Discharge Requirement ("Biosolids General Order"). The fields selected for this study are enrolled in the General Order. Biosolids applications occur annually from April 15 through October 15 on designated parcels.

In 2004 the Solano County Board of Supervisors established a biosolids scientific research and education fee as a per-acre surcharge, charged to the applicator of land applied biosolids, to provide funding for biosolids research in Solano County Code. Revenue generated by this fee was used to fund this research project.

The study area was located in the southeastern portion of Solano County, north of State Route 12, west of State Route 113, west of Rio Vista and east of Fairfield. See **Figure 1** for an overview map. Fields where biosolids applications occur are non-irrigated pasture/rangeland. The study area selected was not mechanically harvested, and has on-going sheep and cattle grazing. As specified in the Biosolids General Order, buffers between the public roads, residences, groundwater wells, riparian areas and areas of shallow groundwater limit where biosolids may be applied.

No planting of forage crops by seed or plugs was performed on the fields studied. The pasture forage species are generally a mix of annual and perennial grasses, with some annual broadleaf species, that grow from an established seedbank. Non-irrigated pasture depends entirely on rainfall for soil moisture. No discing or tillage was done on the study area except to incorporate applied biosolids.

Soil and plant tissue samples were collected and analyzed to assess the agronomic effects, potential carbon sequestration, and determine the fate or movement, if any, of trace metals and salts in soil associated with biosolid applications on rangelands in Solano County. Forage quantity and quality data was used to create a crop agronomic growth model to estimate potential yield resulting from biosolids application and to estimate weight gain of cows grazed on pastures receiving biosolids applications.

Forage and soil data was also used to estimate the potential for enhanced carbon sequestration from biosolids applications.

Results of this study are representative of conditions occurring in these fields, at the particular sites investigated during the study period and may not necessarily characterize the impacts of biosolids application to all lands, generally. Land characteristics including soil type, topography, vegetative community, and microbial community; and climatic conditions including precipitation amount and intensity may have significant impacts on the effects of the application of biosolids to land as a soil supplement. Sampling was performed during the third year of an ongoing drought; below average rainfall conditions were observed. Furthermore, the inherent variability associated with sampling large, non-homogenous areas such as an agricultural field with the number of sites and replicates used in this study may result in data that is not entirely representative of the whole field. An increase of the number of replicates and sites may help reduce inherent sampling variability. The goal of the study was to identify general trends in soil and forage chemistry that may be attributed to the application biosolids.

2.1 Field Selection

A review of the history of biosolids application to Solano County rangeland was conducted to locate suitable sites for soil and forage sample collection. Biosolids application records dating back to 2000 were provided by Solano County Environmental Health. Information in these records included landowner information, Regional Water Quality Control Board Site ID #, and the number of and timing of applications made between 2000 and 2015. Supplemental records for suitable study fields were collected, and consisted of field specific information including field acreages, estimated nitrogen loading rates, carry-over nitrogen concentrations, cumulative lifetime metal loading, 2015 proposed and actual biosolids application rates, and analytical results of nutrient levels and concentrations of metals in biosolids from each of the participating biosolids generators and the approximate contribution of each biosolids generator to the total amount applied.

Field selection criteria included accessibility, cooperativeness of the landowner, similarity of soil type and topography, and history of biosolids applications. Additionally, the site must have received biosolids applications in 2015. The fields chosen were located north of the intersection of California State Route 12 and California State Route 113, west of Rio Vista, CA and east of Fairfield, CA. See **Figure 1** and **Figure 2**. Environmental Health and associated biosolids permitting names for the fields are S04-221 and S04-223, herein referred to as "Field 221" and "Field 223".

The study sites chosen received six (6) biosolids application between 2000 and 2015. The timing of these applications is presented in **Table 1**.

Table 1: Biosolids Application to Selected Fields

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Field 221	-	-	-	-	-	✓	✓	✓	✓	-	-	✓	-	-	-	✓
Field 223	-	-	-	-	-	✓	-	✓	✓	-	✓	-	✓	-	-	✓

- No biosolids application made
- ✓ biosolids application made

Field 221 and Field 223 soil types were mainly Altamont-Diablo clays (48.7%), San Ysidro sandy loam (20.7%), and Antioch-San Ysidro complex (14.5%), with small pockets of Altamont-San Ysideo-San Benito complex (8.4%), Antioch-San Ysidro complex (5.7%), and Solano loam (1.8%). See **Figure 3** for the US Department of Agriculture – Natural Resources Conservation Service (USDA-NRCS) soil survey map of

soil types in the study area. The fields were generally flat or gently sloping with grades less than 10% (Soil Survey Staff, 2015). These soil types are typical of rangelands in Solano County.

The land use for both fields was pasture/grazing, with similar use patterns, root zone depths, and forage species. Critical to the study design were adjacent fields with similar soil type and slope, and the buffer areas where no biosolids are applied due to presence of an active groundwater well, a public road, an ephemeral stream, or groundwater protection zone. The SWRCB requires that buffer zones be established around water supply wells, surface water drainage courses, wetlands, sensitive groundwater zones, and public areas to prevent the transmission of pathogens to the public, as a condition of the Biosolids General Order. These buffers and the adjacent field provided a zone within the study area where no biosolids applications have occurred. Within NBAS areas, no groundwork or tillage occurs and no fertilizer or other soil amendments are applied. See **Figure 4**.

Within both Field 221 and Field 223, four sites were selected for soil and forage sample collection. Two sites were selected where biosolids applications occur, and two sites were selected where no biosolids have been historically applied in each field. The location of these sites were based on ease of access, on-going grazing by sheep and cows, presence forage vegetation representative of the fields as a whole, the same soil type (Altamont-Diablo clay), and topography compatible with the construction of exclosures (*i.e.*, relatively flat, smooth ground).

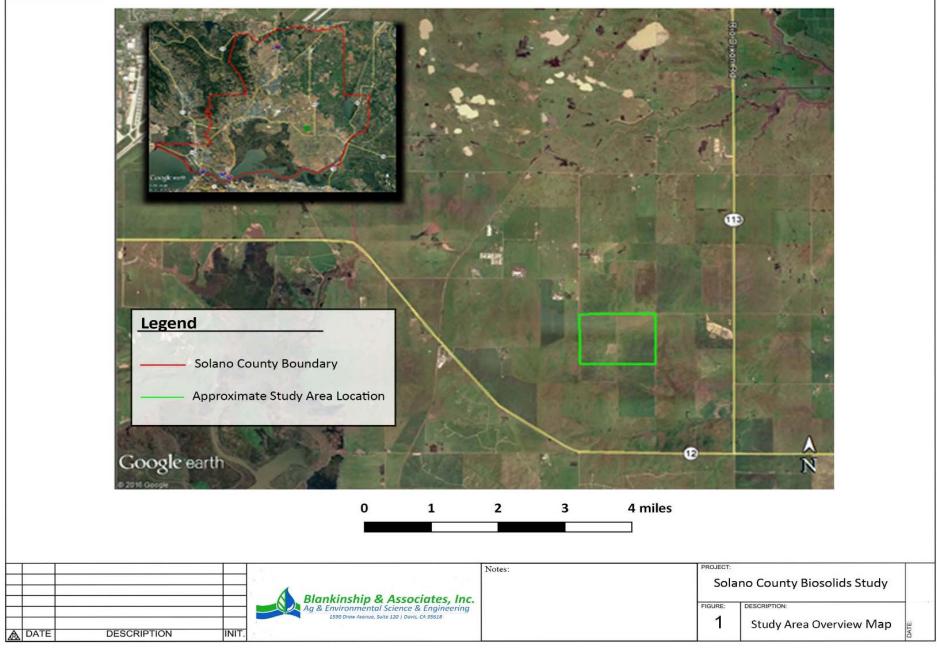


Figure 1: Study Area Overview Map

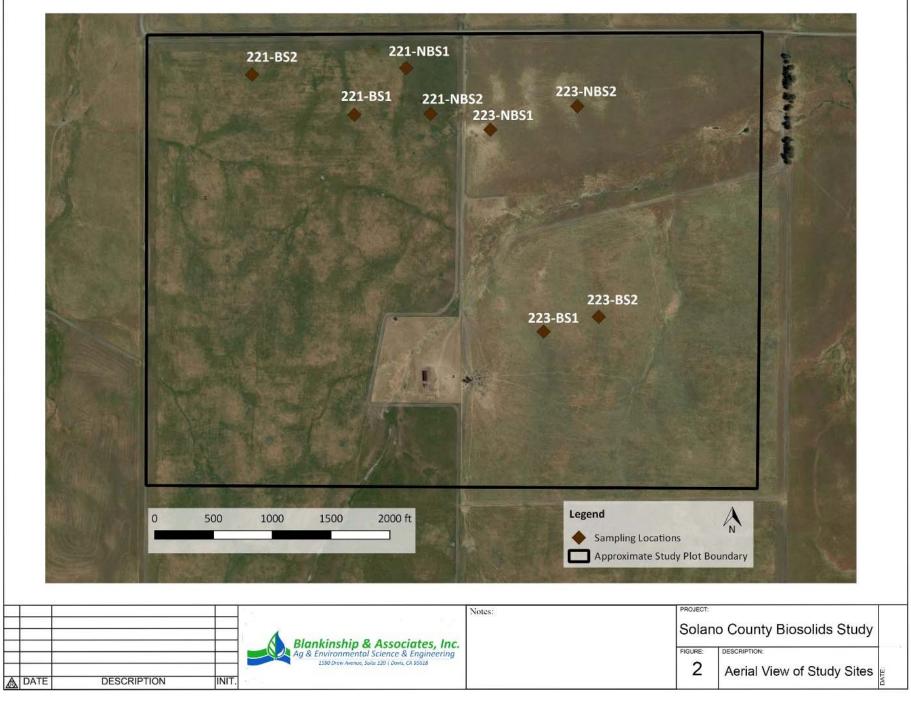


Figure 2: Aerial Map of Study Sites

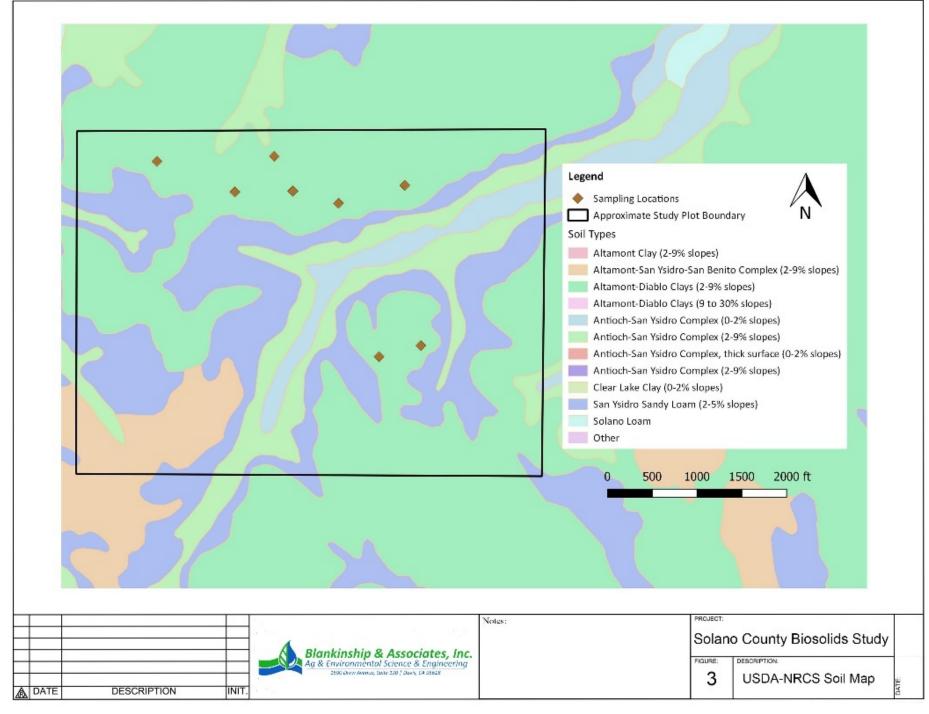


Figure 3: USDA-NRCS Soil Map

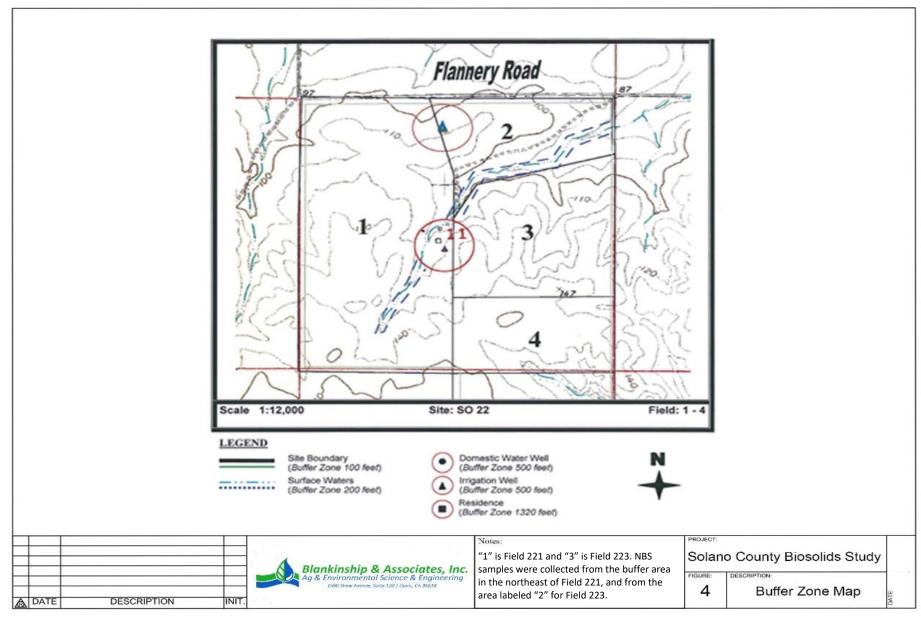


Figure 4: Map of buffer zones for biosolids applications to study fields

2.2 Soil Sampling

Two rounds of soil sampling occurred during the study, one was conducted to establish baseline concentrations in the soil profile of plant essential nutrients, trace metals and salts prior to significant winter rains and the forage growing season ("pre-forage"). Pre-forage soil sampling was conducted on December 17, 2015. "Post-forage" soil sampling was conducted on August 17, 2016, after water infiltration may have moved analytes through the soil profile, and after the forage growth season. The area received approximately 14 inches of precipitation between the two rounds of soil sampling. Most of this precipitation fell between mid-December and mid-March. See **Figure 5**. The study season took place during a drought year where Solano County and the rest of California experienced drier winter conditions and a shorter rainfall season than normal.

During each round of sampling, samples were collected from four (4) depth intervals at each of the eight (8) sites. The selected depth intervals were, in distance below ground surface ("bgs"): 0-1 feet, 1-2 feet, 2-3 feet, and 3-5 feet. These depth intervals were selected to aid in determining the extent, if any, of vertical transport of trace metals through the soil profile. The depths allowed sampling from the A, B and C horizons of the soil profile.

To obtain the samples a backhoe was used to excavate a pit from an area of undisturbed ground, to approximately 5 feet bgs. Once the pit was completed, measuring tape was positioned along the face of the pit and samples were collected by hand using a trowel and, where needed, the pick end of rock hammer. Care was taken to collect samples from beyond the exposed face of the pit, where contamination from the backhoe bucket and/or mixing or smearing of material from the excavation process could have affected sample integrity or disturbed the soil at each depth interval. Samples from each

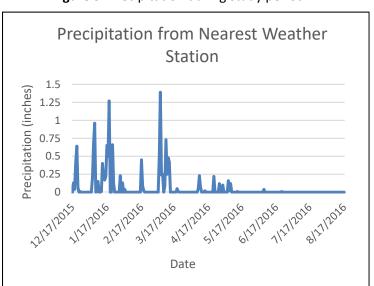


Figure 5: Precipitation during study period.

depth interval were field composited into clean, labeled paper bags and sealed by rolling the top of the bag downwards and securing the bag with tape. The samples were inventoried on a field sheet and remained sealed and under chain-of-custody until delivered to the analytical laboratory. After samples were collected, the pits were filled in with the excavated material and packed down.

2.3 Exclosure Construction

On March 2, 2016, exclosures were constructed at each of the eight (8) sites. The exclosures measured eight-feet by sixteen-feet (8' \times 16'), and were constructed using steel "T-Posts" and galvanized stockade fencing. The exclosures were placed on undisturbed ground adjacent to pre-forage excavation sites that contained a community of forage vegetation representative of the field. The exclosures were necessary to prevent livestock from grazing within the area plant tissue would be collected through the growing season.

2.4 Forage Sampling

Three (3) rounds of forage sampling occurred over the course of three (3) months. Samples of plant tissue were collected on April 5, 2016, May 12, 2016, and June 24, 2016. During each round of sampling, a one-foot by one-foot (1' x 1') plot within the exclosure was selected at random for sampling. The blind-throw technique with an excavation marking flag was used to mark the center of where a 1' x 1' quadrant made of polyvinyl chloride (PVC) pipe would be placed. Throws were rejected if the sampling quadrant overlapped previously sampled areas, was too close to the fencing to allow sampling of the full area.

All individual plants whose crown (part of the root structure from which the stem emerges) fell within the inside edges of the quadrant was included in the sample. In the event that vegetation was windswept, or otherwise deviated from an upright position, and had its crown within the quadrant, the plant was tufted up into the quadrant so that it would be included in the sample. The average height of the vegetation within the quadrant was measured by randomly selecting and measuring a subset of individual plants from ground level to the tip of the furthest extending structure and averaging the values of the subset. Qualitative data was recorded including species present, estimations of species composition, and condition of the vegetation.

To collect the forage sample, hedge shears were used to cut vegetation to one (1) inch above the ground surface. The harvested area was marked with a surveying flag labeled with the collection date so that the area would not be inadvertently resampled during future sampling events. The collected plant tissue was weighed in the field using a My Weigh™ 6001T digital scale with a tested accuracy of ±1 gram and a resolution of 0.1 grams. The samples were transferred to clean, labeled Ziploc® bag(s) and sealed. The samples were inventoried on a field sheet and remained sealed and under chain-of-custody until delivered to the analytical laboratory.

2.5 Analytical Methods

Soil and forage samples were delivered under chain of custody to analytical laboratories for analysis. Forage samples were submitted to:

- Dellavalle Laboratories (DL) in Fresno, CA; a California Department of Food and Agriculture and California Regional Water Quality Control Board approved laboratory,
- BC Laboratories (BC Lab) in Fresno, CA; a full service environmental laboratory with ELAP certification in the State of California, and
- Cumberland Valley Analytical Services (CVAS) in Hagestown, MD; a National Forage Testing Association certified laboratory for Near-infrared spectroscopy(NIR) and chemistry.

Soil samples were submitted to:

- IAS Laboratories (IAS Lab) in Phoenix, AZ; an agronomy laboratory with certifications from the United States Department of Agriculture and a participant in the North American Proficiency Testing Program,
- Dellavalle Laboratories, and
- BC Laboratories

See **Appendix A** for a table of analytical methods, detection and reporting limits, and which lab was used for each analysis.

2.6 Data Analysis

Forage and soil analytical data was assessed using the Student's t-test to identify statistically significant differences in means, using a significance level of 0.05 (p=0.05). Where possible and appropriate, data was grouped together to increase sample size. For example, if pre- and post-forage concentrations of a particular group of analytes were statistically similar, as they were in the case of trace metals concentrations, the pre- and post-forage data was grouped together and used to compare BAS and NBAS soil concentrations. This was not the case for soil nutrients, where pre-and post-forage data were statistically different, so comparisons were made between both BAS and NBAS soil concentrations as well as for pre- and post-forage concentrations.

In many cases, sample sizes were relatively low. Inherent variability associated with sampling non-homogenous materials paired with these small sample sizes tends to return results indicating that there were no statistically significant differences in sample means, even though a trend exists that indicates some degree of difference. Throughout this report, results referred to as 'statistically significant' are used when statistical analysis returned a P value less than 0.05 and was statistically significant; results that are referred to "substantial" indicate that statistical analysis returned a P value greater than 0.05, but that an ecologically or practically relevant trend exists.

3.1 General Soil Characteristics

Soil Classification

The study site is located on the Altamont-Diablo Clay complex according to the USDA-NRCS soil survey. The complex Altamont-Diablo consists of both Altamont and Diablo series characteristics and is dominated by the Altamont series.

The Altamont soil series is characterized by deep, well-drained soil. This soil contains shrink-swell clay soils to a depth of 20 inches or more when the soil is dry (Soil Survey Staff, 2017). These soils are primarily used for livestock grazing and dryland farming and are typical of rangeland in Solano County. The Diablo soil series is characterized by well-drained soils with slow permeability (Soil Survey Staff, 2017). This soil contains shrink-swell clays to a depth of 20-40 inches when soil is dry. These soils are typically used for grazing and dryland farming. See **Table 2**.

Shrink-swell soils contain clay that expands when wet and contracts when dry. Deep cracks emerge in these soils during the dry season. These soils are problematic because as they wet and dry they can tear plant roots and compromise roads or buildings.

Table 2: Soil Series Properties Summary

Soil series	Surface Texture	Drainage	Permeability	Depth	Use
Altamont	Clay (shrink/swell)	Well Drained	Slow	N/A	Grazing/dryland farming
Diablo	Clay (shrink/swell)	Well drained	Slow	Deep	Grazing/dryland farming

Soil Physical Characteristics

Soil was found to be clay textured in all but two samples. At 223-BAS1, the sample collected from the 0-1 foot depth interval was found to be clay loam textured, and at 221-NBAS2, the sample collected from the 2-3 foot depth interval was found to be loam textured.

Bulk density ranged from 1.21 grams/cm³ to 1.59 g/cm³.

Field capacity was determined to be between 20% and 27.9% volumetric moisture content.

At NBAS sites, no groundwork or tillage occurs and no fertilizer or other soil amendments are applied. At BAS sites, the soil is lightly tilled to incorporate the biosolids. This tillage may have an effect on soil physical characteristics.

The soil physical characteristics determined at these sampling sites influence the ability of water to penetrate through the soil, and consequently the ability of nutrients to leach through the soil.

3.1.1 Soil Moisture Retention and Drainage

Soil physical factors influence the ability of water to be held in soil, to infiltrate into soil, and to drain through soil. These physical factors also influence the leachabilty of nutrients through the soil and beyond the root zone. Soil physical properties determine the total volume and distribution of pore space

and the configuration, size and distribution of soil aggregates into soil structure. These factors influence water and nutrient movement within the soil.

Porosity is the total volume of pore space (voids) in a volume of soil. Bulk density is the weight of soil per volume of soil. Bulk density can be used to calculate porosity using the equation: porosity (%) = (1-(β^b/β^d)) x 100, where β^d is the particle density (assumed to be 2.65 g/cm³) and β^b is the bulk density of the soil. Bulk density is dependent on soil organic matter content, soil texture and soil compaction. Farming practices such as tillage can alter bulk density over time. Tillage tends to reduce bulk density and increase porosity. Soil organic matter has very low density, so soils with high organic matter content have lower bulk density. Course textured soils (sands) have larger pores, but less total pore space and greater bulk density than finer (clay) textured soils. See **Table 3** and **Figure 6**. Bulk density for clay textured soils range from 1.25-1.45 g/cm³ (USDA-NRCS, 2017). Bulk density values greater than this in a clay textured soils may indicate soil compaction. Results from this study show that bulk density values are lower and organic matter content levels are higher on the sites where biosolids have historically been applied. The biosolids are tilled in (incorporated) into the soil as they are applied. This tillage may contribute to the decreased bulk density on BAS sites. Some compaction may be present in areas where no biosolids were applied to Field 221 , indicated by high bulk density for a clay textured soil.

Table 3: Soil Physical Properties

	Bulk Density	Porosity	Texture	% Clay	Organic Matter*
Site	g/cm³	%			0-1 ft (%)
223-NBAS1	1.40	47.2	Clay	49	1.97
223-NBAS2	1.46	44.9	Clay	49	2.88
223-BAS1	1.21	54.3	Clay Loam	38	2.84
223-BAS2	1.36	48.7	Clay	42	2.48
221-NBAS1	1.53	42.3	Clay	49	2.41
221-NBAS2	1.59	40.0	Clay	47	2.28
221-BAS1	1.29	51.3	Clay	58	3.45
221-BAS2	1.43	46.0	Clay	52	2.91
BAS Average	1.32	50.1		47.5	2.92
NBAS Average	1.50	43.6		48.5	2.39

^{*}Organic matter data is from pre-forage soil analysis

Average Soil Physical Properties 3 2.5 2 1.5 BAS NBAS 1 0.5 **Bulk Density** Percent Clay Organic Matter* Porosity (% as decimal) (% as decimal) (g/cm3) (%)

Figure 6: Average Soil Physical Properties

Field capacity and permanent wilting point influence the amount of water a soil can hold and how much water is available in the soil for plant uptake. Field capacity is the amount of water a soil can hold after gravimetric drainage (drainage due to the pull of gravity) has occurred. Permanent wilting point is the point at which any remaining water is being held so tightly that most plants are not able to remove more water from the soil. The amount of water available to the plant is the difference between these two numbers and is called "plant available moisture". See **Table 4** and **Figure 7**. Soil texture influences the amount of plant available water in a soil. Clay soils can hold more water than coarser textured soils, however, due do a large volume of very small pores in clay soil, much of the water in the soil is not available to the plant. Thus, the permanent wilting point in clay soils tends to be high and clay soils have a moderate amount of plant available water.

Table 4: Water Holding Properties

	Field Capacity (%)	Permanent Wilting Point (%)	Plant Available Moisture (%)
Site Location	at 1/3 Bar	at 15 bar	(FC-PWP)
223-NBAS1	24.2	8.2	16.0
223-NBAS2	25.4	8.8	16.6
223-BAS1	21.2	6.0	15.2
223-BAS2	20.0	6.0	14.0
221-NBAS1	25.6	9.0	16.6
221-NBAS2	23.6	11.0	12.6
221-BAS1	27.9	10.1	17.8
221-BAS2	25.0	10.5	14.5
BAS Average	23.5	8.2	15.4
NBAS Average	24.7	9.3	15.5

^{*} Organic matter data is from the 0-1' depth interval from pre-forage soil analysis.

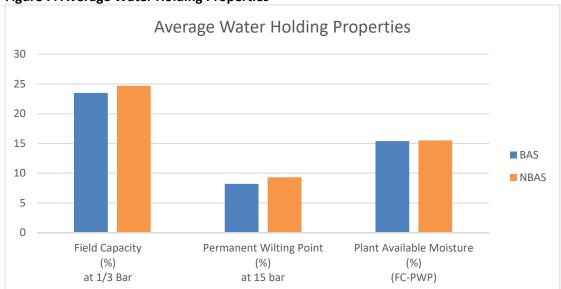


Figure 7: Average Water Holding Properties

There is no substantial difference in field capacity, permanent wilting point or plant available moisture between the BAS and the NBAS sites.

3.1.2 Comparison Between BAS and NBAS Sites

Results of soil physical characteristics show that where biosolids were historically applied, the bulk density is less than in soils where biosolids were not applied - 1.32 g/cm³ compared to 1.50 g/cm³. There is no difference in soil texture or clay content between these two groups, which indicates that the difference in bulk density is due to the increased levels of organic matter and/or tillage in the biosolids applied sites.

Drainage and infiltration are not substantially different between the BAS and NBAS sites. All sites tend to have a very high clay content which causes very slow water infiltration and drainage. Although there was slightly higher porosity and lower bulk density in the BAS sites, there were no substantial differences in permanent wilting point and plant available water content between BAS and NBAS sites.

The application of biosolids was not found to influence water holding capacity or plant available moisture in the soil.

The leaching potential of nutrients at this location is very low due to the high clay content of the soils which typically have slow infiltration and drainage and a complete lack of irrigation water. Shrink-swell clay soils swell in the rainy season which makes it very difficult for water and nutrients to move through the soil profile. In addition, the data show that nitrogen content decreases with depth, and that nitrogen values are very low at the 3-5 foot depth. The leaching potential is very low in both the biosolids and non-biosolids applied sites.

3.2 Soil Chemistry

3.2.1 Nutrients and Salinity

Total salts (as measured by electrical conductivity) are moderate to low in most areas based on crop tolerances without a reduction in yield. Total salts were found to be high in the pre-forage samples collected from the 3-5 foot depth interval at 223-BAS1 and 221-NBAS1. These values (3.68 dS/m and 4.81, respectively) were found to be statistical outliers using the Moore and McCabe quartile value outlier detection method. Post-forage samples collected at the same sites, from the same depths were lower (0.77 dS/m and 2.24 dS/m for 223-BAS1 and 221-NBAS, respectively), although EC in the sample from the 3-5 foot interval at 221-NBAS1 was high compared to values detected at other sites within the same depth interval. A larger sample set is required to assess the significance, define trends or attribute to random variation within these results given the inherent sample variability with non-homogenous soils and variations in horizon depth. Regardless, these salinity levels are unlikely to limit plant growth as they are far below the root zone of forage plants.

Calcium tends to be low; however, calcium levels are greater than sodium in BAS and NBAS areas which is beneficial for soil structure and water infiltration.

Soil nitrogen levels tend to be low and decline with depth. Nitrogen was found to be very high from 3-5 feet in 221-BAS2. However, this measurement was determined to be an outlier using the Moore and McCabe quartile value method, and this high value is likely due to the contamination by manure during the excavation process. In most areas nitrate-nitrogen decreased between the pre-forage and post-forage sampling events.

Soil phosphorous concentrations are considered low for forage crops when below 10 mg/kg (ppm). Phosphorus levels are very low in most areas. Phosphorous levels are adequate to high in 223-BAS2 and 221-BAS2. BAS sites had more phosphorous than NBAS sites in both the pre-forage and post-forage sampling events in both fields. These results indicate that biosolids increase soil phosphorous levels which may increase soil productivity.

Potassium (K) levels are adequate in both BAS and NBAS sites. There are no differences in K levels between BAS and NBAS sites during the pre-forage or post-forage soil sampling events.

Zinc levels are low for forage crops when below 1.0 mg/kg. Zinc levels were adequate in the top foot in both the BAS and NBAS sites. Zinc levels were found to be significantly greater in the BAS sites during both the pre-forage (P = 0.035) and post-forage (P = 0.018) sampling events.

During the pre-forage sampling event, soil organic matter ranged from 1.97% to 3.45% in the first foot and decreased with depth. During the post-forage sampling event, organic matter ranged from 4.43% to 5.4% in the first foot and decreased with depth. Organic matter was much higher in all sites during the second sampling event. There was not a significant difference in soil organic matter content between the NBAS and BAS sites.

3.3 Nutrient and Salt Loading

Nitrate-nitrogen (NO3-N), phosphorus (PO4-P), potassium (K), zinc (Zn), and organic matter (OM) content were compared between biosolids amended soil and non-amended soil samples. Nitrogen, phosphorus, and potassium (N, P, K) are especially important to plant growth because they are

macronutrients - required in large quantities - and have the greatest influence on crop productivity. Soil organic matter is an important property of soil that affects nitrogen availability as well as physical soil properties. Results from samples below the 0-2' depth were not included because analysis determined that there were no significant differences in nutrient content in the 2-3' and 3-5' depth intervals.

Biosolids and Non-Biosolids Applied Sites

In pre-forage soil samples, biosolids-amended soil generally had higher levels of total salts (measured as electrical conductivity or "EC"), nitrogen, phosphorus, and zinc than non-amended soils. However, only differences in phosphorus and zinc were statistically significant (P = 0.002 and P = 0.035, respectively).

A similar trend was observed in post-forage samples. Biosolids-amended soils contained higher levels of EC, nitrogen, phosphorus, and zinc. However, only differences in nitrogen, phosphorus, and zinc were statistically significant (P = 0.021, P = 0.045, and P = 0.018, respectively). See **Figure 8**.

Analysis performed by Synagro on the concentrations of constituents in the biosolids material indicates the highest concentration are of plant available nitrogen (PAN), phosphorous, potassium and zinc, applied at rates of 165, 136 to 139, 21 to 24 and 12 to 13 lbs. per acre respectively. Overall, results show that BAS sites generally have increased soil concentrations of nitrogen, potassium and zinc which are the large quantity nutrients contained in the biosolids. Organic matter content and potassium concentrations do not differ greatly between BAS and NBAS sites.

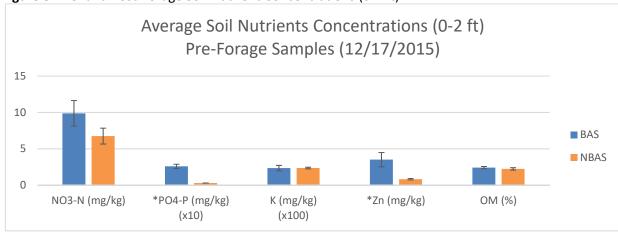


Figure 8: Pre- and Post-Forage Soil Nutrient Concentrations (0-2 ft)

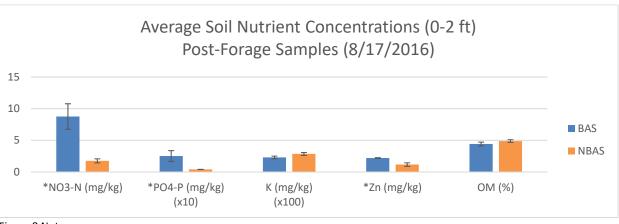


Figure 8 Notes:

Error bar is equivalent to 1 standard deviation

^{*} Indicates statistical significance

Measurements of pH, total salts (measured as electrical conductivity or "EC"), cation exchange capacity ("CEC") were compared between biosolids amended soil and non-amended soil samples collected from the 0-1 foot depth interval. **See Figure 9**.

Soil pH is a measure of the acidity or basicity of a soil, ranging from 0 to 14, with 7 being neutral. Most plants prefer soil pH values between 5.5 and 7.0. Soils pH values are neutral to slightly acidic in the first foot with pH becoming increasingly alkaline with depth. There were no differences in soil pH between the first and second sampling events or between NBAS and BAS sites. There were no substantial differences in the pH values of BAS and NBAS soils in either pre- or post-forage samples, with individual sample measurements ranging from 5.9 to 6.9 and averages ranging from 6.1 to 6.6.

Soil EC is a measure of the soil's ability to conduct an electrical current and correlates with the concentration of total salts in the soil. Elevated salt levels can hinder plant growth, crop yield, crop suitability, plant nutrient availability, and water infiltration. Results suggest salt levels are greater in biosolids-amended soils than in non-amended soils, although differences in EC between BAS and NBAS sites are not statistically significant (P = 0.31). These differences were observed in both pre-forage and post-forage samples. In clay soils, EC levels up to 2.0 dS/m (equal to 200 mS/m) are considered "Non-Saline" (Smith and Doran, 1996).

Soil CEC is the ability of soil to hold exchangeable cations (positively charged ions). Clay soils tend to have higher CECs due to clay's negatively charged particles. High CEC is generally a desirable characteristic, as high CEC can reduce nutrient leaching, helping to hold nutrients in soil layers where they are available to plants. In clay soils, CEC values greater than 30 meq/100 g are typical, and can reach as high as 100 meq/100 g in some clay types (Sonon et al. 2014). There were no substantial differences in CEC between BAS and NBAS soils, with individual measurements ranging from 24.9 meq/100 g to 41.1 meq/100 g and averages ranging from 32.4 meq/100 g to 37.1 meq/100 g. Values indicate that CEC values are typical for the soil type examined. Application of biosolids are not likely to change the soil's CEC at the application frequency and amount.

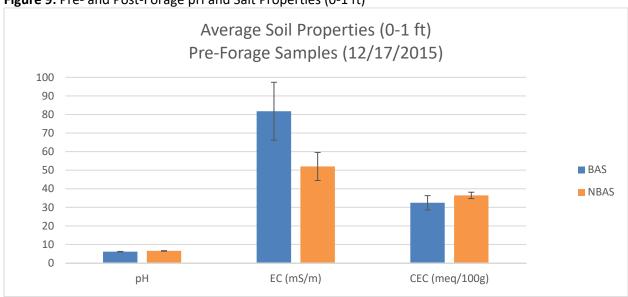


Figure 9: Pre- and Post-Forage pH and Salt Properties (0-1 ft)

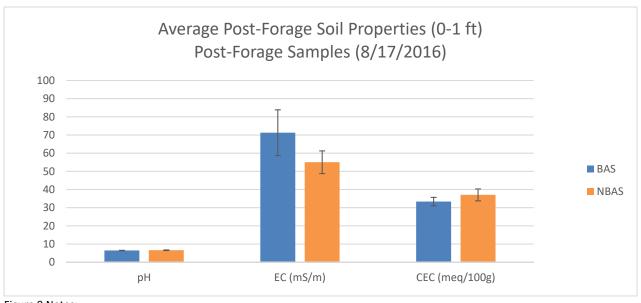


Figure 9 Notes:

Error bar is equivalent to 1 standard deviation

Pre-Forage vs. Post-Forage Soil sampling

When comparing differences between the pre-forage samples and post-forage samples greater levels of organic matter were found in post-forage samples than in the pre-forage samples in both the BAS and NBAS sites, but only differences in the NBAS samples were statistically significant (P = 0.006). In the NBAS sites, nitrate-nitrogen was significantly higher in the pre-forage sample (P = 0.005). In the BAS sites, zinc was higher in the pre-forage than in the post-forage sample, but the difference was not statistically significant (P = 0.135). There were no other substantial differences between pre-forage and post-forage samples.

There were no substantial differences in BAS and NBAS sites during either the pre-forage or post-forage sampling events for soil pH, EC or CEC. See **Figure 9**

Increased organic matter content in the post-forage samples was likely due to increased organic matter accumulation in the 0-2 foot intervals due to plant growth and biomass. Sloughing and exudates by plant roots likely increase the organic matter content of the soil over the forage season. The reduction of nitrate-nitrogen in the NBAS sites indicates that the plants are scavenging for nitrogen and have taken up substantial levels of soil nitrogen between December 2015 and August 2016. See **Figure 10**.

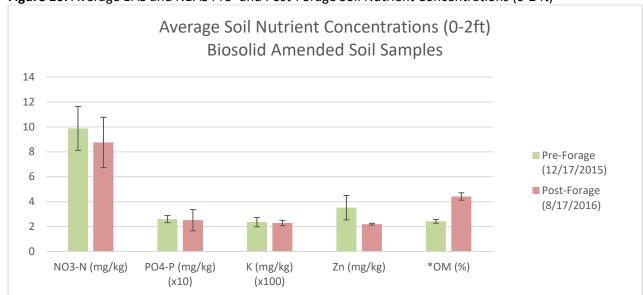


Figure 10: Average BAS and NBAS Pre- and Post-Forage Soil Nutrient Concentrations (0-2 ft)

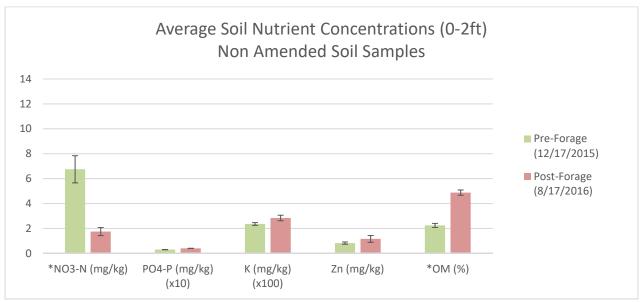


Figure 10 Notes:

Error bar is equivalent to 1 standard deviation

3.4 Trace Metals

Soil samples were analyzed for a selection of relevant trace metals (arsenic, cadmium, chromium, copper, lead, mercury, molybdenum, nickel, selenium, and zinc). Values above the method reporting limit (MRL) were not detected for cadmium, molybdenum, and lead in any sample. Only two data points above the MRL were detected for mercury, and selenium was only detected above the MRL in preforage samples. Due to lack of data for comparison, data is not presented for trace metals that were at or below the MRL. All analytical results are presented in **Appendix D**.

Analytical results for samples collected from 0-1 foot of soil were used to calculate average concentrations from BAS and NBAS sites. Differences in trace metals concentrations were evaluated for each analyte. See **Figure 11**. Statistically significant differences were observed in copper, chromium,

^{*} Indicates statistical significance

nickel, and zinc. Copper and zinc concentrations were significantly greater in samples collected from BAS sites (P = 0.001 and P = 0.005, respectively). Chromium and nickel concentrations were significantly greater in samples collected from NBAS sites (P = 0.001 and P = 0.001, respectively).

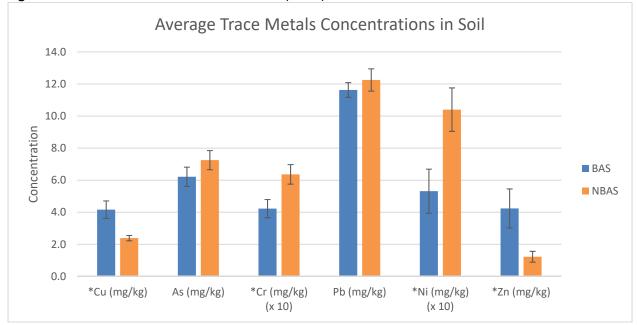


Figure 11: Trace Metals Concentrations in Soil (0-1 ft)

Figure 11 Notes:

Error bar is equivalent to 1 standard deviation

Although significantly higher concentrations of copper and zinc were detected in BAS compared to NBAS soils, with the exception of arsenic and nickel, all metal concentrations were below relevant regulatory screening levels. Average and maximum individual-sample metals concentrations were compared to human and environmental health protective regulatory levels, and average (geometric mean) California soil background concentrations from the University of California Kearney Foundation of Soil Science Special Report (1996). See **Table 5**.

Copper, lead and zinc levels in samples from BAS and NBAS were all below all relevant regulatory levels reviewed. The concentrations of these elements are also less than the average background concentrations found in California soils.

Nickel concentrations were below regulatory levels in samples collected at BAS sites, but were greater than the San Francisco Regional Water Quality Control Board (SF-RWQCB) Tier 1 Environmental Screening Level (ESL) for Soil in some individual samples and on average in samples collected at NBAS sites. The SF-RWQCB ESL for nickel is exceptionally low (86 mg/kg) compared to the other regulatory levels examined, approximately 1/20th of the value of the next lowest regulatory level examined (1600 mg/kg). It should be noted that the SF-RWQCB ESLs selected for comparison are based on a generic conceptual site model that is designed for use at most sites, and uses conservative assumptions. The model uses a residential land use, and assumes the underlying groundwater is used as a drinking water source, a shallow depth to groundwater, and that the soil type is sand. Additionally, "the presence of a chemical at concentrations in excess of an ESL does not necessarily indicate adverse effects on human health or the environment, rather that additional evaluation is warranted" (SF-RWQCB, 2016). Nickel concentrations from both BAS and NBAS sites are elevated compared to the average background concentration found in California soils.

^{* -} Indicates statistical significance

The SF-RWQCB ESL and California Human Health Screening Levels distinguish between the two primary valence states of chromium, chromium (III) and chromium (IV). Chromium (III), or trivalent chromium, is a common, naturally occurring state of chromium that is an essential nutrient. Chromium (VI), or hexavalent chromium, is less common and primarily produced by industrial process. Chromium (VI) is much more toxic than chromium (III) for acute and chronic exposures. In this study, soil was analyzed for total chromium. Concentrations of total chromium in BAS and NBAS samples were all below regulatory limits. With the exception of one sample collected from a NBAS site, all samples were below average background concentrations of total chromium; and on average, both BAS and NBAS soil were below average background concentrations.

Arsenic was detected in BAS and NBAS samples at concentrations higher than the SF-RWQCB Tier 1 ESL, and residential and commercial/industrial CHSSL values, but well below RCRA Hazardous Waste standards. The concentrations of arsenic were also consistently greater than the average background concentration for California soil. Concentrations of arsenic in soil samples are close to expected background concentrations for Field 221 and Field 223 (6.38 mg/kg and 6.29 mg/kg, respectively), as calculated in the Syangro West 2015 Landspreading Notification Report. In Solano County, arsenic in soil and groundwater are thought to be primarily derived from natural sources.

The CHHSSL for arsenic is exceptionally low. A 2011 study by Duvergé assessed sites from the GeoTracker database and found the mean concentration of arsenic in Bay Area soils to be 4.61 mg/kg, indicating that background arsenic concentrations are higher than the environmental screening values derived by the SF-RWQCB. The same study proposed an 11.0 mg/kg upper estimate (99th percentile) for background arsenic within undifferentiated urbanized flatland soils. A 2012 study of the impacts of biosolid application to rangeland in Solano County also reported arsenic concentrations greater than these regulatory limits in both biosolids amended soil samples and control/reference samples. The concentrations of arsenic observed in soil samples were not dissimilar from the concentrations detected in biosolids material tested prior to land application in 2015 (concentrations ranged from 1.0 to 15.6 mg/kg, with an average of 6.6 mg/kg). There was no significant difference between arsenic concentrations in BAS and NBAS samples (P = 0.053), and it is unlikely that the addition of biosolids to these fields substantially increases soil arsenic concentrations.

Table 5: Comparison of Sampling Results, Regulatory Screening Levels, and Background Concentrations for Metals in Soil

	Cu (mg/kg)	As (mg/kg)	Cr (mg/kg)	Pb (mg/kg)	Ni (mg/kg)	Zn (mg/kg)
TTLC Limit ¹	2500	500	2500	1000	2000	5000
Tier I Environmental Screening Level for Soil ^{2,3}	3100	0.067	120000 ⁴ , 0.3 ⁵	80	86	23000
CHHSL Soil-Screening Number for Residential Scenario ^{6,7}	3000	0.07	100000 ⁴ , 17 ⁵	80	1600	23000
CHHSL Soil-Screening Number for Commercial/Industrial Scenario ⁶	38000	0.24	100000 ⁴ , 37 ⁵	320	16000	100000
California Background Concentrations ⁸	24.0	2.8	76.0	21.7	36.0	145
Average in surface NBAS samples ⁹	2.4	7.3	64	12.3	104	1.2
Average in surface BAS samples ⁹	4.2	6.2	42	11.6	53	4.2
Maximum in surface NBAS samples ⁹	2.9	8.6	81	14	140	2.8
Maximum in surface BAS samples ⁹	5.9	7.8	55	13	83	9.2

Table 5 Notes:

- 1 Values for Total Threshold Limit Concentration (TTLC) 40 CFR 261.
- 2 San Francisco Bay Regional Water Quality Control Board, 2016 (Rev. 3).
- 3 Based on a generic conceptual site model designed for use at most sites.
- 4 Chromium III
- 5 Chromium VI
- 6 CHHSL = California Human Health Screening Level (California Environmental Protection Agency Office of Environmental Health Hazard Assessment, 2010).
- 7 Residential use also generally adequate for other sensitive uses (day care, medical facilities, etc.)
- 8 Background as geometric mean from Kearny Foundation of Soil Science Special Report, 1996.
- 9 Surface = 0-1 ft depth

Section 4: FORAGE QUALITY AND QUANTITY

4.1 Forage Quality

As described in the Forage Sampling section, three rounds of forage sampling were conducted in each exclosure. Concentrations of plant forage nutrient values were evaluated on a per cutting basis for each of the three cuttings, and as a complied set of averaged data to determine differences in forage nutritional quality over time and on the whole. For purposes of nutritional value comparison, results were compared on a mg/kg or percent basis. Nutritional values evaluated include nitrogen, phosphorus, potassium, protein, magnesium, copper, ash, and selenium. Results for selenium were low, with many samples retuning values below the laboratory reporting limit ("RL"). Values that were reported were only slightly above the RL, and were not significantly different over time or across treatment areas (i.e. biosolids areas and non-biosolids areas). Selenium values are not discussed, but results may be reviewed in **Appendix D**. Along with nutrient values, acid detergent fiber (ADF), amylase-treated neutral digestible fiber (aNDF), and neutral detergent fiber (NDF) digestibility were evaluated.

In BAS and NBAS sites, ADF and NDF digestibility decreased with each cutting, and aNDF increased with each cutting. aNDF was statistically different (lower) in NBAS areas than BAS areas in the April cutting (P = 0.023), meaning the digestibility of forage from NBAS sites was higher at this time. In all cuttings, NDF digestibility was significantly greater in NBAS areas compared to BAS areas (P = 0.018, P = 0.016, and P = 0.001 for April, May and June, respectively). The lower aNDF and higher digestibility in the NBAS areas may be due to the lower nutrient levels limiting growth rates of forage plants as immature, smaller plants are easier for grazing animals to digest. ADF, aNDF, and NDF at all sites across all sampling events was palatable, resulting in no negative affect on grazing or digestibility.

In biosolids areas, nitrogen, phosphorus, potassium, and protein concentrations were highest in the first cutting, decreased in the second cutting. From the second to third cutting, nitrogen, phosphorus, and protein concentrations decreased. Potassium concentrations exhibited a slight increase between the second and third cuttings. Magnesium, copper, and ash concentrations remained steady in BAS areas in all three cuttings.

Analysis of tissue from the first round of cutting shows that nutrient levels and forage quality is greater in tissue collected from sites with biosolids amended soils for nearly every parameter. Nitrogen, phosphorus, protein, magnesium, copper, ADF, and aNDF are greater in BAS samples than in NBAS samples. Significant differences (greater in BAS compared to NBAS) are exhibited in nitrogen (P = 0.043), phosphorus (P = 0.007), protein (P = 0.043), aDF (P = 0.023). NDF digestibility was greater in NBAS compared to BAS (P = 0.018).

Similar, though less pronounced trends are seen in results from the second round of tissue collection and analysis. Nitrogen, phosphorus, protein, magnesium, copper, ash, ADF, and aNDF are greater in samples collected from sites with biosolids amended soils. The average potassium concentration is greater in samples collected from non-amended soils, and again, NDF digestibility is greater in non-amended soils. NDF digestibility is the only parameter of the second round of forage analysis exhibiting a statistically significantly difference (P = 0.016).

The trend reverses in results from the third and final round of forage tissue collection and analysis. ADF is the only parameter significantly greater in biosolids amended soil (P = 0.008). Nitrogen, potassium, protein, magnesium, and NDF digestibility are greater in samples collected from sites with non-amended

soil. Copper, ash, and aNDF are at similar levels. NDF digestibility again exhibits a statistically significant difference (P = 0.001).

From a grazing animal's perspective, the feed grown in all areas is of sufficient nutritional value and quality that the analytes could be interchangeable for animal growth modeling.

4.2 Forage Quantity

Average forage biomass was consistently greater in BAS fields over NBAS fields. In the first round of cutting, a 64.1% relative percent difference (RPD) is seen in BAS forage relative to NBAS forage, a 9.3% (RPD) in the second cutting, and a 24.6% RPD in the third cutting. When viewed as a seasonal average, there was a 42.4% RPD in forage biomass when comparing BAS forage to NBAS forage, and a 53.8% increase when using NBAS biomass for the initial value. See **Figure 12a** through **Figure 12d**.

When forage biomass amounts are accounted for, the amount of available forage per acre for grazing animals is in BAS than NBAS fields. See **Figure 13**. Given the higher biomass and potential for increased foraging per unit area, potentially detrimental constituents such as copper or selenium are not present at concentrations detrimental to animal health. While from a grazing animal perspective, the concentrations of forage nutrients and digestibility are substantially similar, the benefits of increased forage biomass result in a greater availability of nutrients while retaining adequate digestibility characteristics.

Figure 12(a-d): Relative Percent Difference in Forage Sample Averages



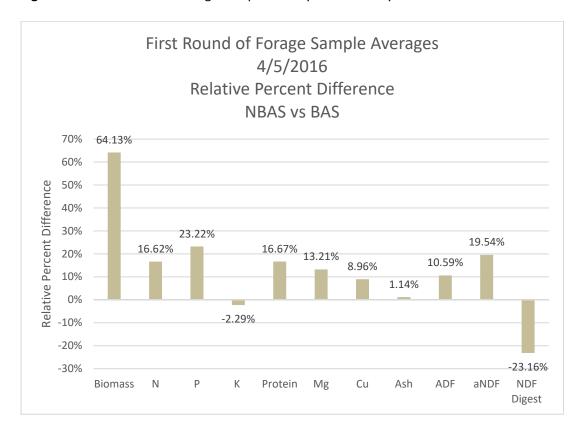


Figure 12b: Second Round of Forage Sample Quality and Quantity Differences

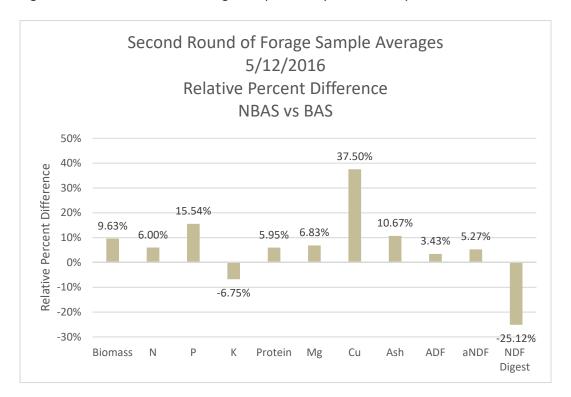


Figure 12c: Third Round of Forage Sample Quality and Quantity Differences

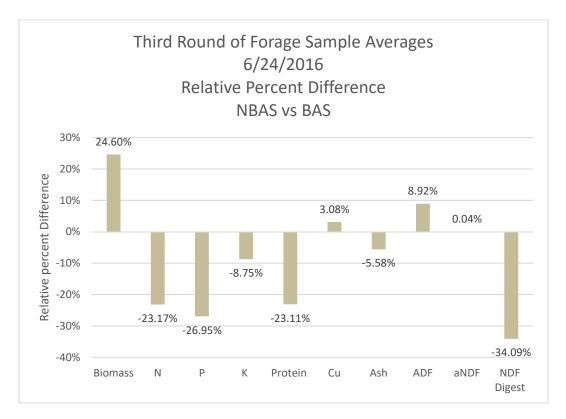


Figure 12d: Third Round of Forage Sample Quality and Quantity Differences

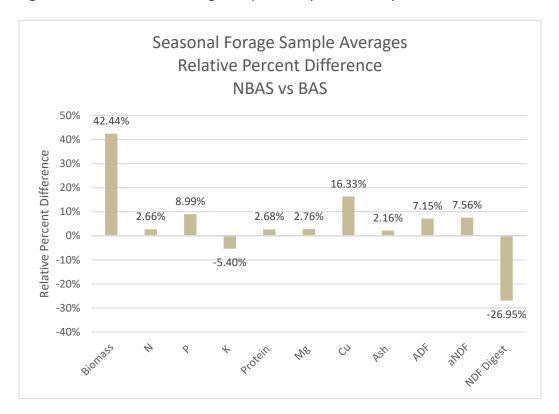


Figure 13: Forage Biomass Averages – NBAS vs BAS

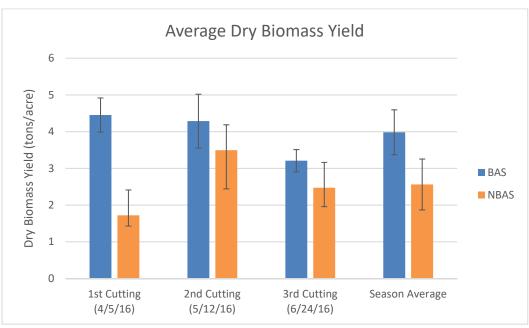


Figure 13 Notes:

Error bar is equivalent to 1 standard deviation

Section 5: Crop Growth Agronomic Model

A crop growth agronomic model allows the determination of the maximum yield that can be expected based on the soil nitrogen that is available to the crop. A crop growth model considers the amount of nitrogen available to the crop and the concentration of nitrogen present in the plant. Crop growth was modeled in two ways. The first is a soil-based model which considers the amount of nitrogen present in the soil. The second is a biosolids applied model and uses the amount of plant available nitrogen (PAN) in the biosolids as the nitrogen available to the crop. Agronomic models are important because they assess the theoretical yield expectations of BAS and NBAS sites and, subsequently, the quantity of forage that will be available to the grazing animals. In both models, the amount of nitrogen removed by the crop was used as a secondary factor in determining maximum yield.

Nitrate-nitrogen content of the soils was used to determine the amount of nitrogen available to the crop in the soil-based model. Organic matter mineralization of manure and/or biosolids in is a process in which non-plant available organic forms of nitrogen are converted to nitrate-nitrogen, a plant available source of nitrogen. The rate at which this conversion occurs is very complex and highly variable. A rule of thumb is that about 20 lbs. of nitrogen is released per 1% of organic matter per growing season (Lamb, Fernandez, Kaiser, 2014). This model accounts for all the nitrate-nitrogen present in the top two feet of soil - the "active root zone" - and 20 lbs. N per 1% OM in the first foot of soil and 10 lbs. N per 1% OM in the second foot. Less organic matter mineralization will occur in the subsurface soil due to less aeration and moisture. The results of the soil samples collected in December 2015 were used to estimate plant available nitrogen since these values more closely represent the nitrogen that will be available to the crop in the following growing season.

The biosolids-based model uses the amount of PAN in the biosolids that were applied in 2015 as the nitrogen that will be available to the crop. According to the Synagro Field Report data, approximately 165 lbs./ac of plant available nitrogen were applied to each of the BAS areas. The model assumes 100% of PAN will be available to the crop during the growing season.

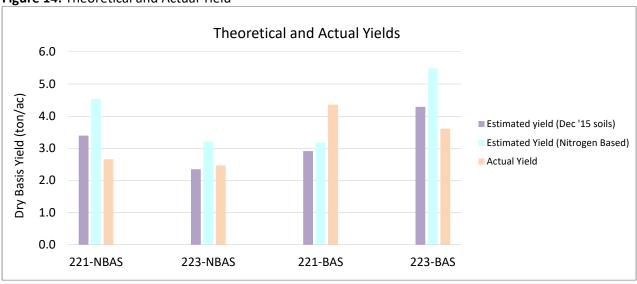
Low nitrogen in the plant tissue is an indication that the plants are under fertilized. See **Table 6**. Nitrogen in plant tissue was very low in all areas, indicating that the addition of nitrogen could further increase overall productivity of the rangeland. Tissue levels were not found to be significantly different between the BAS and NBAS sites. Tissue nitrogen levels tended to be higher during the first cutting and lower in subsequent cuttings. Total nitrogen content (dry weight) ranged from 2.35% to 3.64% at the first cutting, from 0.89% to 2.87% in the second cutting and from 1.09% to 2.75% during the third cutting. These tissue nitrogen concentrations allow the calculation of the amount of nitrogen that is present in per ton of plant biomass using the equation: lbs. N/ton = %N * 2000/100.

Table 6: Plant Nitrogen Concentrations

Tubic of Flame Hill object confections								
	4/5/2016	5/12/2016	6/24/2016					
	Total	Total	Total					
	%	%	%					
Site Location	N	N	N					
221-BAS1	3.64	2.28	2.17					
221-BAS2	2.95	2.71	2.34					
221-NBAS 1	2.63	1.22	1.50					
221-NBAS2	2.65	2.10	1.74					
223-BAS1	3.13	0.89	1.04					
223-BAS2	3.12	2.87	1.09					
223-NBAS1	2.35	2.37	2.75					
223-NBAS2	3.24	2.55	2.39					
Forage Crop								
Whole Plant	Nitrogen (%)							
Low	< 4	< 2.5	< 2					
Optimum	4-6	3-4.5	2.5-4					
High	6+	5+	4.5+					

Using both models, the theoretical yield was greater than actual yield in the non-biosolid amended portion of Field 221 and the biosolids-amended portion of Field 223. Two reasons why actual yield may be less than theoretical yield are that there may be a factor other than nitrogen that is limiting yield; and/or, plant available nitrogen may be overestimated in the models. The biosolid amended portion of Field 221 had a greater actual yield than modeled yield using either the soil-based or biosolids application models. See **Figure 14** for a graph of the yields estimated by both models. "Dec '15 soils" estimated yields are based on the soil nitrogen content, and "Nitrogen Based" yields were estimated based on the amount of PAN from the biosolids applications.

Figure 14: Theoretical and Actual Yield



The NBAS site data shows that actual yields are lower than theoretical yields. This indicates that nitrogen is limited in the NBAS sites, and that the application of biosolids, and the nitrogen associated with it, can increase yields in these areas.

Section 6: ANIMAL GROWTH MODEL

To estimate use of pasture feed on growth in cattle and sheep after biosolids application, a model predicting cattle growth was used. The Beef Cattle Nutrient Requirements Model, Version 1.0.37.9, created by the National Academies of Sciences was used to estimate animal growth and body condition maintenance levels (2016). The model is more robust, with well-defined inputs and well validated growth prediction equations based on compositional analysis of feeds, live growth and carcass composition than estimating weight gain based on available forage quantity using other available animal growth models. The National Research Council (NRC) model is a conservative estimate of growth based on breeds, feeds, activity and other environmental inputs (National Academies of Sciences, 2016). This is a static model with fixed inputs and assumes fixed rates of accrual for body condition maintenance, pregnancy, lactation, growth and reserves noted in the top central panels of the model, and as seen in the screenshots found in **Appendix C**. The NRC model assumptions are based on tens of thousands of trials examining feeds and growth responses in feeding trials here in the United States and globally, particularly Europe.

6.1 Model Inputs

The model input variables were based on quantitative forage quality and yield data, and stocking information from interviews with the rancher. A comparison of the upper and lower 95% limit bound of the forage quality parameters indicate the forage from BAS and NBAS sites are substantially similar. As such, the forage nutrition and quality data is considered interchangeable for purposes of the NRC model. Forage yield per cutting described in Section 4 was used to estimate available feed for grazing animals.

Fields that receive biosolids applications were stocked at a density of 1 cow per 13 acres, and 1 ewe (sheep) per acre for the full year. NBAS pastures were stocked at a rate of 1 cow per 26 or 39 acres, and 1 ewe per 2 or 3 acres. Put simply, the potential stocking rate of animals on BAS fields is two to three times higher than NBAS fields. The rancher also indicated a seasonal need to supplement feed on NBAS areas. The NRC modeling used to estimate weight gain and body condition changes was completed using mean forage quality values from BAS sites. The stocked animals are assumed to be 100 pound ewes and 1100 pound (500 kg in the model) cows with calves. The modeled weight gain and body condition includes metabolic requirements for lactation as well.

Animal intake rates vary with available forage type and quality, body weight, pregnancy, lactation and body condition maintenance. Assuming standard intake rate averaged across the year, one cow and 13 ewes consume approximately 88 pounds of dry matter (DM) forage per day, or approximately 2675 pounds of feed per month. The annual intake amount of one cow and 13 ewes is approximately 16 tons.

From the forage quantity data described in Section 4, average forage annual availability (dry weight) is estimated to be:

BAS 3.98 t/ac 51.8 t/13 ac NBAS 2.56 t/ac 33.3 t/13 ac

The t/13 ac value is presented to match the stocking density of 1 cow and 13 ewes per 13 acres. This leaves a theoretical forage residue of approximately 2.7 t/ac in BAS fields and 1.3 t/13 ac in NBAS fields. Interviews with the rancher indicate residual forage may be grazed down by sheep to remove most standing biomass prior to the next growing season.

In addition to BAS fields increasing feed production compared to NBAS fields, analytical results of plant tissue collected in the three cuttings showed no evidence that the concentrations of the trace metals analyzed exceeded values expected to be injurious to grazing animals.

6.2 Predicted Intake and Performance

Intake and performance was modeled using the NRC model for the three months forage samples were collected. These results may be extrapolated to the whole year because sufficient quantity and quality residual forage was available for grazing after the June sampling event. The amount of residual feed would allow animals at the stocking density to graze the field until the next growing season.

Forage consumption in April was predicated on a dry matter intake of 11.16 kg DM/day. Intake rates for May and April were 14 kg DM/day and 15.4 kg DM/day, respectively. Dry matter intake rate was used because it reflects what is actually ingested and digested, and is comparable among all feed types. DM intake rates were adjusted to account for changes in the percent moisture in the feed measured during each forage sampling event. At this level of DM feed intake, feed moisture would not limit intake quantity.

For each modeled month, the initial body weight is assumed to be 500 kg, and 522 kg at the end of the month. This becomes the basis for the required protein and energy to support the estimates for body maintenance, pregnancy, lactation, and growth (body weight gain) in these cattle, based on the NRC prediction equations. Body condition is maintained for each month modeled; energy needs for lactation, growth, pregnancy and reserves are met. The estimated daily weight gain is 0.73 kg/day, or 1.6 lbs./day on BAS fields. To achieve this weight gain in NBAS fields, the acres required per animal would increase by two- to threefold because of differences in available forage.

Forage yields are the primary driver on potential stocking density. Evaluation of feed quality of BAS and NBAS fields indicates there are no substantial differences from an animal nutrition perspective. The NRC model indicates sufficient quantity and quality of forage for grazing animals exists at the stocking density of 1 cow and 13 ewes per 13 acres to allow for maintenance of body condition and other metabolic needs. Given the lower yield of NBAS fields, the stocking density must be less. Application of biosolids allows more animals to be grazed per unit of land.

Section 7: CARBON SEQUESTRATION

Rangelands comprise 36% of the total land area in the United States. In California, rangelands account for approximately 62% of the land area, and in Solano County account for an estimated 38% of the total land area, and 57% of all agricultural land (Solano County, 2009). As a major component of the carbon cycle, rangelands store 10% of the terrestrial biomass of carbon and up to 30% of the global soil organic carbon (SOC) (Schlesinger, 1997, Scurlock and Hall, 1998). SOC represents the largest carbon pool in terrestrial ecosystems and is a major factor in the productivity of agricultural soils. Due to the large land area of rangelands, their cumulative potential to sequester carbon is significant, despite the generally low sequestration rates observed compared to other land types. Rangeland soils have the potential to provide a significant sink for carbon. Management techniques to increase production and fertility and reduce energy costs have been studied for decades; however, knowledge of the role of soil amendment with biosolids and the capacity of rangelands to sequester carbon is limited. Many factors affect the ability of land to sequester carbon including climate, soil type, microbial community, vegetation, grazing patterns, tillage and other land management practices.

Land can sequester carbon in two basic ways, through the production of biomass and in the storage of carbon in soil as SOC and inorganic carbon (carbonate minerals). SOC generally comes from either incidental inputs of soil organic matter (SOM) from crop litter residue or animal wastes, or from the deliberate addition of organic matter such as manure, compost, or biosolids. The incorporation of organic materials to soil represents a direct input of organic carbon into the soil profile and also contributes important nutrients to the soil. In addition to increasing SOC and nutrients, land application of biosolids has been shown to result in soil improvements including: decreases in soil bulk density, and increases in soil pore size, aeration, root penetrability, water holding capacity, and biological activity, all of which may be reflected in an increase in crop yields (Torri, 2014). Increased crop yields translate to increased sequestration capacity as plants incorporate carbon from atmospheric CO₂ into their tissue. For grass and herbaceous plants that dominate Solano County rangelands, storage of carbon in aboveground biomass is short-term, however, long term storage of carbon is observed in belowground biomass (root biomass), especially in well managed land.

Rangelands in Solano County are generally not fertilized. When receiving biosolids applications, material is applied at agronomic rates based on plant available nitrogen. In the study fields, 1610 dry tons and 670 dry tons of biosolids were applied to fields 221 and 223, respectively, to achieve an estimated nitrogen concentration of 200 lbs. N/acre. The carbon content of the biosolids applied to the fields prior to sampling were not reported, however, biosolids are typically 40-70% organic matter, with organic carbon content ranging from 20-50% (Christensen, 2001, Sullivan et al., 2007). Dry (dewatered) biosolids "cake" material consists of approximately 20% total solids (See **Table 7**). Using these assumptions, it can be estimated that the total organic carbon load applied to the study fields in 2015 was between 64.4 to 161 tons to field 221 and 26.8 to 64 tons to field 223. Using median values from these ranges for organic carbon loading (112.7 tons to field 221 and 45.4 tons to field 223) and field acreages, the organic carbon loading rate can be estimated. Field 221 has an area of 282 acres, resulting in an estimated organic carbon

by generator from 2015 Landspreading						
Notification Report						
	Total Solids					
Generator	(%)					
Calistoga	20.3					
Burlingame	14.4					
Central Marin	20.3					
Daly City	28.8					
Delta Diablo	24.7					
El Dorado Hills - Deer Creek	13.1					
Milbrae	21.3					
Pacifica	28.3					
San Mateo	22.9					
SF - Oceanside	15.8					
SF - Southeast	25.4					
Silicon Valley Clean Water	24.5					
Sunnyvale	19.1					
Union Sanitary District	24.5					

Mean

Table 7: Percent total solids in biosolids

loading rate of 780 lbs./acre; field S04-223 has an area of 138 acres, resulting in an estimated organic carbon loading rate of 640 lbs./acre.

Carbon from biosolids applications is bound in SOM, of which there are varying rates of decomposition rates or turnover times. During decomposition, SOM is broken down into various components including carbohydrates, amino acids, proteins, nucleic acids, lipids, tannins, and humus. The makeup of SOM can be categorized into three "pools" in mineral soils, which range from the most labile to least labile (Parton et al., 1987):

- Fast pool: Characterized by a short turnover time, with fast decomposition (e.g. daily to annual). Also referred to as the labile or active pool.
- Slow pool: Characterized by a longer turnover time, with slower decomposition (annual to decadal). Also referred to as the stable or humus pool.
- Passive pool: Characterized by a much longer turnover time (decadal to centennial or millennial). Also referred to as the refractory or recalcitrant pool.

Fast pool components of SOM are the primary source of food and energy for soil microorganisms, and are readily mineralized, releasing much of the carbon back to the atmosphere as CO₂. To enhance carbon sequestration, it is especially desirable to increase the total amount of carbon in the slow and passive pools that break down slowly, since content in the fast pool is more vulnerable to loss if conditions change. Recycled organic materials that have already undergone a decomposition process (e.g., anaerobically digested biosolids) contain organic carbon that is present in a relatively higher recalcitrant fraction (Xiao, 2015). This notwithstanding, not all of the carbon contained in biosolids will remain sequestered in the soil. A portion of the carbon will be lost to processes including erosion, mineralization, and oxidation. The degree to which carbon is lost to mineralization and oxidation is directly related to the makeup of the SOM.

A comparison of total organic carbon in samples collected from the top 1-foot of soil, looking only at differences in samples of BAS versus NBAS soil indicated that biosolids amended soils did have a slightly higher organic carbon content. However, analysis indicated that this difference is not statistically significant (P = 0.255). When assessed as separate sampling events, the difference is greater in samples collected in pre-forage samples, but lacks statistical significance (P = 0.088). Samples collected post-forage showed nearly no difference in total organic carbon content, and any difference has no statistical significance (P = 0.919). A summary of the data is presented in **Table 8**.

In addition to storage of carbon in soil as SOC, land productivity also contributes to its carbon sequestration capacity. Plants capture CO₂ from the atmosphere and incorporate the carbon into tissue. The carbon stored in plant material is utilized by animals as

Table 8: Total organic carbon (as %) of soil samples collected from top 1 foot of soil.									
	All So	amples		Collected De	cember 2015		Collected A	ugust 2016	
	Biosolids	Non		Biosolids	Non		Biosolids	Non	
	Amended	Amended		Amended	Amended		Amended	Amended	
	1.59	0.81		1.59	0.81		1.01	1.01	
	1.25	1.49		1.25	1.49		1.36	1.43	
	1.41	1.04		1.41	1.04		1.14	0.76	
	1.62	1.13		1.62	1.13		0.75	1.14	
	1.01	1.01							
	1.36	1.43							
	1.14	0.76							
	0.75	1.14							
Mean:	1.27	1.1		1.47	1.12		1.07	1.09	
P (two-tail):	tail): 0.255			0.088			0.919		

food or returns to the soil and atmosphere as it decomposes. Increasing the productivity of agricultural land increases the rate at which the plants can capture and store carbon from the atmosphere. Traditionally, increasing the productivity of land comes at the expense of carbon debits. For example, the production of nitrogen fertilizer is estimated to require 0.86 to 1.08 kg of carbon per kg nitrogen fertilizer (West and Marland 2002, Izaurralde et al. 1998). The production of biosolids that are suitable

for land application is also an energy intensive process. However, large waste water treatment plants must dewater and refine sludge to some degree to dispose of the material in landfills, and the process to further refine the sludge into a material suitable for land application or other beneficial reuse is minimal when compared to commercial fertilizer production. While commercial fertilizer amends soils with specific nutrients (typically nitrogen), amendment of agricultural land with biosolids may increase other desirable soil qualities, which may also contribute to increased production of plant biomass. Further, agronomic and environmental benefits, in addition to the potential for enhanced carbon sequestration, attained through applying biosolids to agricultural land may be substantially greater than if the biosolids are disposed of in a landfill.

A comparison of biomass production from biosolids amended land and non-amended land during three sampling events over the course of three months indicates that biosolids amended land is more productive than non-amended land. Analysis confirms that the difference is statistically significant (P = 0.014). The difference is the greatest in samples collected during the first round of forage sampling

(April 2016), and is also apparent in the two subsequent sampling events (May 2016 and June 2016), although to a lesser degree. The decrease in the degree of difference over time is likely attributable to a shift from nutrient limitation during the rainy season to soil moisture limitation as precipitation and soil moisture decreased during the study period. A summary of the results is presented in **Table 9**.

Table 9: Forage Sample Dry Biomass								
	Biosolids Amended			Non-Amended				
		Dry Biomass			Dry Biomass			
Date	Site ID	(g)		Site ID	(g)			
4/5/2016	221-BAS1	76.4		221-NBAS1	24.1			
4/5/2016	221-BAS2	94.7	1 [221-NBAS2	63.6			
4/5/2016	223-BAS1	94.4	1 [223-NBAS1	21.3			
4/5/2016	223-BAS2	105.5	1 [223-NBAS2	34.2			
5/12/2016	221-BAS1	148.6	1 [221-NBAS1	41.5			
5/12/2016	221-BAS2	73.4	1 [221-NBAS2	87.8			
5/12/2016	223-BAS1	44.7	1 [223-NBAS1	53.8			
5/12/2016	223-BAS2	90.3	1 [223-NBAS2	107.9			
6/24/2016	221-BAS1	53.8	1 [221-NBAS1	44.8			
6/24/2016	221-BAS2	97.7	1 [221-NBAS2	70.3			
6/24/2016	223-BAS1	64.6	1 [223-NBAS1	47.2			
6/24/2016	223-BAS2	51.1		223-NBAS2	43.7			
Mean:		82.9	1		53.4			
P (two-tail)	P (two-tail) 0.014							

The carbon content of vegetation is surprisingly

constant across a wide variety of species. Most of the information for carbon estimation described in literature suggests that carbon constitutes between 45 to 50% of dry matter (Schelsinger, 1991), and it can be estimated by multiplying the dry biomass of plant matter by 0.475. Using this assumption, the average carbon content of the biosolids amended soil forage sample is 39.38 grams of carbon, and the average carbon content of non-amended soil forage sample is 25.37 grams of carbon. Samples from all sites were collected using a 1-foot by 1-foot quadrant, representing carbon content in the aboveground portion of plant material at 39.38 g C/ft² and 25.37 g C/ft² (equal to 1.89 tons C/acre and 1.22 tons C/acre), for BAS and NBAS forage respectively. Assuming that by the time the last sample was collected plant community growth had shifted from nutrient limitation to soil moisture limitation, it is reasonable to conclude that the nutritional benefits of biosolids amendment is negligible for the duration of the dry season. Observations of limited regrowth of vegetation at harvested sites during the study period support this conclusion, as does the typical growing patterns observed in and around the study area.

Using this data, and assuming equal productivity in amended and non-amended land outside of the rainy season, it can be estimated that plants growing in biosolids amended soil have the capacity to capture approximately 55% more carbon than plants growing in non-amended soil. The above-ground "shoot" portion of grass biomass removes carbon from the atmosphere, but is not considered long-term storage. Much of the shoot biomass is consumed by grazing livestock. The remaining shoot biomass dies and the residual material ('litter") is reincorporated to the soil as SOM. The residual litter experiences

the same SOM fate discussed above, with a larger portion of the material falling into the 'fast pool' of SOM compared to biosolids material. This bulk of this material is rapidly decomposed, and much of its carbon content is released to the atmosphere.

In grasses, most of the carbon that enters the SOM pool does so through root growth and death. Root-shoot ratios describe the relationship between the dry weight of the plant tissue that have supportive functions (roots) and growth functions (shoots). Root-shoot ratios vary depending on plant type, soil type, nutrient availability, climate, grazing pattern, land use history, and other conditions. Root-shoot ratios also change over the course of a plant's growth cycle. Due to the variation and lack of long-term site specific data, it is difficult to assign an estimate for root-shoot ratios for Solano County rangelands. A 2002 study of root biomass and root-shoot ratios in perennial forage crops indicated that there was little difference in root-shoot ratios observed among the perennial grass species selected for the study. In the first year of production, a root-shoot ratio between 0.58 and 1.0 was observed, and a root-shoot ratio of between 1.15 and 2.32 was observed in subsequent years (Bolinder et al., 2002). Using the median value of ratios for established rangeland vegetation, the root-shoot ratio of Solano County rangeland forage vegetation can be estimated to be 1.735.

When a plant is pruned back, as with grazing, a roughly equivalent amount of root biomass dies off because the shoot portion of the plant can no longer photosynthesize enough to support the entire root system. Over the course of many years, this cycle of root growth and sloughing continually adds carbon to the soil. When roots have a healthy community of mycorrhizal fungi colonizing around the roots, a coating of glomalin is formed. Glomalin is an insoluble, hydrophobic glycoprotein that is resistant to microbial decay. The formation of glomalin around roots protects the root biomass from rapid microbial decay and creates SOC that has a high proportion of more recalcitrant carbon. It can be estimated that approximately 20% of root biomass will be converted to long-term carbon storage in soils (Burkholder, 2017).

Using site specific values for above-ground biomass in biosolids-amended and non-amended soils, root-shoot ratios from Bolinder (1.735), and assuming equal proportions of carbon in shoot and root structures; it can be estimated that the amount of carbon allocated to below-ground biomass is 3.28 tons C/acre and 2.12 tons C/acre in biosolids amended and non-amended soils, respectively. Approximately 20% of the carbon is in the recalcitrant pool of carbon that is considered long-term storage, so a sequestration rate of 0.656 tons C/acre per year and 0.424 tons C/acre per year can be estimated for BAS and NBAS fields, respectively. **Table 10** below summarizes these calculations.

Table 10: Sequestration calculations							
	Biosolids amended soils	Non-amended soils					
Dry aboveground (shoot) biomass (g/ft²) _a	82.9	53.4					
Dry aboveground (shoot) carbon content (g C/ft²) _b	39.38	25.37					
Dry aboveground (shoot) carbon content (tons C/acre) _c	1.89	1.22					
Dry belowground (root) carbon content (tons C/acre) _d	3.28	2.12					
Belowground (root) long term carbon storage (tons/acre) _e	0.656	0.424					

- a Field measured
- b Estimated using assumption from Schlesinger 1991 (carbon content is 47.5% of biomass)
- c Conversion from g/ft² to tons/acre
- d Estimated using assumption from Bolinder 2002 (root-shoot ratio is 1.735)
- e Estimated using assumption from Burkholder 2017 (20% of root mass carbon content is converted to long term carbon storage)

Applying results from the study plots to the fields shows that Field 221 could sequester an additional 58.378 tons of carbon annually and Field 223 could sequester an additional 27.876 tons of carbon

annually through the application of biosolids, based on belowground plant biomass carbon storage alone. Assuming similar conditions exist throughout Solano County, applications of biosolids to all of the available rangeland (approximately 201,040 acres as of 2008), has the potential to increase the amount of carbon sequestration in that land by an additional 46,940 tons of carbon annually. Solano County has restrictions on applications of biosolids to land in proximity to surface water, drainages, homes, groundwater wells, and other sensitive areas, so it is not practical to assume that applications could be made to all of the rangeland acreage. At 50% coverage, carbon sequestration rates could be increased by 23,470 tons of carbon per year.

There are many other aspects to investigate in assessing the potential impact of biosolids application to rangeland's effects on carbon sequestration, which could not be completed during this study. Although available literature indicates that applications of organic material, including biosolids, increases SOC and contributes to carbon sequestration in soils, the data gathered in the study do not support this assertion. To determine the extent of SOC increase from biosolids application further studies would be necessary. This notwithstanding, it is clear from data collected during this study that biosolids application increases forage production and has a significant effect on the lands ability to sequester carbon.

This study not only looked at the effects of biosolids applications on carbon sequestration, but also sought to determine effects to forage production, forage nutrient value, animal growth, vertical transport of trace metals, and soil quality. Future investigations focusing solely on the effects of biosolids application to carbon sequestration could provide a much greater wealth of information by excluding the collection of data irrelevant to carbon sequestration. Gathering data on the amounts and types of carbon in plant tissue and soil, isolating root biomass and carbon content, and collecting a greater number of samples from the top foot of soil more frequently and over a greater time period would provide a more robust site specific data set. It would also remove many assumptions made in this investigation, and yield more accurate results. Because the cycling of carbon in soils takes place on both a short and long time scale (labile pool vs recalcitrant pool), a long-term monitoring program for fields receiving regular applications of biosolids may be required to determine the cumulative effects of regular applications to SOC. Since SOC fluctuates throughout the year as a function of plant growth, death and decomposition, regular sampling intervals would remove temporal effects and yield more relevant data. Geologic carbon cycling is a highly dynamic process that is affected by a multitude of factors, some of which can be manipulated (e.g., management practices) and others which are uncontrollable (e.g., weather). To overcome these variables, it is critical to develop a large data set.

Carbon stored in terrestrial soils represents the largest pool of terrestrial carbon, constituting approximately two-thirds of the total carbon in terrestrial ecosystems (Torri 2014). Globally, soils contain about 3 times more carbon than the atmosphere, and 4.5 times more carbon than in all living things. Therefore, a relatively small increase in the carbon content of soils can make a significant contribution to reducing atmospheric CO_2 levels (Xiao 2015). Historically, agricultural practices have led to a decline in the carbon content of soils through erosion, oxidation from excess tillage, and poor land management. The Ohio State University Carbon Management and Sequestration Center estimates that the worlds cultivated soils have lost between 50 and 70 percent of their original carbon stock, and there is growing concern about the increase in atmospheric CO_2 and other greenhouse gasses. In response to this concern, researchers are assessing sequestration sources for atmospheric carbon. Soils, especially degraded soils, offer a great potential sink for carbon. The addition of carbon to soils in the form of biosolids offers to solve two problems: how to increase carbon content in soils, and how can the residual sludge produced by wastewater treatment plants can be put to beneficial use. The potential for using biosolids application for both agricultural and environmental benefit is a promising solution, and warrants further investigation.

Section 8: Future Research Recommendations

Potential future research and feasibility analyses that could be conducted to expand the current beneficial use of biosolids in Solano County include:

- Feasibility analysis to conduct test applications of biosolids to more productive land
- Potential use of biosolids to condition soils in currently unproductive, fallow or unused land
- Conduct "Cradle to Grave" analysis of benefit of biosolids applications instead of disposal:
 - Research and quantify biosolids energy costs including generation, trucking, landfill disposal direct and indirect costs, greenhouse gas emissions
 - Research and quantify biosolids environmental benefits including carbon sequestration, conventional fertilizer offset (if applicable), increased land productivity, deferral of material from landfills for beneficial uses
 - o Benefit of recycling of nutrients instead of sending them to landfill
 - Offset from conventional fertilizer application if applicable to the system where biosolids are being applied
- Carbon sequestration
 - Consider a Carbon Offset program for biosolids generators similar to King's County (see http://www.kingcounty.gov/services/environment/wastewater/resource-recovery/Loop-Biosolids/Carbon.aspx)
 - Investigate potential use of biosolids to grow tree crops for their significantly higher ability to sequester carbon
 - Economic feasibility of claiming greenhouse gas emission credits for beneficial use of biosolids
- Investigate the potential to apply biosolids to areas with subsurface drip irrigation. This would
 minimize offsite movement/runoff potential while allowing for irrigation in biosolids applied areas.
 Potential to grow feed corn, but this would still require additional synthetic fertilization for optimal
 yields and plant nutrient requirements.
 - Work with NRCS staff to develop list of potential crops that could be grown in a subsurface drip system while allowing for incorporation of biosolids material.
- Conduct groundwater monitoring under biosolids applied fields to see if N movement to groundwater is of concern in areas with higher soil permeability.
- Partner with Dixon RCD to research growing cover crops using fertilization from biosolids in areas
 where crops aren't currently grown for human consumption. Consider benefit to increasing
 groundwater recharge, reducing stormwater runoff and increase infiltration. Analysis would have to
 include evaluation of sites with recharge potential and fit the requirements of the biosolids
 regulations.
- Evaluate potential yields and benefit of biosolid applications to seeded and actively farmed grazing land, and what forage species are optimal
- When proposing new research project, focus on long-term monitoring to track trends over time.
 Need sufficient budget to allow for increased replicates and sample event frequency to allow for more options with statistical analysis and power.
 - o Focus on narrow scopes of study.
 - o Attempt to focus on constituents shown to be significantly between treatments and avoid analytes or topics where there will be lots of non-detects or no significance.

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