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UNIVERSITY OF NORTHERN COLORADO

Greeley, Colorado

The Graduate School

POTENTIAL ENVIRONMENTAL IMPACTS OF OIL  
AND NATURAL GAS PRODUCTION ON  
A SHORTGRASS STEPPE

A Dissertation Submitted in Partial Fulfillment  
of the Requirements of the Degree of  
Doctor of Philosophy

Randi Corrine Lupardus

College of Natural Health Sciences  
School of Biological Sciences  
Biological Education

August 2017

This Dissertation by: Randi Corrine Lupardus

Entitled: *Potential Environmental Impacts of Oil and Natural Gas Production on a Shortgrass Steppe*

has been approved as meeting the requirement for the Degree of Doctor of Philosophy in College of Natural Health Sciences in School of Biological Sciences, Program of Biological Education

Accepted by the Doctoral Committee

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Accepted by the Graduate School

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## ABSTRACT

Lupardus, Randi Corrine. *Potential Environmental Impacts of Oil and Natural Gas Production on a Shortgrass Steppe*. Published Doctor of Philosophy dissertation, University of Northern Colorado, 2017.

Federal lands of the Arapaho and Roosevelt National Forests and Pawnee National Grassland (PNG) lie on the Niobrara play and bring high profits to the State of Colorado. Natural gas development, production, and associated processing activities; however, can be a substantial source of air pollution. Common fugitive emissions on typical PNG Oil and Natural Gas (O&NG) production sites include volatile organic chemicals (VOCs), such as benzene, toluene, ethylbenzene and the xylenes (BTEX). These VOCs can then deposit (wet or dry) onto or transfer (via soil or water) into surrounding vegetation. Minerals, including heavy metals, are also released during the production phase of O&NG development and can deposit near the emission source. There are also impacts beyond pollutants, including habitat loss, fragmentation and the alteration of vegetation communities due to O&NG construction and associated structures. The current study presents novel data related to (1) the ambient levels of common fugitive emissions on typical O&NG production sites (Chapter II) (2) the deposition of these emissions (BTEX) onto proximate flora (Chapter III) (3) the impact on mineral content in proximate flora (Chapter IV) and (4) reclamation success and shifts in plant community structure (Chapter V). In Chapter II, Volatile Organic Compounds (VOCs) were quantified in real-time and used to determine the spatial and temporal windows of exposure for proximate flora and fauna. We found that VOC concentrations

generally increased during the 6 hr. day and were predominately the result of O&NG production and not vehicle exhaust. Thirteen of 24 VOCs had statistically significant differences in ambient levels between production groups, frequently above reference standards and thus at biologically relevant levels for shortgrass steppe flora and fauna. The most biologically relevant VOCs found at concentrations exceeding time weighted average permissible exposure limits (TWA PEL), were benzene and acrolein.

Generalized Estimating Equations (GEEs) were used to measure the relative quality of statistical models predicting benzene concentrations on sites. For Chapters III-V sites were grouped according to status (PA or PR) and production date (spud date). Groups were as follows: PA = Plugged and abandoned in the 1980s, PR1 = Producing since 1980-1990, PR2 = Producing since 2000-2005, and PR3 = Producing since 2006-2013.

We also measured the effects of Distance in all chapters with a maximum distance of 100 m from the wellhead. In Chapter II, *Bouteloua gracilis* (blue grama) and *Bouteloua dactyloides* (buffalo grass) leaves were collected and BTEX were quantified in plant tissue. Deposition and accumulation of BTEX onto proximate flora significantly decreased with production age (PA sites). Newer wells and sites with active pumpjacks had significant concentrations of benzene and toluene in vegetation. BTEX were present on every site except one plugged and abandoned site. The average concentration of toluene on all sites combined was 2.32 ppbv. The average concentration for benzene on all sites combined was 13.18 ppbv, but concentrations were as high as 176 ppbv. These concentrations are arguably biologically relevant as organisms within 100 m of O&NG production sites are likely breathing, and if grazing, consuming high levels of BTEX. In Chapter III, concentrations of minerals in *Bouteloua* leaves were quantified and their

effects on foraging quality were determined. Of the macro minerals, K, P, and S were significantly higher in vegetation found at 25 m and 50 m than in those at 100 m. Calcium was highest in vegetation near PA sites, while P and K were highest in vegetation near PR1 and PR2 sites. Shoot concentrations of Cu, Br, Cr, Pb, Sr and Ba were higher further from the wellhead (100 m), indicating impact further than previously expected. There were still impacts near the wellhead, as Hg was significantly higher in vegetation at 25 m. Concentrations of Mn, Fe, and Ba were all highest in vegetation at PR3 sites, whilst Br was highest on PR1 sites and Sr was highest on PA sites. Concentrations of micro minerals in shoots were in the following order Fe > Cl > Pb > Br > Mn > Sr > Ba > Zn > Cu > Se > Ni > Hg > Cr. Concentrations of Se (5.67 ppm), S (0.33%), and K (1.21%) had the potential to exceed max tolerable concentrations for cattle (based on 2 kg daily mass intake). All other nutrient shoot concentrations were potentially appropriate for grazing cattle, depending on specific cattle and grazing characteristics. Toxic elements such as Br (54 ppm) and Sr (46 ppm) were present in shoot samples far below maximum tolerable levels, while concentrations of Hg (1.54 ppm) and Pb (83 ppm) were beyond daily maximum tolerable levels for cattle when considering a 2 kg DM diet. We also compared shoot nutrient levels to data collected by Fresquez et al. (1991) and concentrations of micro minerals were comparable to *Bouteloua* grown in sludge treated soils, indicating a substantial impact from O&NG production. This impact has had a lasting effect on soils and vegetation as seen with Pb levels on PA sites reclaimed over 30 years ago. In Chapter IV, we characterized proximate vegetation cover, diversity and functionality during well production and following abandonment. In general, PA 100 m sites were distinctly different from all PR

sites. As expected, at 20 m and 50 m, sites had substantially more bare ground and introduced plant species than at 100 m and PR3 sites had the highest percentage of bare ground. There were 16.5% introduced plant species on all plots combined and 2% of plant species sampled were invasive. Satisfactory reclamation was achieved at 50 m on PR1 and PR2 sites as vegetation was at 80% total cover when compared to 100 m. Our PA sites were the highest in plant diversity indices and PR3 were the lowest. With the high cover scores and diversity indices on PA sites it seems recovery over time is possible. We did not find high plant functional redundancy on our O&NG sites; instead, we found high plant species diversity and high functional diversity on PA sites. These disturbed plant communities with greater spatial heterogeneity than blue grama-dominated sites are shifting in community structure. We found that novel intensities of O&NG disturbances along with other synergistic disturbances promoted species and functional shifts in vegetation. The PNG has had an exponential increase in O&NG drilling and extraction in the last decade and results indicate production has caused a novel and biologically relevant impact on native flora and fauna.



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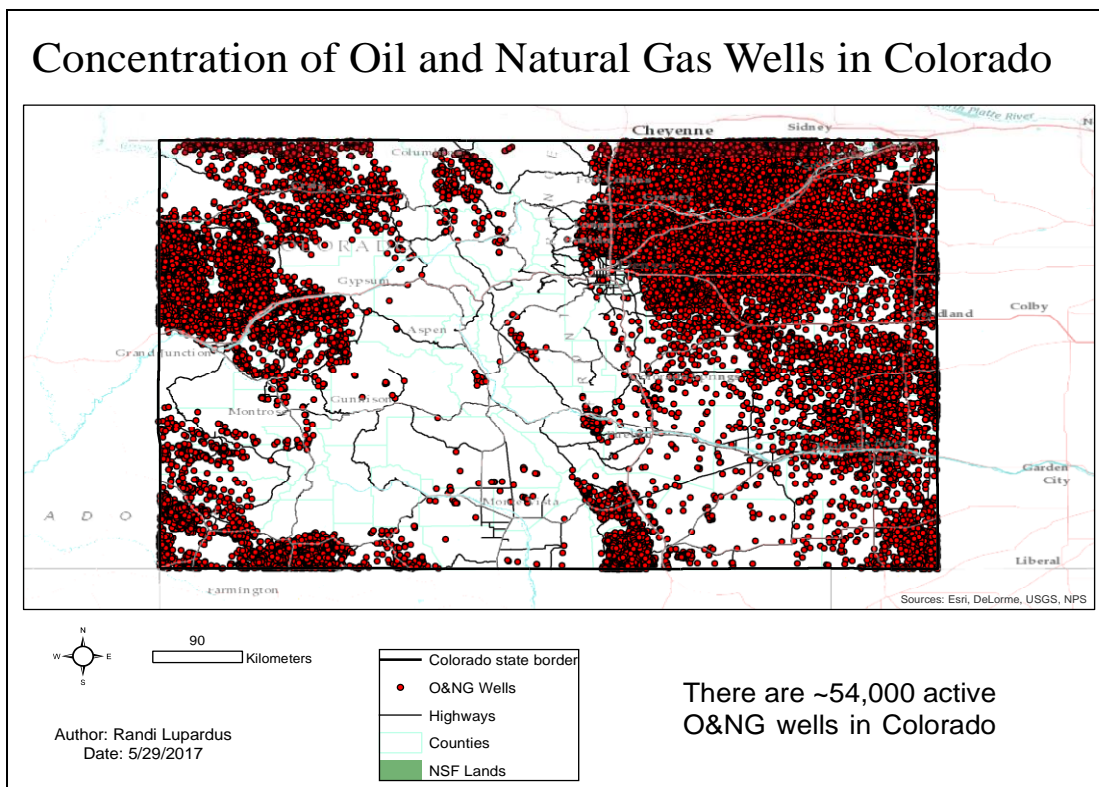
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**CHAPTER I**  
**INTRODUCTION AND OVERVIEW**  
**Oil and Natural Gas in Colorado**

In the last decade, crude oil production in Colorado has quadrupled to 8,261 thousand barrels and natural gas production rose by 51% to 1,704,836 million cubic feet (Mcf; U.S. Energy Information Administration [EIA] 2015a). As a state, we consumed ~ 1,477 trillion british thermal units (BTUs) of natural gas in 2014, exceeding coal, gasoline and renewables. Our total carbon dioxide emissions were 92 million metric tons and our petroleum expenditures exceeded 12 trillion dollars (U.S. EIA 2015a). Over 70% of heating in Colorado comes from natural gas. It is clear that our current system demands are high and have caused production to escalate, largely unregulated and without considering the trade-offs. Our national agencies have made most production decisions without relevant data on ecological impact (Allred et al. 2015). Over 87% of Colorado's 54 thousand active O&NG wells are located in six counties (Weld, Garfield, Yuma, La Plata, Las Animas and Rio Blanco) with 23 thousand oil wells and 90% of Colorado production in Weld county (Swain 2017; Figure 1). Weld was also the highest producing county in Colorado with over a half a trillion cubic feet of natural gas. Some of the top O&NG producers in Weld County include Bonanza Creek Energy Operative Company, Barrett Corporation Bill, Extraction Oil & Gas LLC, Kerr McGee Oil & Gas Onshore LP, PDC Energy Inc., Encana Oil & Gas (USA) Inc., PCD Energy Inc., Whiting Oil & Gas Corporation and Noble Energy Inc. (Swain 2017). Today, producers use

the newest technologies: high volume, slick horizontal hydraulic fracturing from multiwell pads vertically and then horizontally drilling for up to two miles.



*Figure 1.* Concentration of oil and natural gas wells in Colorado.

This work did not examine the impacts of slick horizontal hydraulic fracturing, a single phase in the lifecycle of a well with which most individuals are familiar. The fracturing process includes inserting 2-5 million gallons of fluid (e.g., water proppant, surfactants and other chemicals) into a well at high pressure to fracture shale rock and typically extract a mix of gases and fluids, including methane (U.S. Environmental Protection Agency [EPA] 2011). The current research specifically focuses on the environmental impacts of the O&NG production phase, which begins after well completion and continues for the life of the well. Impacted land is typically restored or

reclaimed once the well enters the production phase. Maintenance activity continues on site, but the overall level of activity on site declines in the production phase. Depending on the type of extraction on site, infrastructure could include condensate tanks, flare stacks, pump jacks, evaporation pits, and a number of other semi-permanent structures (Figure 2).



*Figure 2.* Example oil and natural gas site infrastructure on the Pawnee National Grassland. A = Full site, B = pumpjack, C = condensate tanks, D = Evaporation pit, E = older tank and stack.



*Figure 2, Continued*

### **The Pawnee National Grassland (PNG)**

For the current research, we studied the potential effects of O&NG production in a specific region of Northeastern Colorado where there is a growing prevalence of

production, the Pawnee National Grassland (PNG). The PNG covers 193,060 acres (79,876 ha) and lies east of the Rockies at an elevation of 1,500 to 1,800 meters (Desalme et al. 2011). The climate is typical of mid-continental semiarid temperate zones but is somewhat drier because of a strong rain shadow effect of the Rocky Mountains to the west. Annual precipitation, and its seasonal distribution, profoundly influences this semiarid grassland. Precipitation-induced changes cascade through the ecosystem, causing fluctuations in vegetative structure, the abundance and species composition of biotic communities, and ecosystem functions such as net primary productivity, nitrogen mineralization and trace gas fluxes. Average temperatures range from 15.6 °C in summer to 0.6 °C in winter and mean annual precipitation is 310 mm. In Colorado, temperature has increased by 1°C since systematic measurements began in 1895 (Stohlgren et al. 2008). Annual variation in temperature and precipitation has been significant and has fluctuated at irregular intervals between warm-dry years and cool-wet years (Stohlgren et al. 2008).

The PNG is classified as a shortgrass prairie region, also known as a shortgrass steppe. The shortgrass steppe is distinguished by the height of its dominant grasses, which includes *Bouteloua gracilis* (blue grama) and *Bouteloua dactyloides* (buffalo grass). Less than 50% of the ground in the PNG is covered by vegetation (Hazlett 1998). Soils on the site consist of 90% stoneham fine sandy loam from 0 to 13 cm, clay loam from 13 to 20 cm and loam from 20 to 36 cm, with 0 to 6% slopes (U.S. Department of Agriculture [USDA] 2014a). In some regions erosive forces have worn away loam to reveal shale, sandstone and siltstone (Crabb 1981). The stratigraphy of the region includes carboniferous to tertiary sedimentary rocks. Layers of cretaceous include

Laramie formation, Fox Hills sandstone, Pierre shale, Niobrara formation, Benton shale and Dakota Group (Crabb 1981). These shales contain commercial quantities of oil. The PNG is used extensively for irrigated agriculture and livestock and is a patchwork of private and government land.

The PNG is administered by the U.S. Forest Service District Ranger in Ault, Colorado and is managed under the Arapaho-Roosevelt National Forest, which officially encompasses the PNG. Congress establishes the laws for Forest management, the Department of Agriculture establishes regulations and the Forest Service make decisions on site, deciding on specific policies and management goals such as O&NG restoration success. Mineral leases have been profitable on the PNG since the 1980s, when there was a large boom in O&NG development (Duram 1995). The Forest Service is a land surface agency, thus the Bureau of Land Management (BLM) handles subsurface leases, but the Forest Service can disapprove a mineral lease and have done so in the past. Specifically, in 1990 the Forest Service halted new leases on the PNG because the shortgrass steppe provides habitat for the mountain plover, a category one protected species, and O&NG production had the potential to destroy plover nesting sites (Duram 1995).

The PNG is divided into East and West landmasses, with available O&NG leasing on all public portions except the Crow Valley Campground and the Pawnee Buttes (Figure 3). Although the newly approved Environmental Impact Statement for PNG Oil and Gas Leasing has stipulations to protect the surface of the PNG from O&NG infrastructure, flora and fauna are likely impacted beyond surface disturbance (U.S.Department of Agriculture [USDA] 2015). The Environmental Impact Statement



did not require quantification of impact on air quality (specifically VOCs), waters, soil or vegetation, but instead used existing data to quantify impacts.

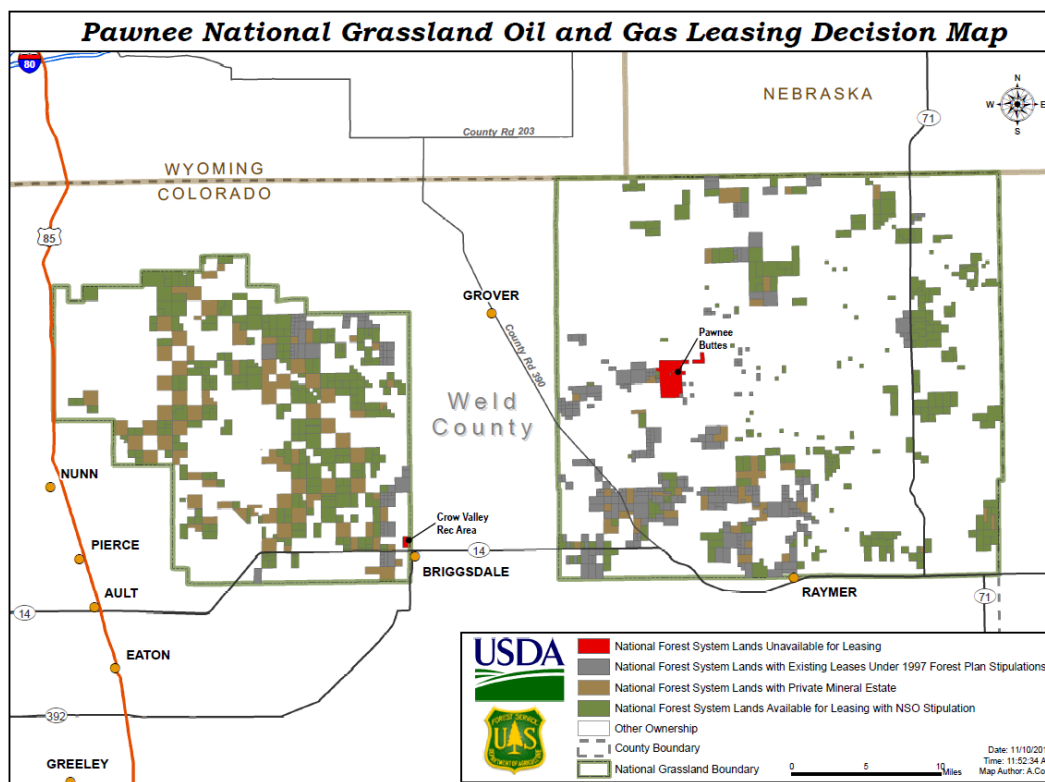


Figure 3. Pawnee National Grassland oil and gas leasing decision map. Source: USDA (2015).

### Potential Impacts on the Pawnee National Grassland Shortgrass Steppe

When I began my literature search on the potential impacts of oil and natural gas in 2012, there was very little existing data. Many individuals of the public, including myself, were fearful of the potential human and environmental impacts of O&NG development. Mainstream media demonized the O&NG industry and especially new slick horizontal hydraulic fracturing techniques. The fear came from a very real place, a lack of

solid environmental and human health analysis from the scientific community. As a citizen, I wanted to make an informed decision on whether or not to support production, but the data simply did not exist. As a scientist, I knew there was a gap in knowledge and so for the sake of federal management and regulation, I decided to conduct this politically driven, but necessary research.

At the time, researchers were making efforts to characterize emissions from O&NG development and to estimate the potential risks to human biotic and environmental health. Shale gas development, production, and associated processing activities were potentially a substantial source of air pollution (Archuleta and Adlhoeh 2009; Katzenstein et al. 2003). In Texas, blood and urine samples taken from household residents near shale wells revealed that toluene was present in 65% of those tested and xylene present in 53% (Rahm 2011). Fracturing fluids contained harmful chemicals such as carcinogenic BTEX: benzene, toluene, ethyl benzene, xylene as well as naphthalene, formaldehyde and silica, yet shale companies had been granted exclusions from the Clean Air and Clean Water Acts that regulated these chemicals (Colborn et al. 2011). McKenzie et al. (2012) found that cumulative cancer risks were 10 in a million and 6 in a million for residents living  $\leq 1/2$  mile and  $> 1/2$  mile from wells in Colorado, with benzene as the major contributor to the risk. Produced (i.e., recovered) waters contained hydrocarbons, technologically enhanced naturally occurring radioactive materials (TENORMS), and heavy metals (Howarth et al. 2011) with concentrations of radon (Ra) and barium (Ba) commonly hundreds of times the US drinking water standards (Haluszczak et al. 2013). Only 10% of produced water was making it to the surface (Vidic et al. 2013) and was typically reinjected into deep wells (Maloney and Yoxtheimer 2012). Most fluid was left

underground with unknown movement and consequences. Increased seismic activity, (Frohlich and Brunt 2013) fragmented habitats and other abiotic effects were largely unknown, but increasingly associated with energy development and infrastructure (i.e., roads, pipelines, waste pits, storage, processing facilities and drill pads; Weiler et al 2002; Doherty et al 2008; Sawyer 2009; Holloran et al 2010). The expansion of O&NG production across the grasslands was shown to exacerbate degradation, fragmentation and habitat loss (Nasen et al. 2011; Slonecker et al. 2012) and had the potential to change landscape dynamics, ecosystem functionality, vegetation communities (Smith et al. 1988; Simmers and Galatowitsch 2010) soil structures (Rowell and Florence 1993) and wildlife populations (Naugle 2011). Impacted populations included birds (Ingelfinger and Anderson 2004; Aldridge and Boyce 2007; Gilbert and Chalfoun 2011; Hamilton et al. 2011; Ludlow et al. 2015) and ungulates (Sawyer et al. 2006; Sawyer et al. 2009; Beckmann et al. 2012). Reclamation success on the grassland had not been studied (long-term) and it was unknown if adequate grazing lands have been reduced on the PNG. Chemicals released from active and producing wells potentially had the ability to deposit (e.g., wet or dry) to surrounding plants (Karl et al. 2010, Rodriguez et al. 2012), soils (Bloomfield et al. 2012) or waters (Hayes 2009), but empirical data were lacking. This deposition had the potential to affect species and ecosystem health of proximate flora.

### **Project Description**

Ecosystem flora, fauna and processes change across space and time. Management plans, therefore, must be adapted with continual revisions of goals based on experimentation and monitoring. This research was conducted using new knowledge, technology and inventory to best assess the true environmental impacts of O&NG

production on the PNG shortgrass steppe. The first objective of the proposed research was to quantify types and amounts of VOCs in the atmosphere, released from production sites. These transient compounds were quantified in real-time and were used to predict the temporal window of exposure for proximate flora and fauna. The second objective was to quantify the deposition and accumulation of BTEX onto shoots of proximate flora (i.e., *Bouteloua gracilis* and *Bouteloua dactyloides*). This deposition might affect species and ecosystem health of proximate flora and fauna that utilize that flora in some way. Shale drilling could include any number of negative effects on the flora at an organism level (Arif and Verstraete 1995, U.S. EPA 2010, Desalme et al. 2011, Taulavuori et al. 2012) and many of these are indirect effects of pollutant overload. The third objective of the proposed research was to quantify micro mineral values, including heavy metals, in *Bouteloua gracilis* and *Bouteloua dactyloides* leaf tissues. This is yet another index of how oil and gas extraction effects mineral (including heavy metal) accumulation and causes a reduction in growth, performance, and yield (Chibuike and Obiora 2014). The fourth objective was to characterize proximate vegetation diversity and functionality indices, to estimate reclamation status during well production and following abandonment of O&NG sites and to assess potential species and community shifts. The impact of O&NG on water and soil were not assessed in the current study due to permitting complications, although they are necessary to understanding cumulative impacts on the PNG.

## CHAPTER II

### AIR QUALITY

#### Abstract

Federal lands of the Arapaho and Roosevelt National Forests and Pawnee National Grassland (PNG) lie on the Niobrara play and bring high profits to the State of Colorado. Natural gas development, production, and associated processing activities, however, can be a substantial source of air pollution. The current study presents some initial results attempting to quantify the ambient levels of common fugitive emissions on typical PNG Oil and Natural Gas (O&NG) production sites. Volatile Organic Compounds (VOCs) were quantified in real-time and used to determine the spatial and temporal windows of exposure for proximate flora and fauna. Our hypotheses were that (1) VOC concentrations would significantly differ among Pump Groups (2) VOC concentrations would be dependent on wind direction (3) VOC concentrations would decrease from 8:00 a.m. to 2:00 p.m. (4) VOCs are from O&NG and not from other sources, and (5) VOC levels frequently exceed reference standards. Eleven O&NG sites on the PNG in Northern Colorado were randomly selected and grouped according to production, along with 13 control sites from three geographical locations. At each site, samples were collected 25 m from the wellhead in NE, SE and W directions. In each direction, two samples were collected with a Gaset DX4040 gas analyzer every hour from 8:00 a.m. to 2:00 p.m. (6 hours total), July-October, 2016 ( $N = 864$ ). PERMANOVA results indicated that Pump group was a significant predictor variable

( $F = 26.9$ ,  $p < 0.001$ ), while Direction was not ( $F = 1.3$ ,  $p = 0.261$ ). The VOCs were found on all sites, including controls, generally increased during the 6 hour day and were predominately the result of O&NG production and not vehicle exhaust. Thirteen of 24 VOCs had significantly different levels between groups, frequently above reference standards, and thus, at biologically relevant levels for shortgrass steppe flora and fauna. The most biologically relevant VOCs, found at concentrations exceeding time weighted average permissible exposure limits (TWA PELs), were benzene (a known carcinogen) and acrolein. Generalized Estimating Equations (GEEs) were used to measure the relative quality of statistical models predicting benzene concentrations on sites. The data not only confirms that O&NG emissions are impacting the region, but also that this influence is present at all sites, including controls.

### **Introduction**

As of December 28, 2016 there were 86,054,487 barrels of oil (bbl) produced and 525,517,737 thousand cubic feet (McF) of coalbed and natural gas produced in Weld county (Colorado Oil and Gas Conservation Commission [COGCC] 2016). The exact quantity of recoverable oil in the Niobrara shale is unknown, but it has been estimated that there could be close to seven billion barrels. Of the approximately 54, 000 producing wells in the State of Colorado, 22,774 are located in Weld County (Weld County 2016). There are hundreds of oil and natural gas (O&NG) operations located on private parcels within the Pawnee National Grasslands (PNG) in Northeastern Colorado, yet only approximately 60 O&NG sites and 20 associated production facilities are on Bureau of Land Management (BLM) owned lands. The Arapaho and Roosevelt National Forests and Pawnee National Grassland increased oil and gas leasing in December 2015 to

include all remaining available land for leasing. Although production companies on these new leases must abide to a no surface occupancy stipulation (prohibiting surface disturbance on the leasehold), existing and future operations still have an impact on air quality.

Fugitive emission sources include leakage from wells, well-site treatment facilities, storage tanks and facilities, pipelines, flare stacks, pneumatic devices, compressors and other associated temporary and permanent structures (Armendariz 2009). As production increases, so will concentrations of volatile organic compounds (VOCs), yet there are limited quantitative data of VOC emissions, making their impact nearly impossible to quantify or regulate. From 2010 to 2015, the number of producing natural gas wells in Colorado increased from 28,813 to 46,322, placing Colorado in the country's top five for natural gas (NG) well density (U.S. EIA 2015a). In 2015 Colorado produced 1,704,836 Mcf of natural gas from gas, oil, coalbed, and shale wells (U.S. EIA 2015a), and three out of every 100 barrels of U.S. crude oil production came from Colorado (U.S. EIA 2015b). Greenhouse emissions in Colorado are projected to increase by 10 % for the period from 2010 through 2030. This compares to an increase of approximately 56% during the 20 year period from 1990-2010 (Arnold et al. 2014). Production of O&NG in Colorado is expected to increase during the next few decades, but there are little data regarding the impact of O&NG sites on VOC concentrations. The goal of this research is to quantify VOC emissions from O&NG production sites to determine if levels meet regulatory standards.

Energy-related activities including O&NG production in the United States account for 75% of anthropogenic greenhouse gas (GHG) emissions, and a major portion

of emissions include carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>), which are a focus of this study. O&NG systems were the second largest stationary source of emissions in the U.S. in 2015, resulting in 231 million metric tons of greenhouse gas emissions (U.S. EPA 2016a). Reported emissions for 2015 were 1.6 percent lower than 2014, but 4.1 percent higher than 2011 (U.S. EPA 2016a). Emissions from natural gas are consistently underestimated using current inventory methods and empirical concentrations are double the estimated values (Brandt et al. 2014; McKain et al. 2015).

There is minimal understanding of O&NG production impact on air quality. Estimated emissions on the PNG and related values have been developed for a select few volatile species, including nitrogen oxide (NO<sub>x</sub>), lead, carbon monoxide, ozone, particle pollution (PM<sub>2.5</sub>) and sulfur dioxide. These compounds are studied on the regional scale by government agencies, because they are regulated under the National Ambient Air Quality Standards.

Although unregulated under the clean air act, one of the major air pollutants of O&NG production is methane, which has been measured at levels well beyond regulatory emission inventories (Brandt et al. 2014; Pétron et al. 2014). Under the EPA's Greenhouse Gas Reporting Program (GHGRP), the Sterling Energy Investments LLC Centennial Gas Plant near the PNG reported their total facility emissions by gas in metric tons for 2015 as: Carbon Dioxide (CO<sub>2</sub>) 32,501, Methane (CH<sub>4</sub>) 3,986, Nitrous Oxide (N<sub>2</sub>O) 16 (U.S. EPA 2017). Nearly one-third of methane emissions in the United States come from O&NG production, transmission and distribution (U.S. EPA 2016a). If President Obama's Climate Action Plan remains in place, methane emissions could be reduced by 40 to 45 percent by 2025 with the first-ever federal standards requiring



companies to monitor and limit emissions (U.S. EPA 2016b). The new regulations not only will reduce methane emissions primarily, but will also reduce ozone-forming VOCs. Ozone is another major surface air pollutant that adversely affects human health and vegetation (Jerrett et al. 2009; Anenberg et al. 2010). Tropospheric ozone is produced as a result of a complex relationship between VOCs and oxides of nitrogen (NO<sub>x</sub>). In the presence of light, NO<sub>x</sub> and VOCs can interact on site to produce ozone, which can cause a series of physiological problems, such as reduced biomass or immunodeficiency in flora and fauna (Bernard et al. 2001; Levy et al. 2001; Godish et al. 2015). In the summer, when temperatures and traffic are at their highest on production sites, tropospheric reaction rates are greatest, and thus the formation of tropospheric ozone is greatest. This is complicated by the fact that VOC/NO<sub>x</sub> ratios generally increase as an air mass moves downwind from major NO<sub>x</sub> sources, which makes quantifying ozone complicated. Generally, VOCs are highest in winter months while ozone is at its lowest and vice versa during summer months. Although VOC levels are lower in the summer, they are still present on O&NG sites and there is increased potential for ozone formation, if not at the wellhead, downwind from the source. Helmig et al. (2014) analyzed surface and vertical profiles of VOCs in the Uintah Basin, Utah, during the winter of 2013 and found that concentrations of VOCs such as benzene and toluene were 5-10 times higher values reported over major US cities. Elevated non-methane hydrocarbons such as alkanes have been reported in air samples collected using aircraft over O&NG production sites (Katzenstein et al. 2003; Pétron et al. 2012). However, there is still a need for in-depth, on-the-ground, real-time monitoring at production sites to quantify daily exposure to flora and fauna.

A well can produce oil, natural gas or, as with most of the O&NG sites in northeastern Colorado, wet or associated gas (i.e., natural gas coproduced with oil). Development activities are a source of air pollutants during all stages of well development and extraction for each of these three scenarios (Brown et al. 2015; Field et al. 2014; Olaguer 2012; Roy et al. 2013). Brown et al. (2015) found maximum 6 hr peak values of VOCs on unconventional (i.e., horizontal) natural gas sites varied according to source of emissions. During well development, VOC sources include: drill rigs, fracturing pumps, truck traffic and well completion. During gas production, sources include: production fugitives, pneumatics, wellhead compressors, blowdown venting, heaters and condensate tanks. Midstream sources include dehydrators, compressor stations, and fugitives from transmission and processing (Roy et al. 2013).

For sites with pumpjacks that produce oil or a combination of O&NG, the types and concentration of air pollutants can vary depending on the infrastructure at each site and the activities present. For example, during the drilling phase, rigs and pumps powered by large diesel engines emit oxides of nitrogen (NO<sub>x</sub>), fine particulate matter (PM 2.5 μm) and VOCs (U.S. Environmental Protection Agency [EPA] 2004; 2013a 2013b). Trucks are used to transport materials to and from the sites, not just during drilling phases, but sometimes throughout the life of the well (i.e., 30-50 years). These diesel-powered trucks emit the same NO<sub>x</sub>, VOCs and PM 2.5 (U.S. Environmental Protection Agency [EPA] 2005). In the current study, traffic (i.e., number of roads within 1 km of a site), well density (i.e., number of wells within 1 km of a site), quantity of oil and gas produced the month of data collection, as well as month of data collection (i.e., time) are variables that we have accounted for in our analyses. Completion venting is

another large contributor to VOCs (Bar-Ilan et al. 2008; Armendariz 2009). Although each of the study sites in the current research have a vapor recovery system and a VOC combustor attached to the venting stacks, these are not 100% effective, and therefore some release of VOCs is expected. Natural-gas-fired compressors, used to maintain gas pressure during production, emit VOCs and NO<sub>x</sub> (Bar-Ilan et al. 2008; Grant et al. 2009). Flashing emissions also release VOCs each time oil or liquid condensate is transferred from the on-site separator into an on-site storage tank (Pétron et al. 2014). Sites with a combination of the above activities will likely have higher levels of VOCs (Table 1).

Speciation profiles such as the U.S. Environmental Protection Agency's (U.S. EPA 2014) SPECIATE database U.S. and other VOC reports (e.g., Hendler et al. 2009) provide estimates of emission composition and are used in air quality models and emission inventories. They are helpful when creating speciation profiles for VOCs on O&NG sites. Some categories of VOCs and sources include: alkenes (diesel engines), aromatics (diesel engines), alkanes (diesel engines, venting, and fugitives) and aldehydes (diesel- and natural-gas-fired engines; see Table 1).

Table 1

*Volatile Organic Compound (VOC) Categories By Source*

VOC category	Gasmeter DX4040 VOCs	VOC Source
alkenes	1,3-butadiene	compressor engines (Roy et al. 2013)
aromatics and cyclic hydrocarbons	benzene, toluene, ethylbenzene, xylene, 3-ethyltoluene, 1,3,5-trimethylbenzene, cyclohexane	compressor engines (Roy et al. 2013) raw natural gas and flashing emissions from storage tanks (Pétron et al. 2012) produced water tanks, crude oil and condensate loading and transportation, natural gas dehydration and processing operations, flares (Pétron et al. 2014)
alkanes	<i>n</i> -pentane, methane, hexane, heptane, <i>i</i> -pentane,	condensate tanks, oil tanks (Armendariz 2009) compressor engines, venting and fugitives (Armendariz 2009; Roy et al. 2013) raw natural gas and flashing emissions from storage tanks (Pétron et al. 2012) pneumatic devices and pumps, equipment leaks (Pétron et al. 2014)
aldehydes	acetylaldehyde, formaldehyde, acrolein	diesel- and natural-gas-fired engines, cleaning agent (Roy et al. 2013)
sulfides	carbondisulfide, carbonyl sulfide (precursors to hydrogen sulfide)	fugitive emission (production additive; Agency for Toxic Substances and Disease Registry [ASTDR] 2012)
ketones	methyl ethyl ketone	fugitive emission (solvent, cleaning agent; U.S. Environmental Protection Agency [EPA] 1994a)
oxygenated species (GHGs)	nitrous oxide, carbon dioxide, carbon monoxide	venting and fugitives, condensate tanks, oil tanks, compressor engines (Armendariz 2009) heaters, wellhead compressors (Roy et al. 2013)

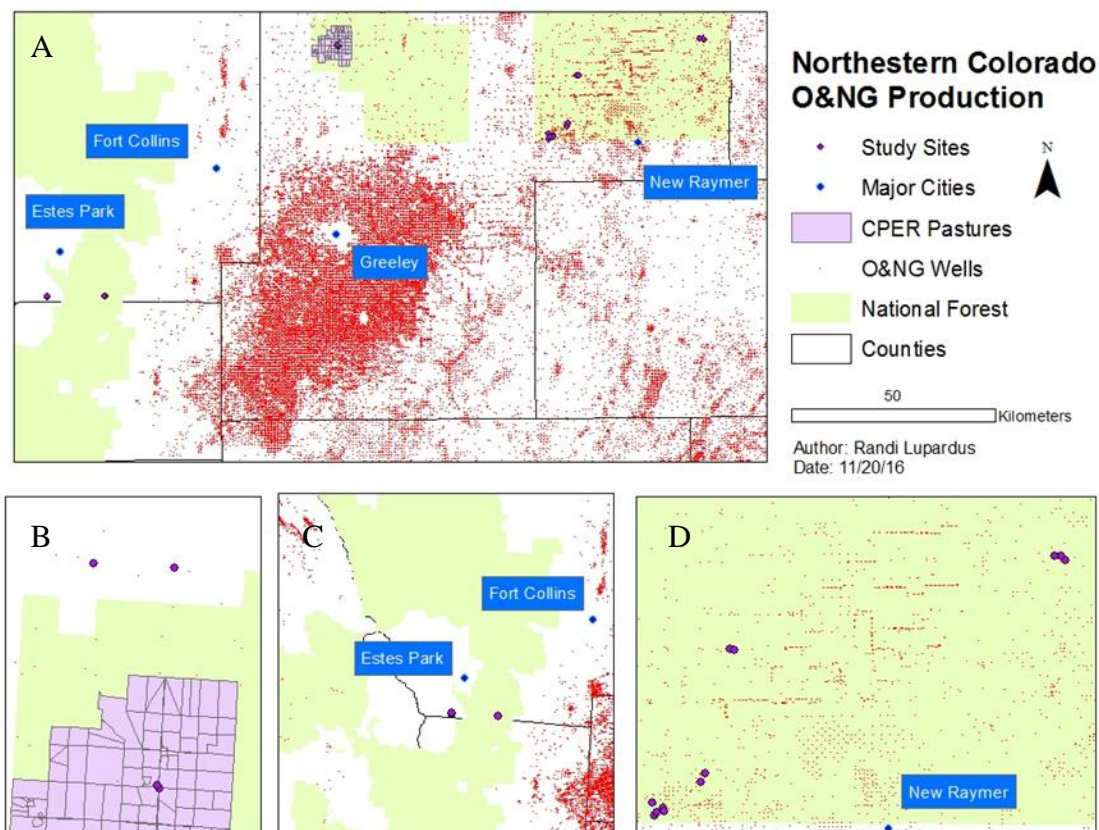
The objective of this study was to quantify daily fugitive emissions of VOCs, 25 m from the source (wellhead), in three directions (NE, SE, and W), and on three different types of production sites (Pumping, Nonpump, and Nojack). We compared concentrations of VOCs on production sites to background concentrations of VOCs on control sites from three geographical locations including western PNG (WPNG), eastern PNG (ControlE), and Roosevelt National Forest (Mountain). These transient compounds were quantified in real-time and used to determine the spatial and temporal windows of exposure for proximate flora and fauna. Our hypotheses were that (1) VOC concentrations would significantly differ among groups (2) VOC concentrations would

be dependent on wind direction (3) VOC concentrations would decrease from 8:00 a.m. to 2:00 p.m. (4) VOCs were from O&NG and not from other sources, and (5) VOC levels frequently exceed reference standards.

## **Methods**

### **Site Selection**

A majority of the study locations ( $n = 21$ ) were on the Pawnee National Grassland (PNG) in Northeastern Colorado (Figure 4). The PNG covers 193,060 acres (79,876 ha) and lies east of the Rocky Mountains at an elevation of 1,500 to 1,800 meters. The PNG is classified as a shortgrass prairie region, also known as shortgrass steppe. The three-decade averages of climatological variables (1981-2010) include an annual average temperature of 6.4 °C (43.6 °F) and mean annual precipitation of 42.62 cm (16.78 in; National Oceanic and Atmospheric Administration [NOAA] 2017a). Four of the control study sites were located in the Roosevelt National Forest near Estes Park, Colorado, which has an annual average temperature of 5.7 °C (42.4 °F) and mean annual precipitation of 45.74 cm (18.01 in; NOAA 2017b)



*Figure 4.* Air quality study locations. Of the 25 study sites (purple dots), there were four control sites near Estes Park, Colorado (C) six on West PNG near the CPER (B), and 15 on the Eastern PNG (D) including Pumping sites and Control sites. Red dots indicate actively producing oil and natural gas sites in the State of Colorado.

The PNG producing sites were narrowed down to only those on National Forest Service land for permitting purposes. O&NG sites were randomly selected and grouped according to production, which we called Pump Groups. There were three pump groups, Pumping, Nonpump and Nojack. The Pumping group ( $n = 5$ ) were sites with wet production (i.e., oil and natural gas produced) and an active pumpjack. The Nonpump group ( $n = 3$ ) were similar to the pumping group, in that they had wet production, yet the pumpjack was inactive (i.e., non-pumping). The Nojack group ( $n = 3$ ) had dry production (i.e., natural gas) with a wellhead, but no pumpjack. This analysis represents typical well

production over the life of the well, beyond an initial production peak. All wells in the study had been producing gas for at least three years at the time data were collected in the summer of 2016; therefore, long-term yearly production rates should be similar. The next three groups, WPNG, Mountain and ControlE, do not have O&NG production and should be considered control sites lending background levels from three geographical regions. Six grassland sites, at least 1km from O&NG production, were randomly selected near the USDA Central Plains Experimental Research Station (CPER) on the PNG (WPNG;  $n = 6$ ). These sites were selected to represent ambient VOC (i.e., background) concentrations on the western land mass of the PNG (Figure 4). Three sites were randomly selected in northeastern PNG where shale production is less dense than any other area on the PNG (ControlE;  $n = 3$ ) and four control sites were randomly selected near Estes Park Colorado in the Roosevelt National Forest (Mountain  $n = 4$ ).

#### **Analysis of Volatile Organic Compounds by Gaset Portable FTIR Gas Analyzer**

From July-October 2016, samples were collected at 25-meter distances from the well in NE, SE and W directions, assuming generally westerly winds. At each of the 25 sites, two or more samples were collected with a Gaset DX4040 gas analyzer every hour in each direction, for 6 hours total, from 8:00 a.m. to 2:00 p.m.. Hourly sample quantity was limited by instrument power (i.e., battery life). To account for lower temperatures and photoreactivity, samples were collected from 10am-4pm on the Roosevelt National Forest sites. Each measurement is a 60 sec average with 10 scans / sec (10 Hz) totaling 120 scans/hour in each direction.

The Gaset DX4040 gas analyzer combines fourier transform infrared (FTIR) spectrometer, rhodium-gold coated sample cell, built-in sample gas pump and signal

processing electronics in a compact, portable unit (see Appendix A for Technical details). Before choosing the Gaset DX4040 for the experiment, multiple measurement methods were experimented to determine the best method of VOC collection and analysis. Over radiello® passive/diffusive samplers and canister samples, the field-based FTIR gas analyzer was chosen for several reasons. Conventional technologies, such as adsorption cartridges, summa canisters and stationary automated gas chromatographs, limit researchers in the field. The DX4040 has the ability to follow real-time gas concentration changes and gives the researcher the ability to analyze and study results within minutes of collection. With access to real-time results, the operator can modify the experimental design whenever needed and troubleshoot on site if there is an anomaly or impact from extraneous variables such as weather. The nondestructive FTIR analyzer is capable of rapid measurements for several gases simultaneously on-site and only required zero calibration with nitrogen gas twice daily (i.e., at 7:30 a.m. and 12:00 p.m.). Both the passive filters and canisters allow for high sensitivity and low detection limits (ppb), but they are costly and time exhaustive methods. Fortunately, the concentrations found on each of the active sites were in the ppm range, which was ultimately the deciding factor when choosing this method and instrument for analysis.

The analyzer came with a gas application library pre-programmed to measure 25 gasses, five of which were preset: Carbon dioxide, methane, nitrous oxide, carbon monoxide and water vapor. The other 20 gases were selected by the researchers based on literature review and preliminary data collection confirming VOC presence on O&NG sites: benzene, toluene, ethyl benzene, m-xylene, o-xylene, p-xylene, acrolein, acetaldehyde, formaldehyde, 1,3-butadiene, *i*-pentane, *n*-pentane, hexane, heptane,



3-ethyltoluene, 1,3,5-trimethylbenzene, cyclohexane, methyl ethyl ketone, carbon disulfide, carbonyl sulfide (Swarthout et al. 2013). These gases were then grouped according to their chemical description (i.e., VOC category) and source (i.e., VOC source; see Table 1). Ozone and hydrogen sulfide were not available in the library. Carbon disulfide hydrolysis forms hydrogen sulfide and so it was chosen to indicate a potential source of hydrogen sulfide on sites. Hydrolysis of carbon disulfide forms the reactive carbonyl sulfide intermediate. This reacts with a water molecule, ultimately forming hydrogen sulfide. The potential for formation of toxic, gaseous hydrogen sulfide increases with precipitation (i.e., water vapor levels). Researchers purposefully avoided collecting samples on days with exceptionally high winds and or rain.

### **Statistical Analysis**

Analyses were performed using SAS 9.4, PCORD 6.0, RStudio 1.0.136 and R package Openair 2.0. Values were averaged over the six hours for each direction (W, NE, SE), yielding three values per site, one for each direction. A Box's M-test for Homogeneity of Covariance Matrices indicated equal variance across sites ( $p = 1$ ). A Henze-Zirkler's Multivariate Normality Test indicated that the data are not multivariate normal, thus a permutational multivariate analysis of variance (PERMANOVA) model was used to distinguish differences in the position and/or spread, in a multivariate space, of the compared groups' VOCs. This nonparametric test is preferred to MANOVA, as it is distribution free, allows for fewer samples than variables in the model, is insensitive to excess zeros and is robust to violations of homogeneity of variance-covariance matrices.

To test if the six hour average VOC levels would be similar among production groups, a PERMANOVA was run including all chemicals as response variables and two

independent factors: Production Group and Direction. Based on significance, follow up analyses included multiple ANOVAs, one for each dependent variable, using the average of samples for all six hours. For models with  $p < 0.05$  in the ANOVA global test, Student-Newman-Keuls post-hoc tests indicate significant between-group differences. The independent variable used in the analysis was Pump Group, which had six levels (Pump  $n = 4$ , Nonpump  $n = 3$ , Nojack  $n = 3$ , WPNG  $n = 6$ , ControlE  $n = 3$ , Mountain  $n = 4$ ) and the dependent variables were VOCs (ppm). Each of the VOCs were tested for homogeneity of variance and only four (i.e., benzene, acetaldehyde, heptane, hexane) needed to be log transformed to meet homogeneity assumptions.

The PERMANOVA determined wind direction was not a significant predictive variable in the model, although we interpreted trends in daily, directional concentrations; Thus, data were analyzed using descriptive statistics. We created a wind rose to show proportion of wind speed and direction over the entirety of the study. To create the wind rose, data from the National Ecological Observatory Network (NEON) Data Portal (2017) were used. The Central Plains Experimental Research (CPER) station tower data were used for all sites except the Mountain sites, where data were from Rocky Mountain National Park. Mean wind speed and mean direction data were matched to the minute with samples collected over the 6-hour period on each site. In addition, regression analysis was used to compare hourly, site, VOC concentrations (i.e., from 8:00 a.m. to 2:00 p.m. or 10:00 a.m. to 4:00 p.m.) to determine if there were hourly trends (see Appendix A).

To determine if VOC source was from O&NG and not other sources, such as traffic, *i*-pentane to *n*-pentane ratios for all sites for the entire 6-hour sampling period

were calculated. A ratio of *i*-pentane to *n*-pentane, falling at or below one was interpreted as VOC release from O&NG (Swarthout et al. 2013). Ratios above one were interpreted as background conditions, from mainly automobile emissions and fuel evaporation (e.g., Russo et al. 2010). To address concerns that VOC levels could be impacted by other, extraneous variables, such as the number of major roads within a kilometer of the site, the number of wells within a kilometer of the site or average quantity of natural gas or oil produced the month data were collected, these variables were tested in the model.

Principle Component Analysis (PCA) and Indicator Species Analysis (ISA) were run using PC-ORD (6.08). PCA and ISA were used to visually examine suites of VOCs in relation to pump groups and to determine indicator species of groups. A Monte Carlo test of significance was run on observed maximum indicator values for VOCs.

We were interested in comparing empirical estimates with model-based estimates. Generalized Estimating Equations (GEEs) were used in the current study as they provide a semi-parametric approach to longitudinal analysis of categorical or continuous response measurements for clustered (non-independent) data. The fit of a GEE model was assessed using residuals and summary measures. The QIC summary measure was used to select the most appropriate working correlation structure (e.g., independent, exchangeable, autoregressive or unstructured). After selecting a structure, QICu's and variance inflation factors (VIFs) were used to select appropriate predictive variables in the GEE general linear model. To predict average benzene concentrations (ppm) we used quasi-likelihood estimation with gamma distribution (due to skewness) and link = "log". Estimate Standard Errors (SEs) were determined using a sandwich estimator.

Full Model:

$$\begin{aligned} \log(\text{Benzene}_i) = & \beta_0 + \beta_1 (\text{Nojack}_1) + \beta_2 (\text{Nonpump}_2) + \beta_3 (\text{NG}_i) + \beta_4 \\ & (\text{Direction}_1) + \beta_5 (\text{Direction}_2) + \beta_6 (\text{Hour}_3) + \beta_7 (\text{Hour}_4) + \beta_8 (\text{Hour}_5) \\ & + \beta_9 (\text{Hour}_i) + \beta_{10} (\text{Hour}_i) + \beta_{11} (\text{Roads}_i) + \beta_{12} (\text{Wells}_i) + \beta_{13} (\text{Oil}_i) \end{aligned}$$

## Results

The PERMANOVA results indicated that Pump group was a significant predictor variable ( $F = 26.9, p < 0.001$  \*\*\*), while Direction was not ( $F = 1.3, p = 0.261$ ). ANOVA results indicate carbon dioxide, methane, carbon monoxide, benzene, butadiene, hexane, trimethylbenzene, MEK, carbon disulfide, ethyltoluene and o-xylene had significantly different concentrations of VOCs among treatment groups (Table 2).

Table 2

*Analysis of Variance (ANOVA) Results for Volatile Organic Compounds (VOCs)*

Variable	Average	Treatment	F	p > F
Carbon Dioxide	Log(Average)	Global	2.64	<0.0032**
		Pump	6.71	<0.0001***
Methane	Average	Global	2.32	0.0093**
		Pump	7.7	<0.0001***
Nitrous Oxide	Average	Global	1.08	0.3953
		Pump	2.82	0.0242
Carbon Monoxide	Average	Global	3.20	0.0005***
		Pump	7.71	<0.0001***
Benzene	Log(Average)	Global	15.1	<0.0001***
		Pump	50.62	<0.0001***
Toluene	Average	Global	0.86	0.6242
		Pump	2.82	0.0241
Ethylbenzene	Average	Global	1.40	0.1730
		Pump	4.23	0.0024
m-Xylene	Average	Global	0.77	0.7187
		Pump	2.29	0.0578
Acrolein	Average	Global	1.19	0.2987
		Pump	3.76	0.0052
Acetaldehyde	Log(Average)	Global	1.41	0.1646
		Pump	4.05	0.0032
Formaldehyde	Average	Global	1.51	0.1255
		Pump	4.35	0.0020
1,3 Butadiene	Average	Global	1.92	0.0350*
		Pump	5.92	0.0002***
Isopentane	Average	Global	1.55	0.1109
		Pump	2.83	0.0238
Pentane	Average	Global	0.80	0.6879
		Pump	1.96	0.0985
Hexane	Log(Average)	Global	2.12	0.0178*
		Pump	6.47	<0.0001***
Heptane	Log(Average)	Global	1.31	0.2203
		Pump	4.03	0.0034
1,3,5 Trimethylbenzene	Average	Global	4.65	<0.0001***
		Pump	14.29	<0.0001***
Cyclohexane	Average	Global	0.65	0.8323
		Pump	1.74	0.1403
MEK	Average	Global	2.03	0.0242*
		Pump	4.57	0.0014**
Carbon disulfide	Average	Global	1.87	0.0406*
		Pump	1.72	0.1453
Carbonyl sulfide	Average	Global	1.41	0.1654
		Pump	2.88	0.0219
Ethyltoluene	Average	Global	3.05	0.0008***
		Pump	9.32	<0.0001***
o-Xylene	Average	Global	2.22	0.0128*
		Pump	7.41	<0.0001***
p-Xylene	Average	Global	0.80	0.6863
		Pump	2.54	0.0381

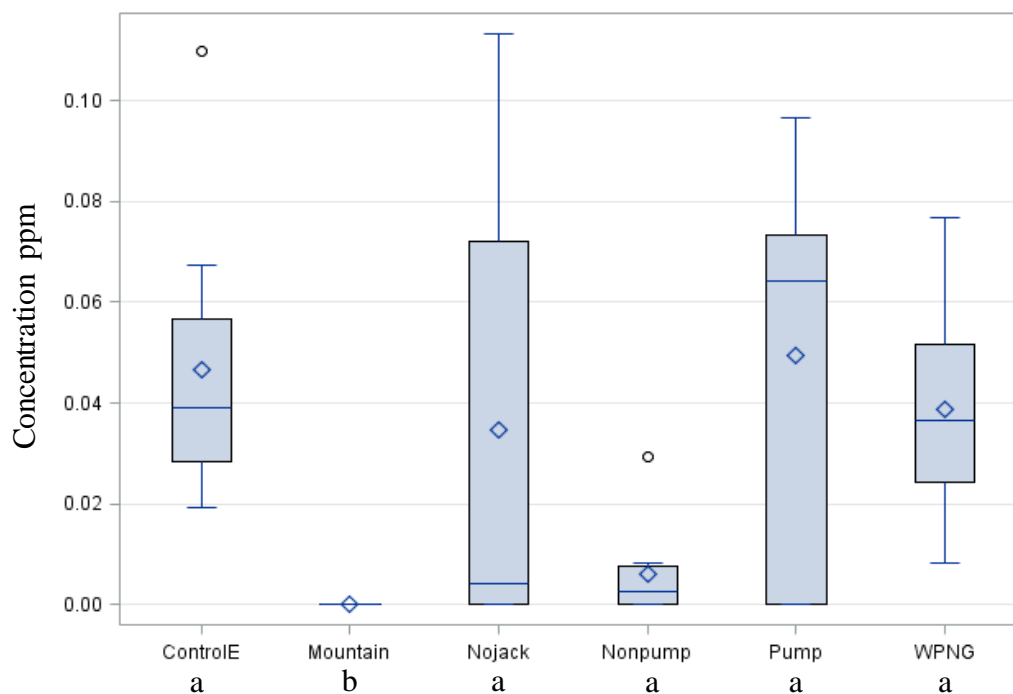
*Note.* Results include *F* statistics and *p* values for Average global and pump tests. Log indicates that the variable was log transformed. Global indicates the omnibus test and Pump indicates between treatment comparisons.

\*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$

ANOVA results for average concentration of individual VOCs in ppm by chemical category (i.e., alkenes, aromatics and cyclic hydrocarbons, alkanes, aldehydes, ketones, oxygenated species) are shown in Figures 5 A-W. For the alkenes, mean butadiene concentrations were significantly lower in Mountain and Nonpump groups (Figure 5A). For the aromatics and cyclic hydrocarbons, o-xylene mean concentrations were significantly higher for Nonpump and Nojack groups while p-xylene levels were significantly higher in Nonpump (Figures 5C and 5D). Ethyltoluene mean concentrations were significantly higher in WPNG and ControlE than other groups, whereas ethylbenzene concentrations were significantly higher on Nojack sites (Figures 5G and 5H). Benzene levels were significantly higher in the Nojack group (Figure 5I). Of the alkanes, heptane was significantly higher in the WPNG group, while methane was significantly lower in the Mountain group than all others except ControlE (Figures 5J and 5M). Of the aldehydes, acetaldehyde was significantly higher in the Mountain group (Figure 5O) and acrolein was significantly higher in the Nojack group (Figure 5Q). Of the sulfides, carbon disulfide was significantly lower in the Mountain group than all others (Figure 5R). MEK was the only ketone observed and was highest in production groups (i.e., Nojack, Nonpump, Pumping; Figure 5T). Of the oxygenated species, carbon dioxide and carbon monoxide mean concentrations were significantly higher in WPNG and ControlE groups and carbon dioxide was significantly lower in the Mountain group (Figures 5U and 5W).

## Alkenes

## A. 1,3 Butadiene



*Figure 5.* Volatile Organic Compound (VOC) boxplots. Plots (A-W) include average concentrations of VOCs in ppm for each chemical category (i.e., alkenes, aromatics and cyclic hydrocarbons, alkanes, aldehydes, ketones, and oxygenated species), and for each production or control group (i.e., ControlE, Mountain, Nojack, Nonpump, Pump, and WPNG). For models with  $p < 05$  in the omnibus test, Student-Newman-Keuls post-hoc tests are shown under the x-axis. Means with the same letter are not significantly different.

## Aromatics and cyclic hydrocarbons

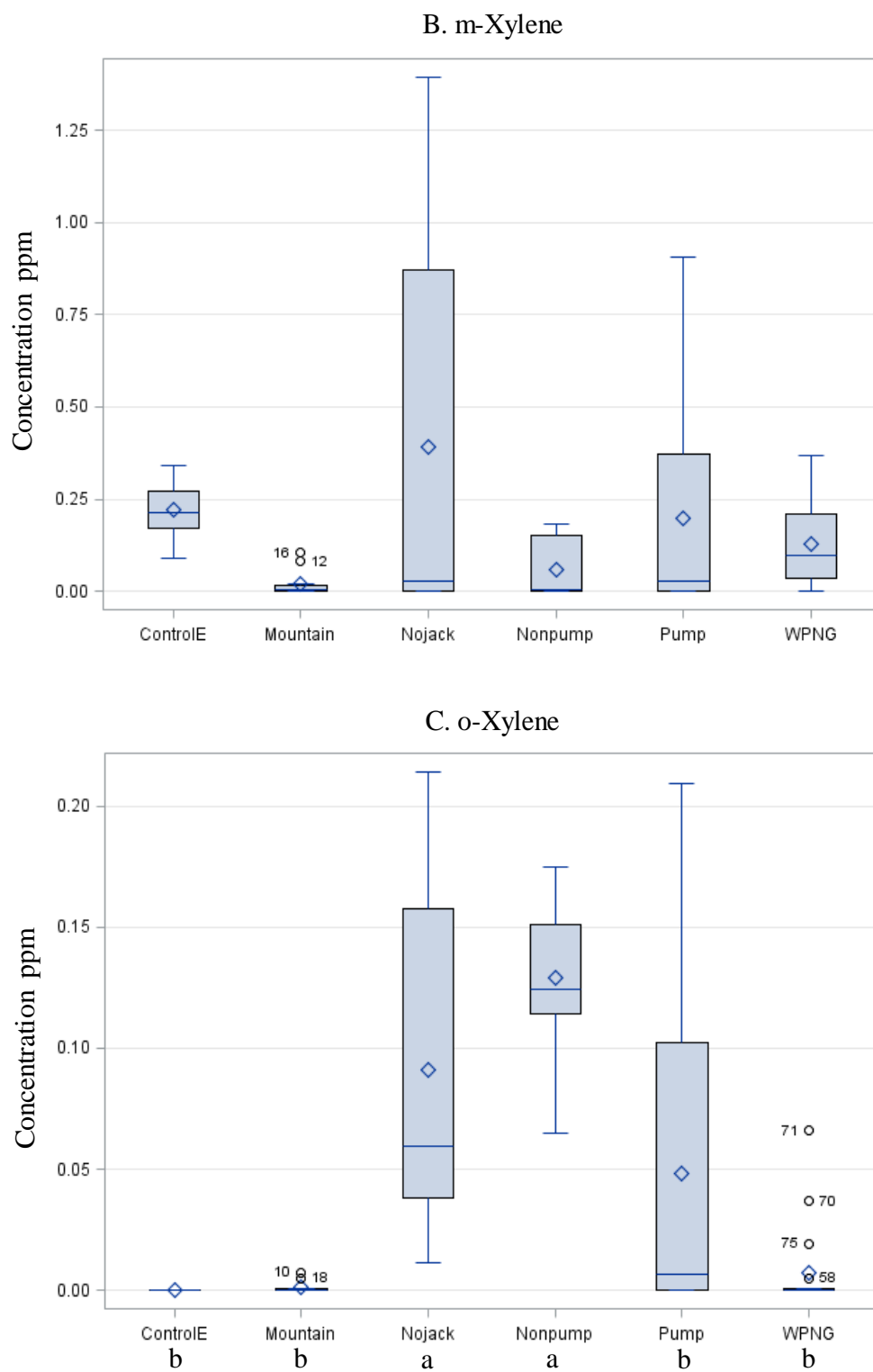


Figure 5. Continued.



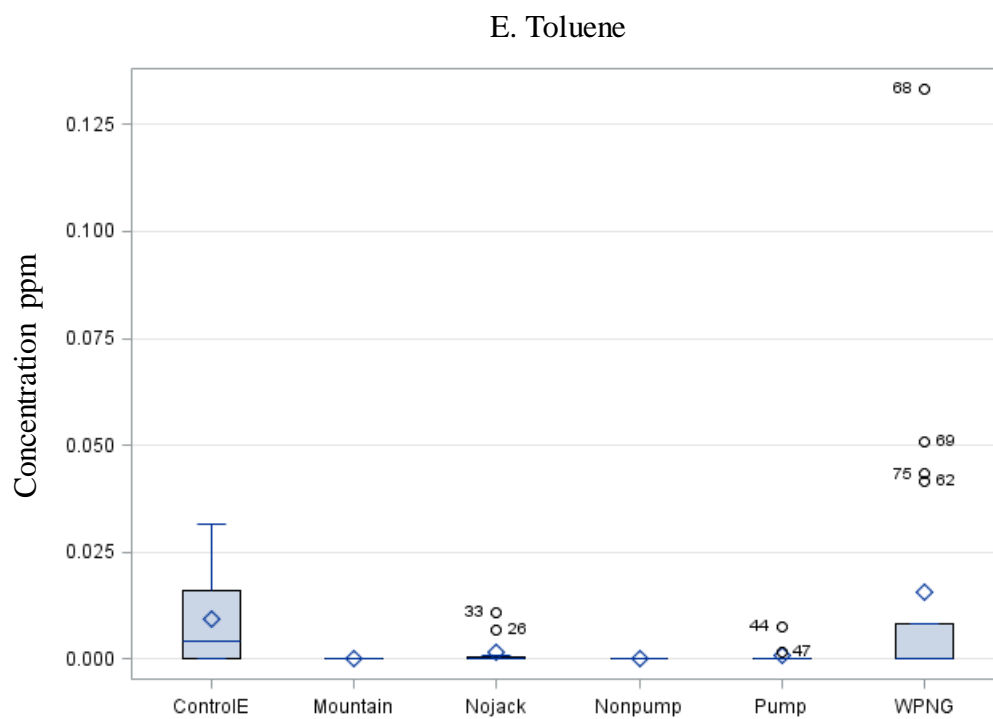
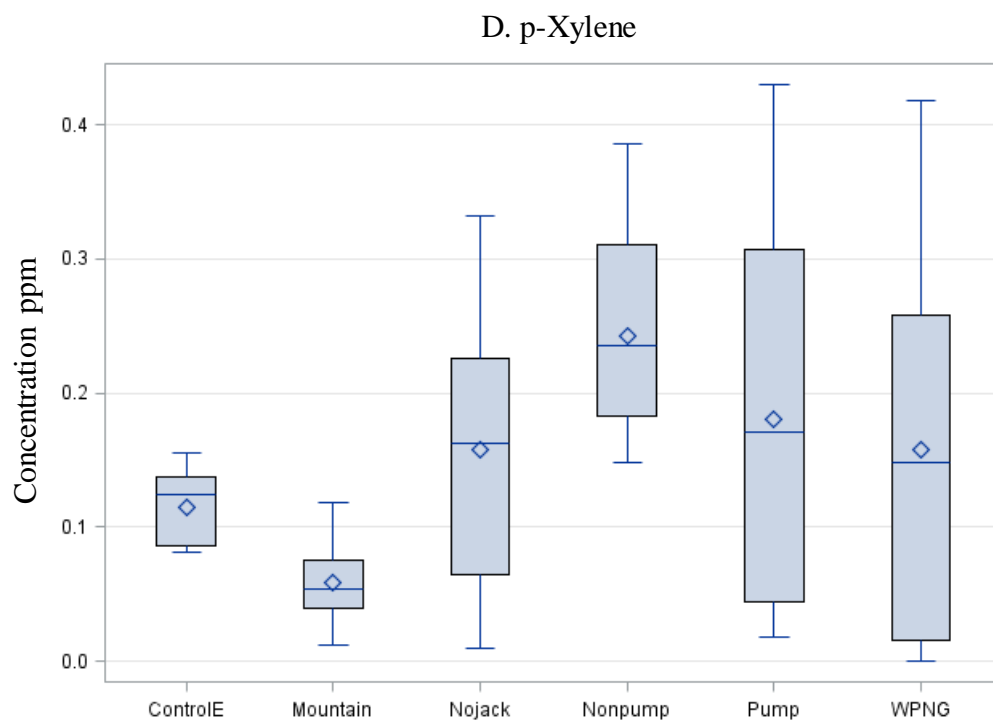
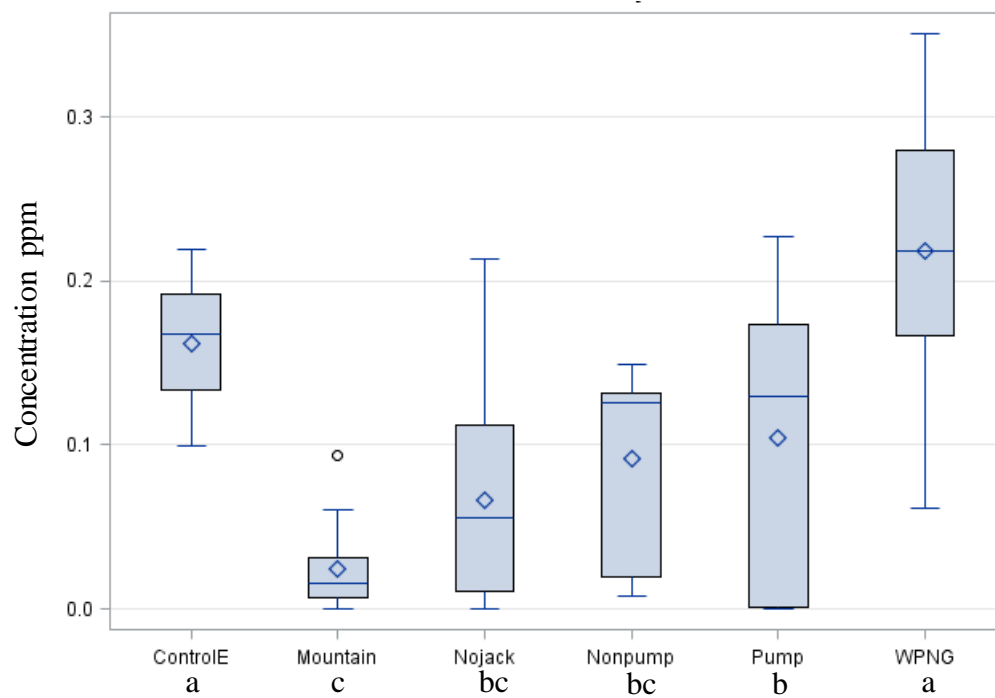


Figure 5. Continued.

## F. 1,3,5 Trimethylbenzene



## G. Ethyltoluene

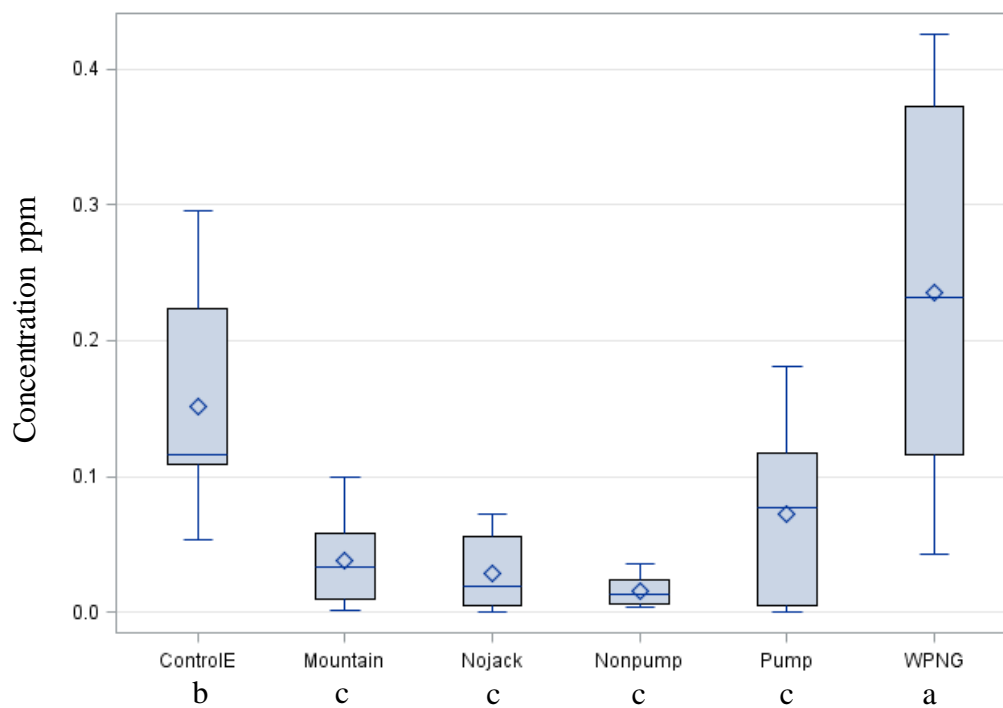
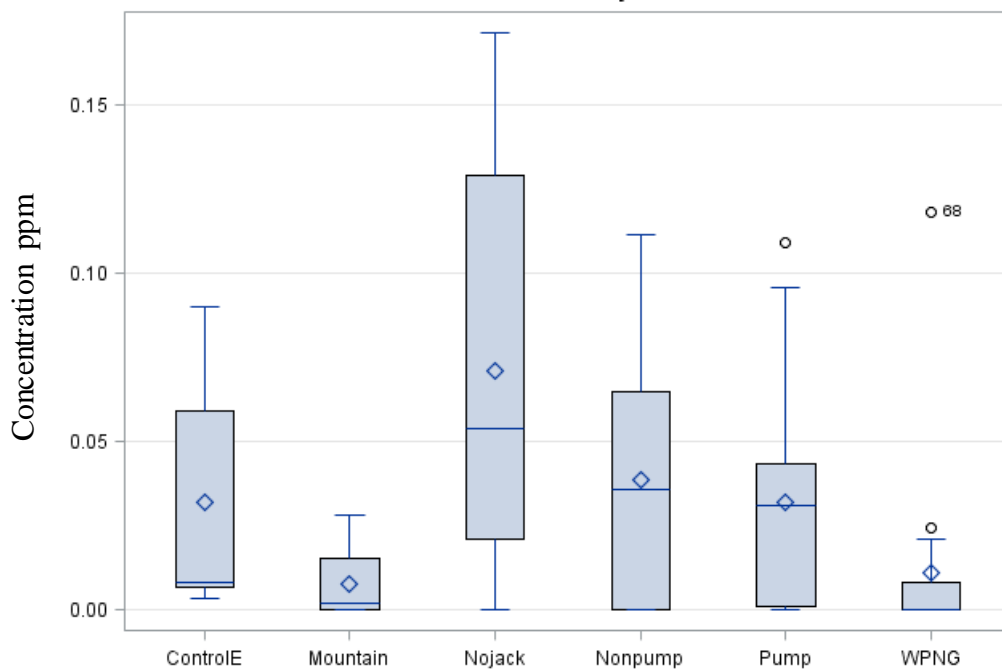


Figure 5. Continued.

### H. Ethylbenzene



### I. Benzene

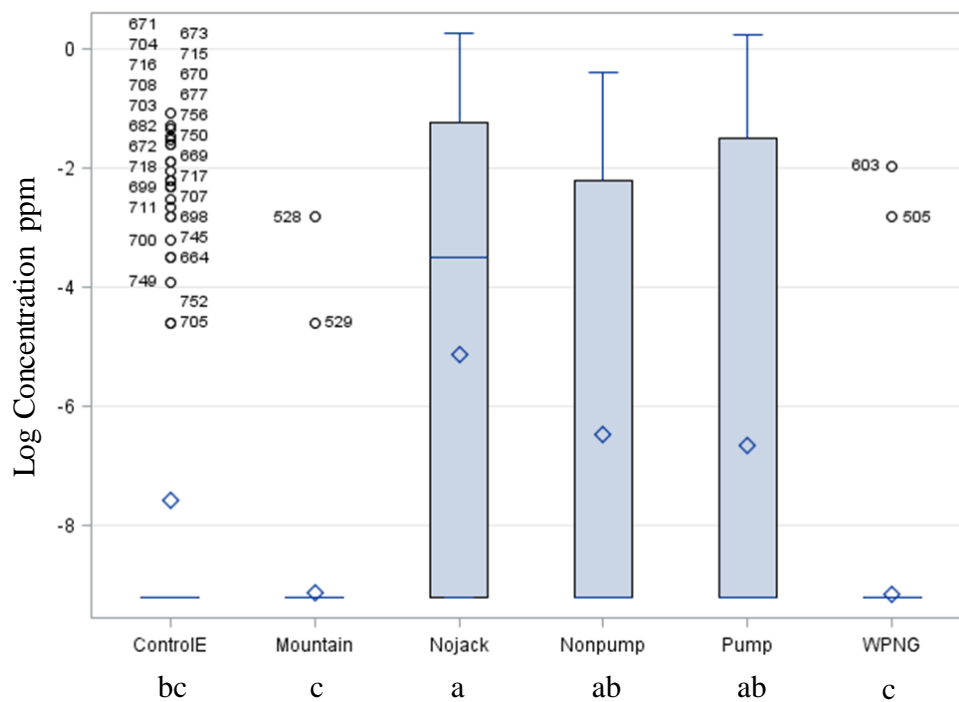


Figure 5. Continued.

## Alkanes

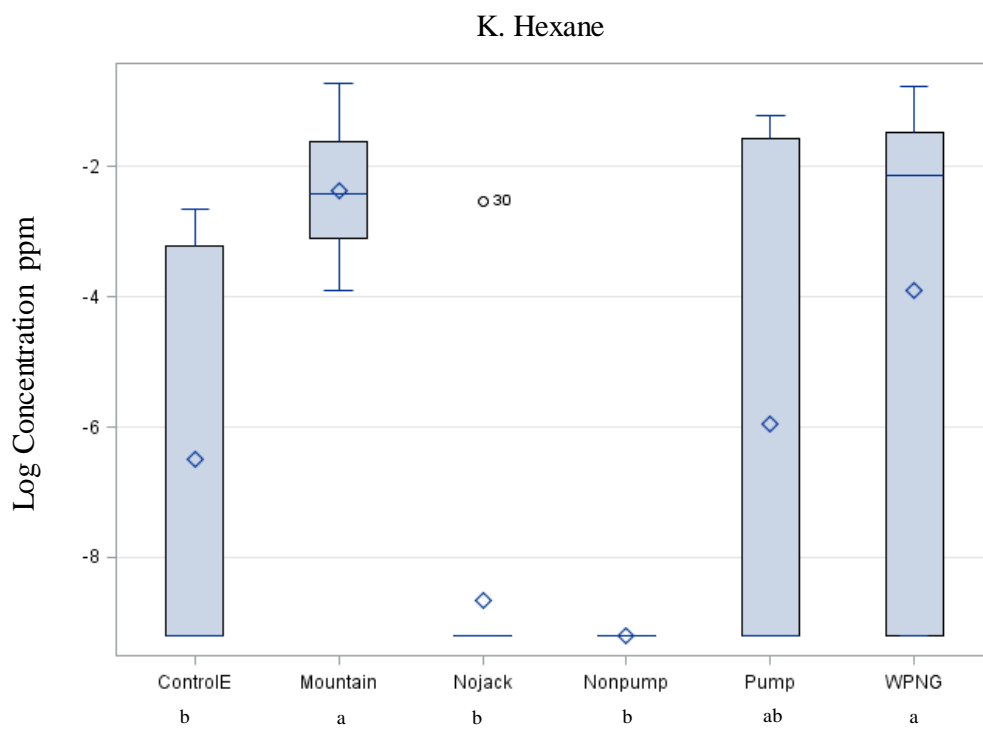
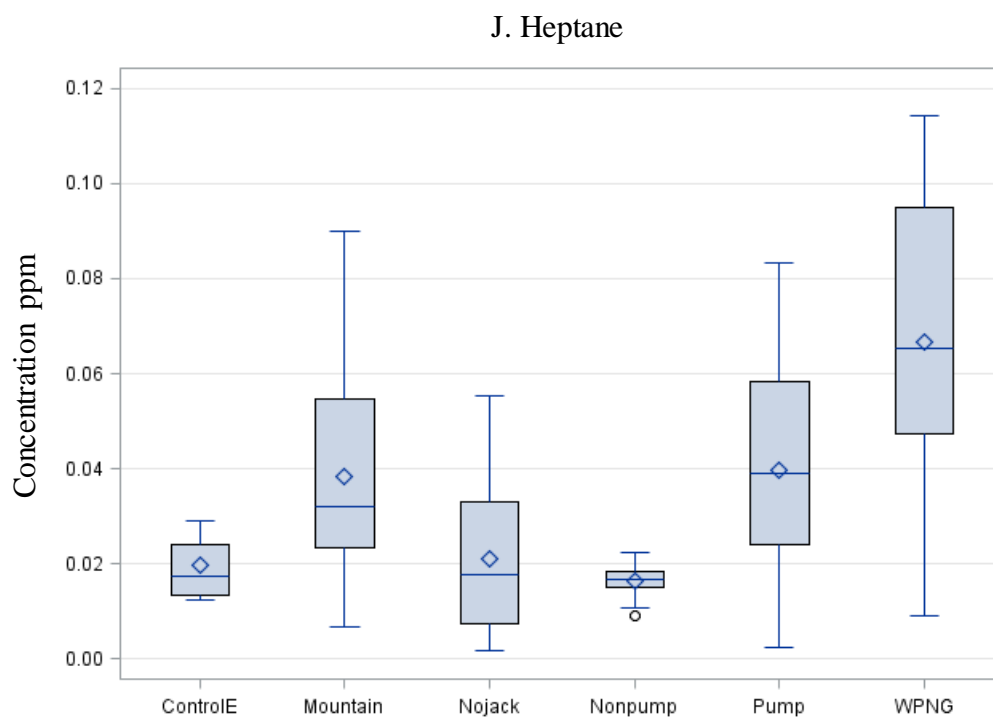


Figure 5. Continued.

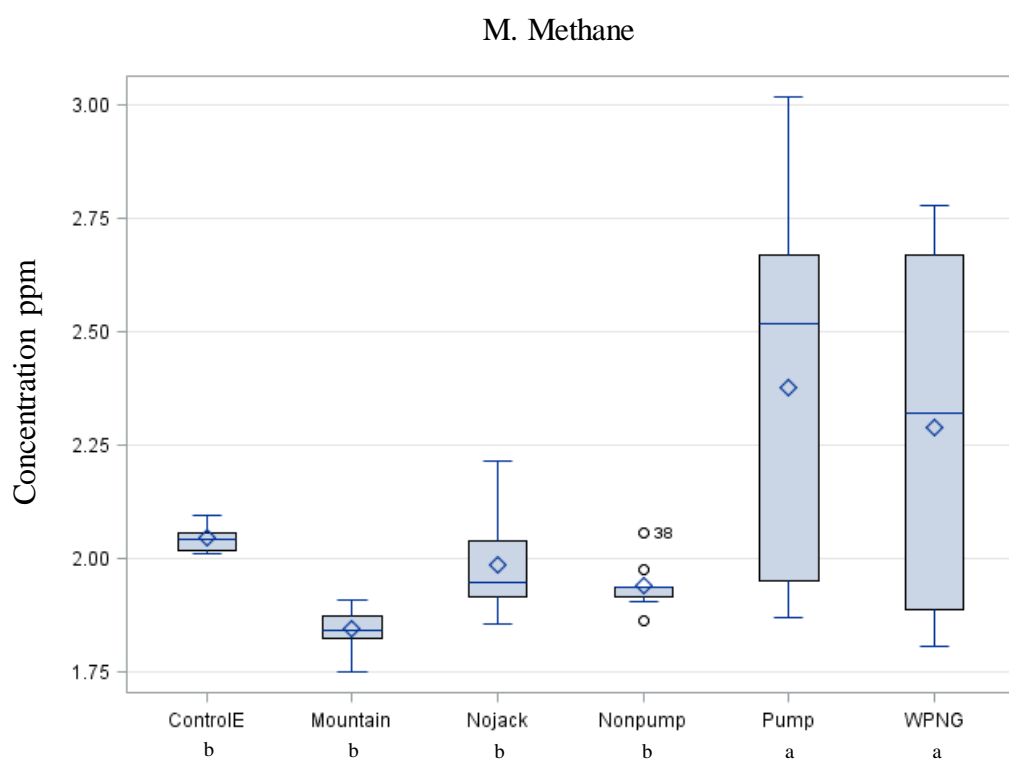
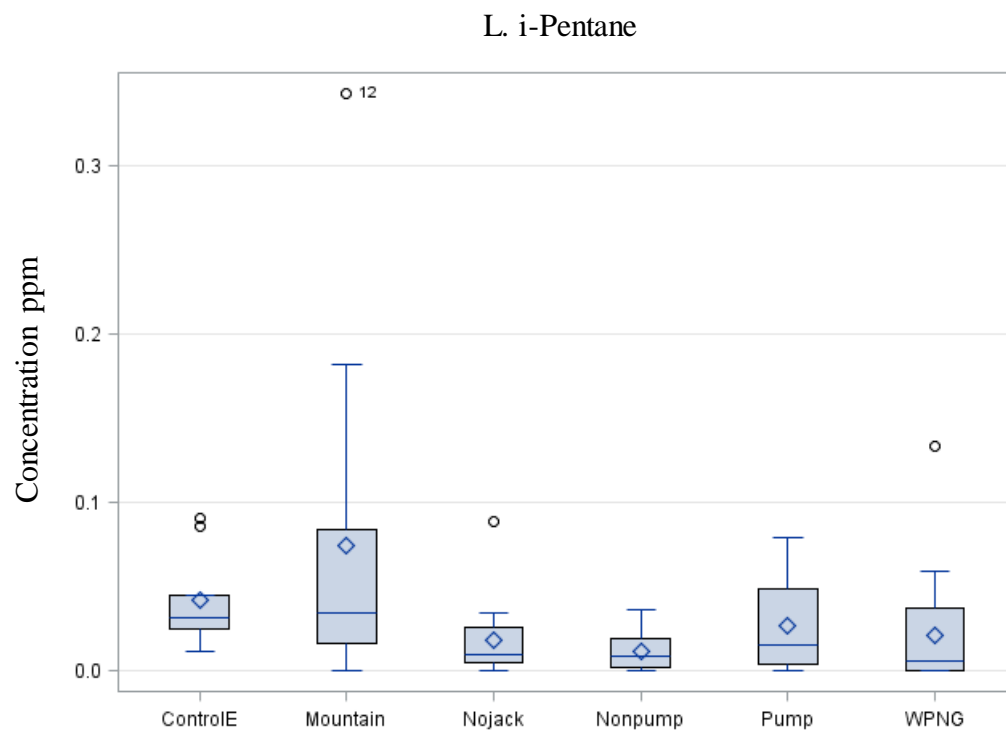
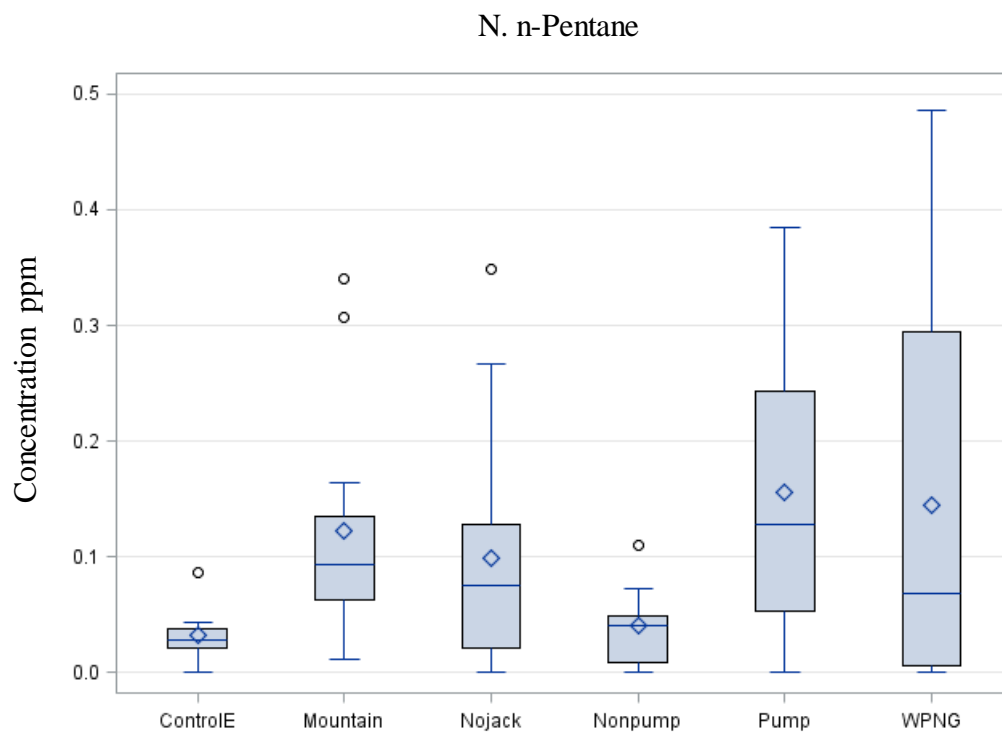


Figure 5. Continued.



Aldehydes

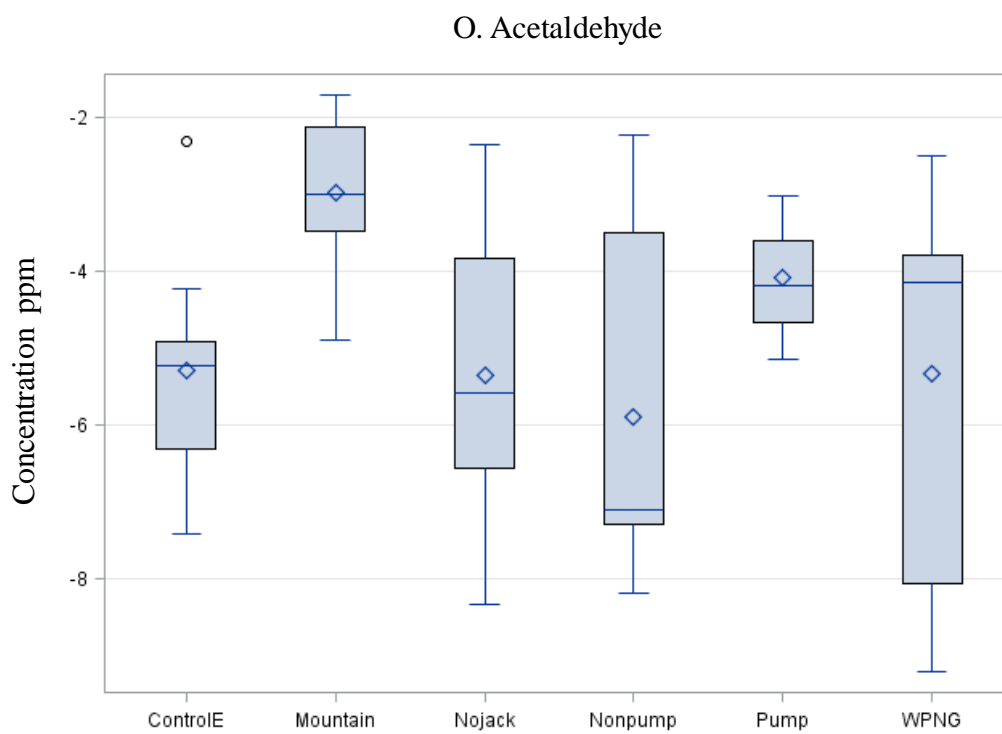


Figure 5, Continued.

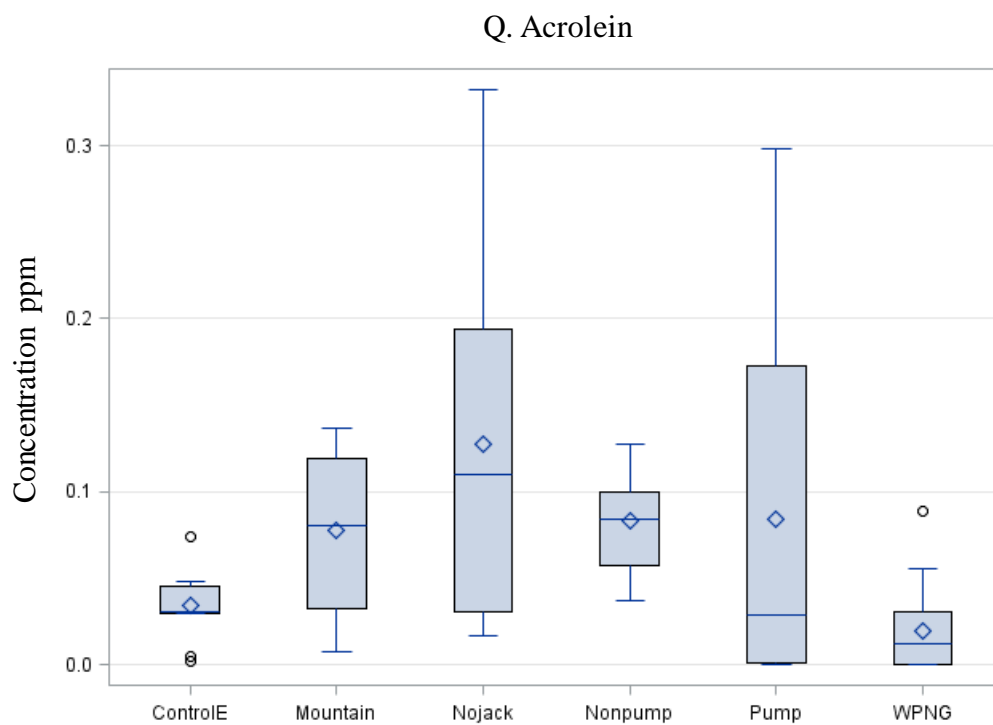
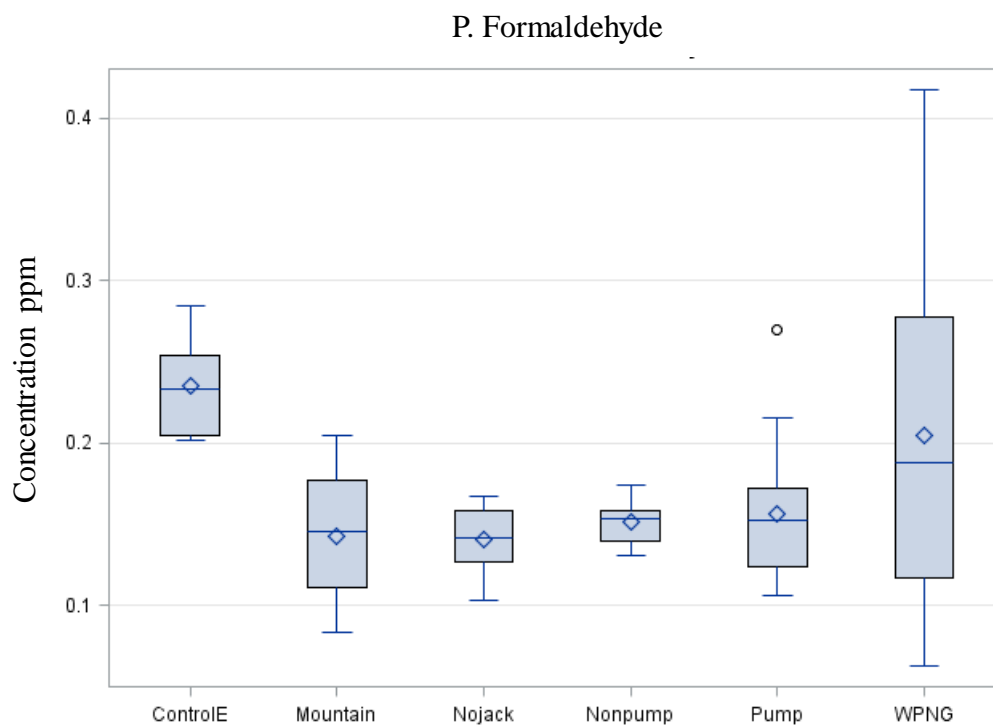


Figure 5. Continued.

## Sulfides

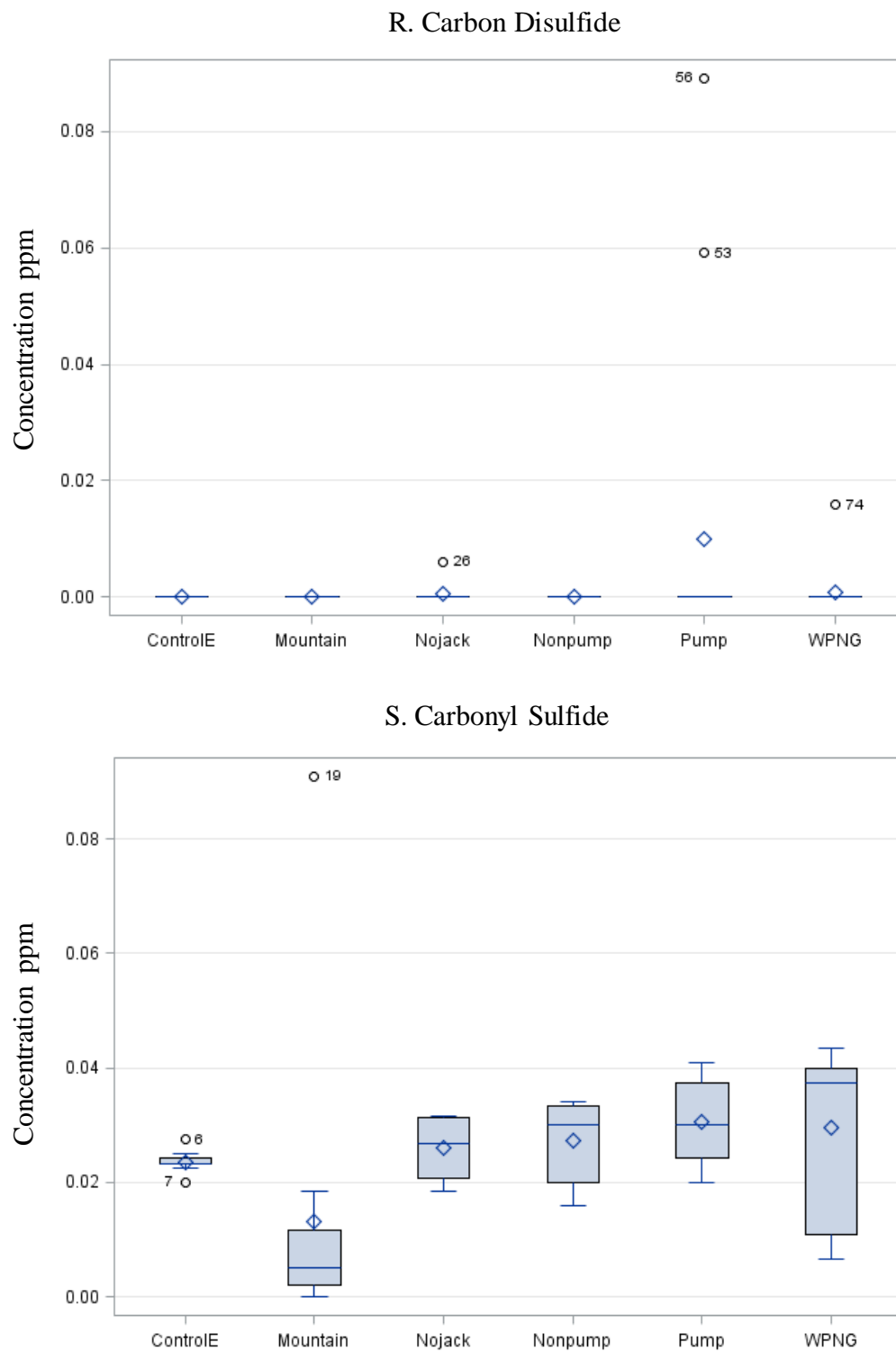
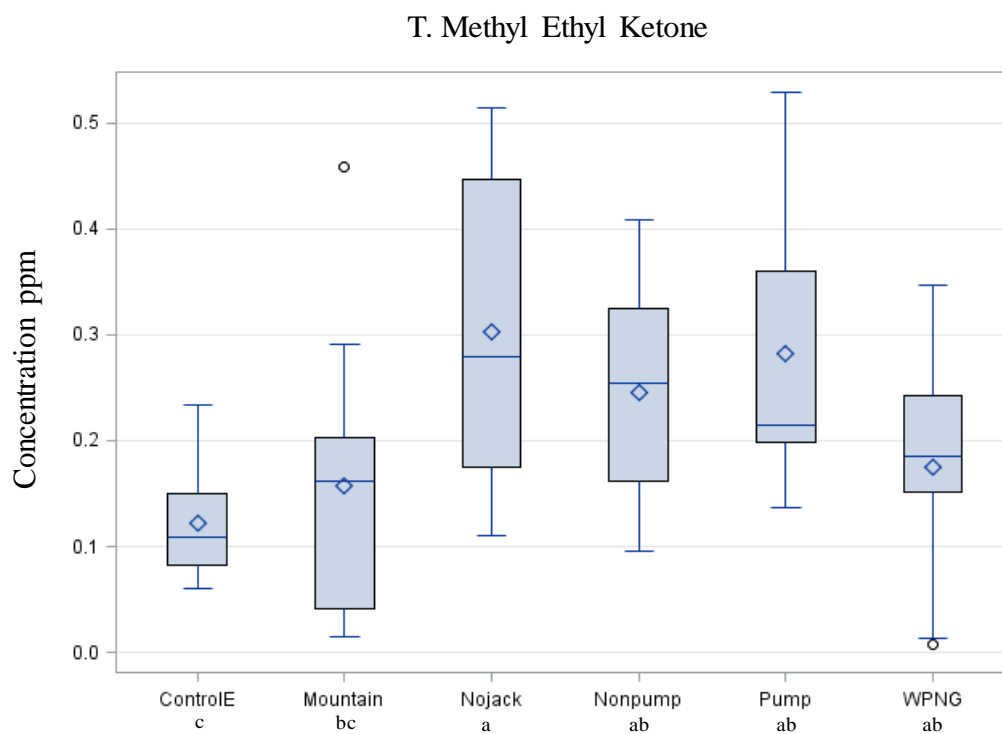


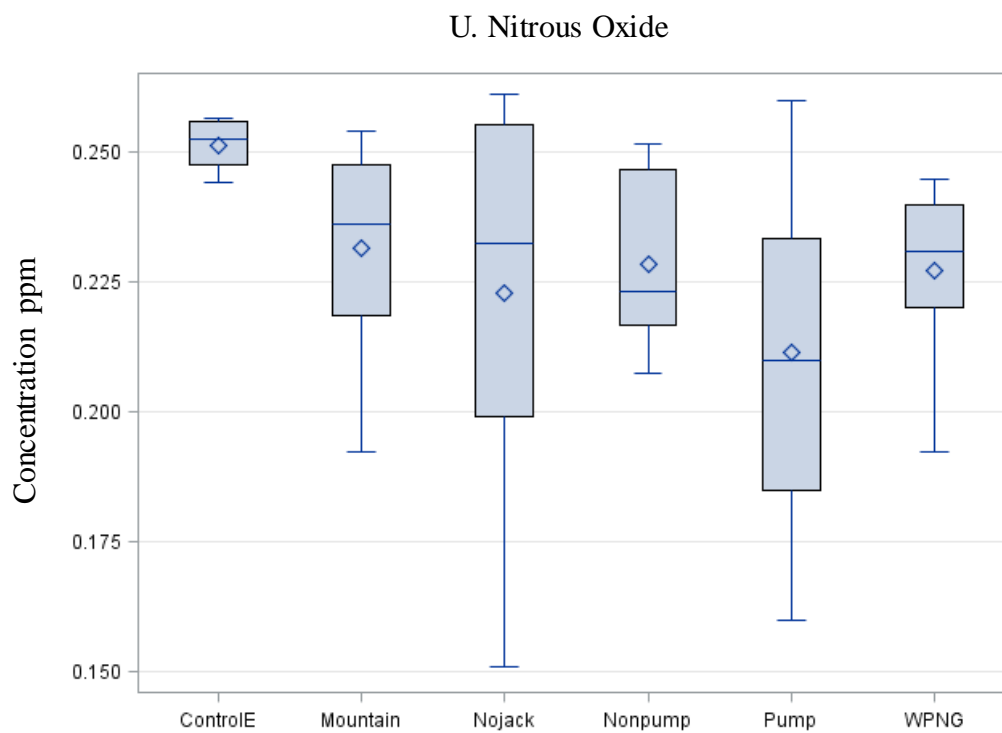
Figure 5. Continued.



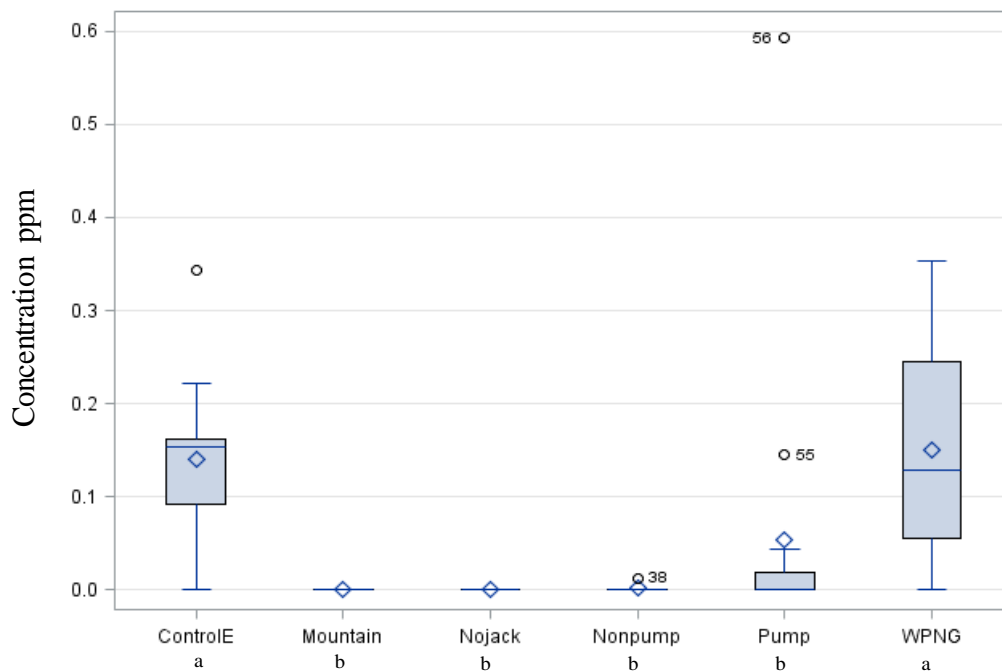
## Ketones



## Oxygenated species

*Figure 5. Continued.*

## V. Carbon Monoxide



## W. Carbon Dioxide

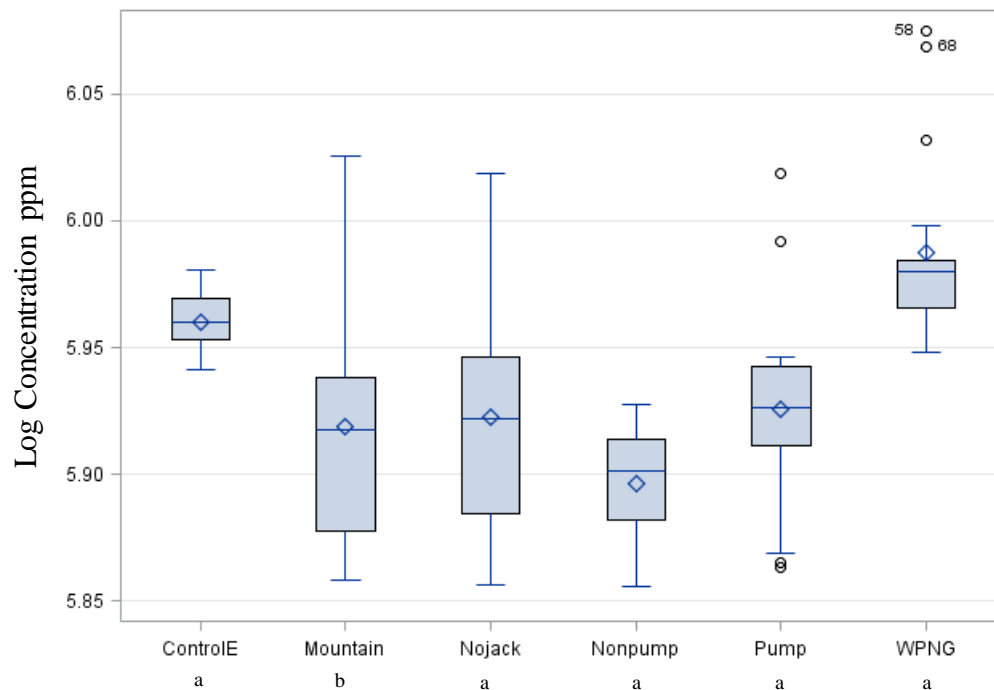
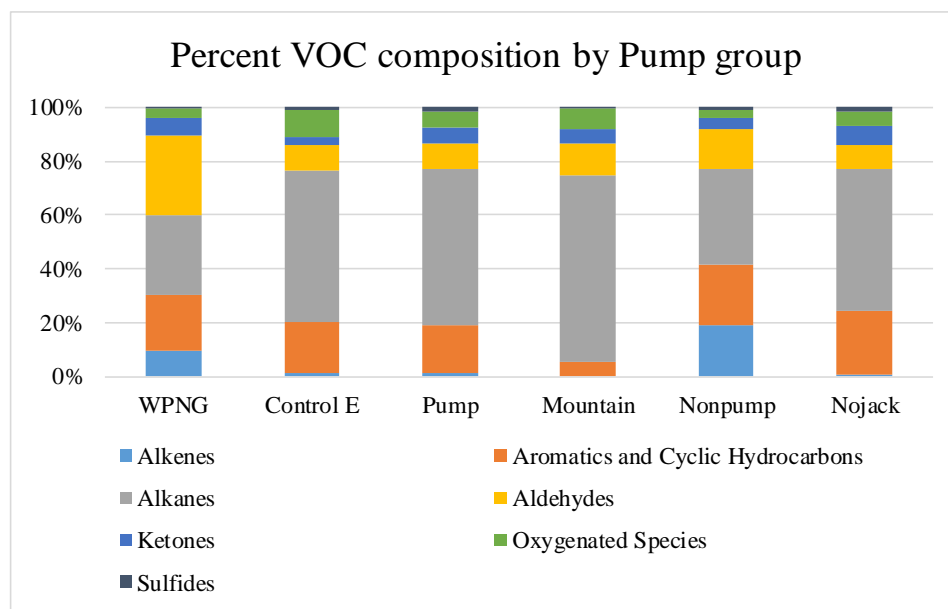
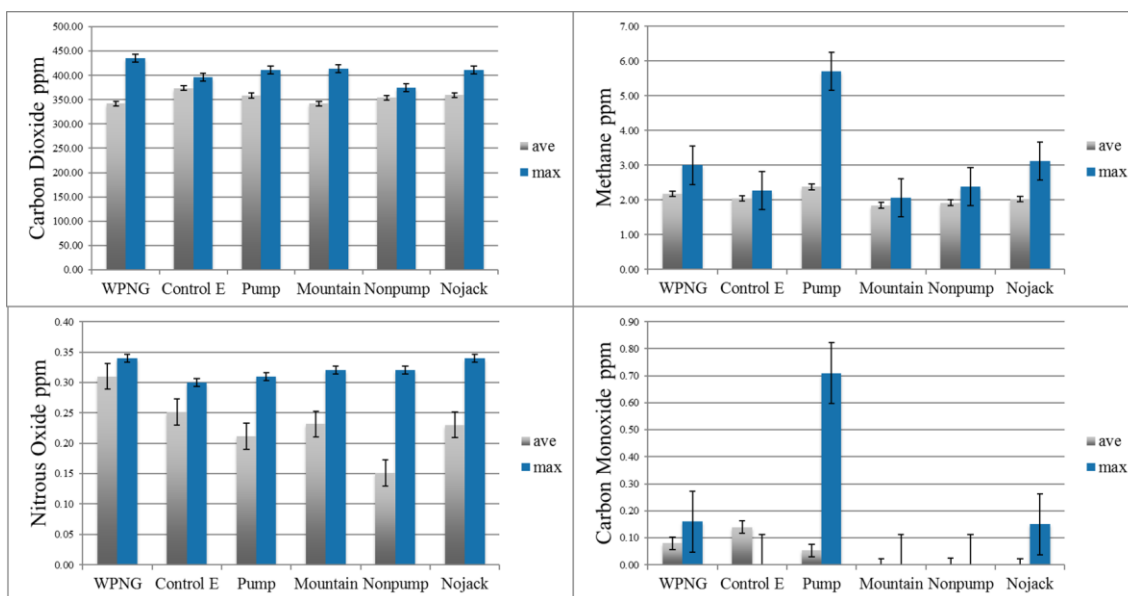


Figure 5. Continued.

Percent VOC composition by Pump group indicates general trends. There were higher alkenes and lower alkanes in the Nonpump and WPNG groups than all others (Figure 6). Ketones were generally higher for Pump, Nojack and WPNG groups. Aromatics and cyclic hydrocarbons were high in all groups except the Mountain group, which had very low concentrations. Aldehydes were higher on WPNG and oxygenated species were higher on ControlE and Mountain sites than all other groups. Of the critical greenhouse gases (carbon dioxide, nitrous oxide, methane and carbon monoxide) maximum concentrations were far above the averages, indicating fugitive emissions or leaks on sites (Figure 7). The maximum methane concentration for the Pump group was ~6 ppm, whilst other groups were ~2 ppm, and the maximum carbon monoxide level was ~7 ppm in the Pump group, which was six times higher any other group maximum.

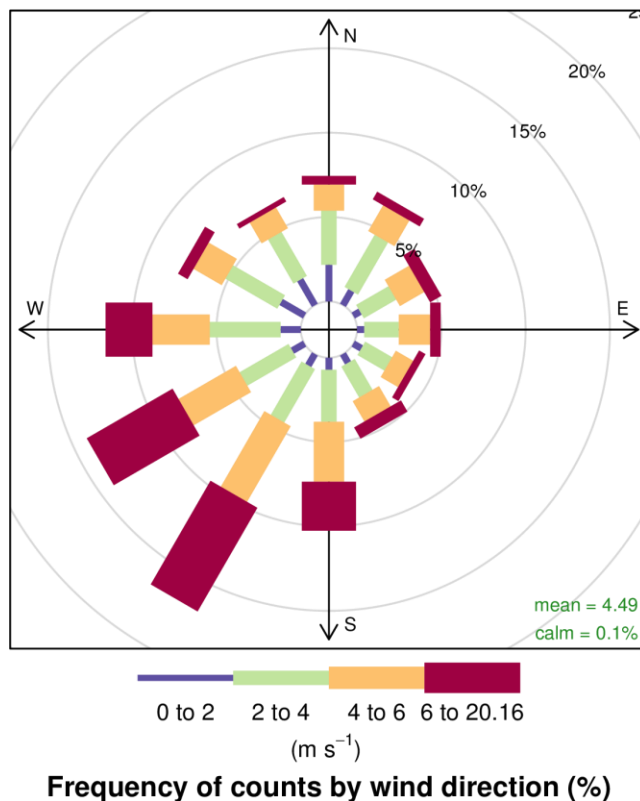


*Figure 6.* Percent Volatile Organic Compound (VOC) composition by pump group. VOCs, excluding carbon dioxide, grouped by chemical category (i.e., alkenes, aromatics and cyclic hydrocarbons, alkanes, aldehydes, ketones, oxygenated species) and by Pump group.

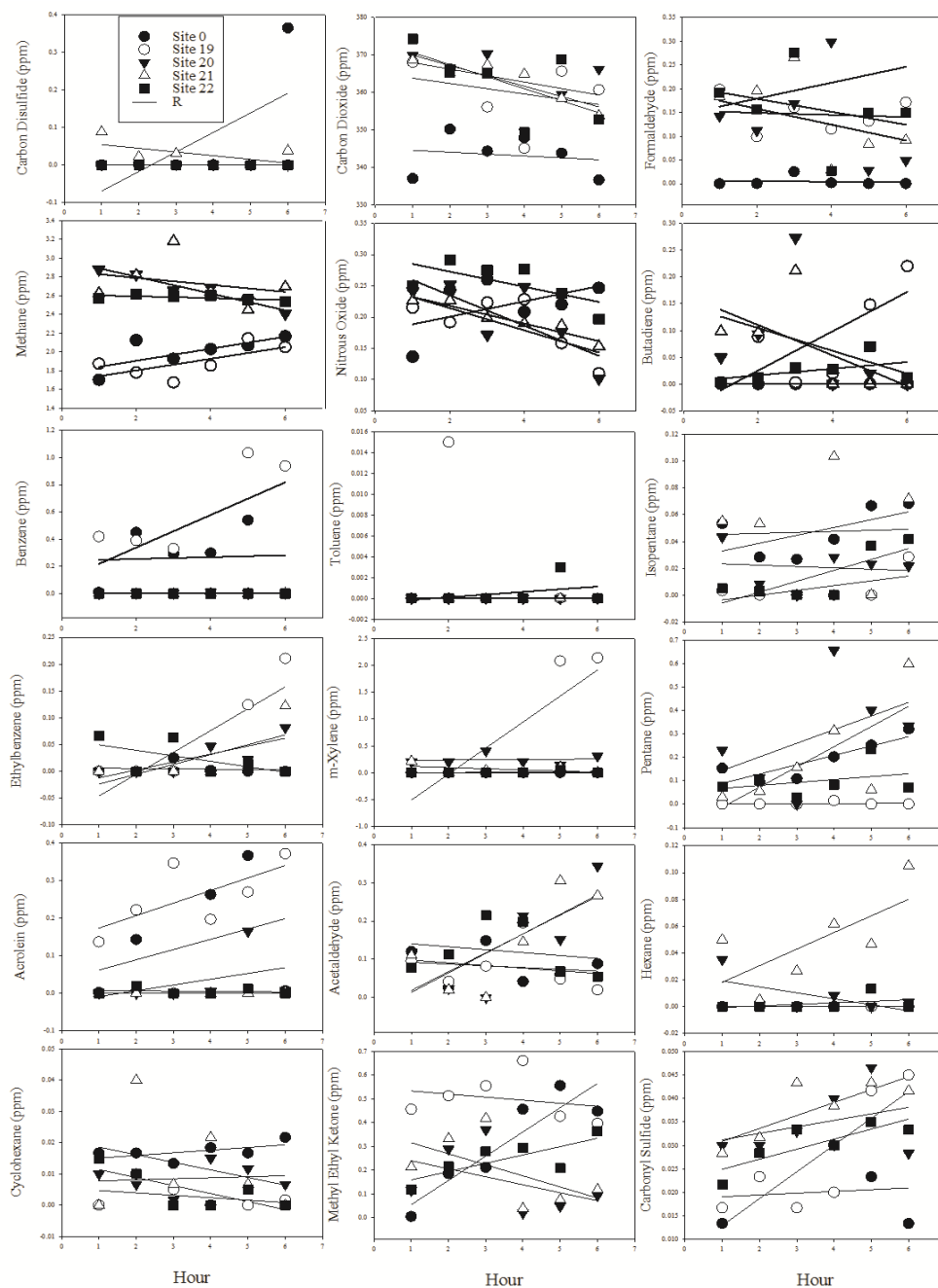


*Figure 7.* Critical greenhouse gases across production and control groups. GHGs include carbon dioxide, methane, and nitrous oxide. These are average and maximum concentrations for each group including control groups (WPNG, ControlE, Mountain) and production groups (Pumping, Nonpump, Nojack).

Direction was not a significant predictor in the model, although we interpreted trends in daily, directional deposition; thus, data were analyzed using descriptive statistics. Mean wind speed and mean wind direction were used to create a wind rose to determine a dominate downwind direction (Figure 8). Wind direction and speed were variable across all sites and times. Approximately 15% of the given time, winds blew from the SW. We likely quantified only a small percentage of total VOCs by sampling in three directions (W, SE and NE) and thus were not able to capture a statistical difference between directions. Concentrations of VOCs did not consistently decrease (i.e., from 8:00 a.m. to 2:00 p.m. or from 10:00 a.m. to 4:00 p.m.; see Appendix A); however, there were trends amongst the four production sites (Figure 9), but only benzene had a significant time effect.



*Figure 8.* Wind rose for all sample times by the minute. Wind speed and direction data for all sites, excluding sites 18 and 19 (data was not available for the corresponding date), were downloaded from the NEON database and matched with time of data collection by the minute. The length of each spoke indicates frequency of wind coming from a particular direction. The percentages indicate how often the wind was blowing in that direction. Spoke color indicates wind speed. The highest speeds appear in red, the lowest in blue. Larger spokes in the SW direction indicate winds predominately blew in the SW direction during data collection.



*Figure 9.* Hourly Volatile Organic Compounds (VOCs) concentrations for Pumping sites. Only oil and natural gas production sites termed “Pumping” were used in the analysis. Each plot contains hourly concentrations for five sites (0, 19, 20, 21, and 22). Hour represents the six-hour collection period.

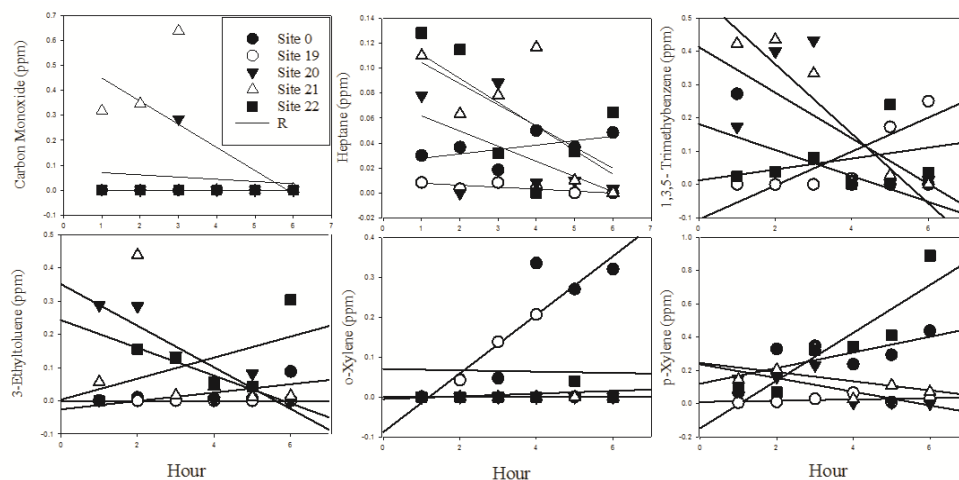
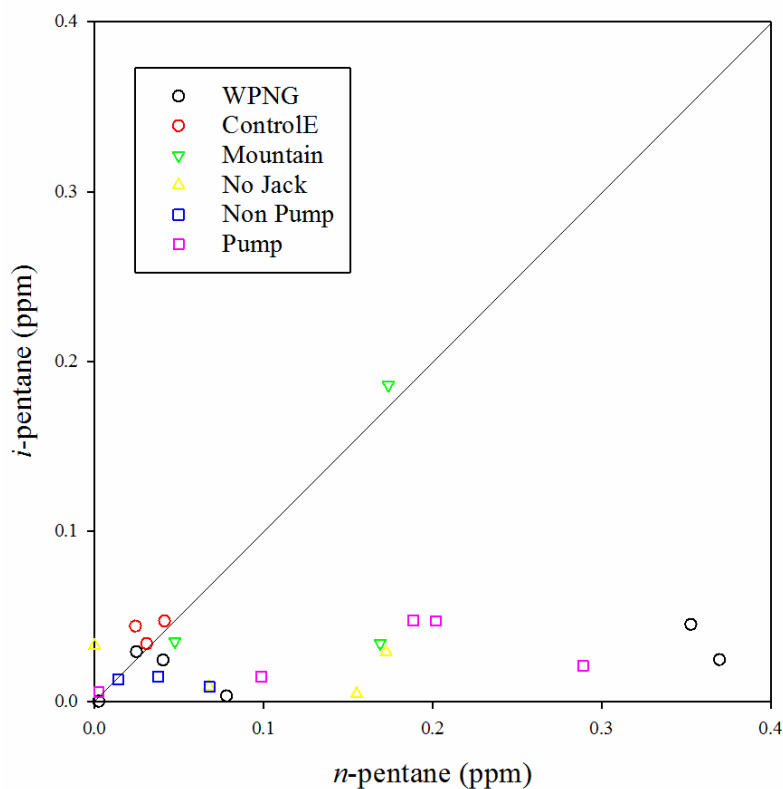


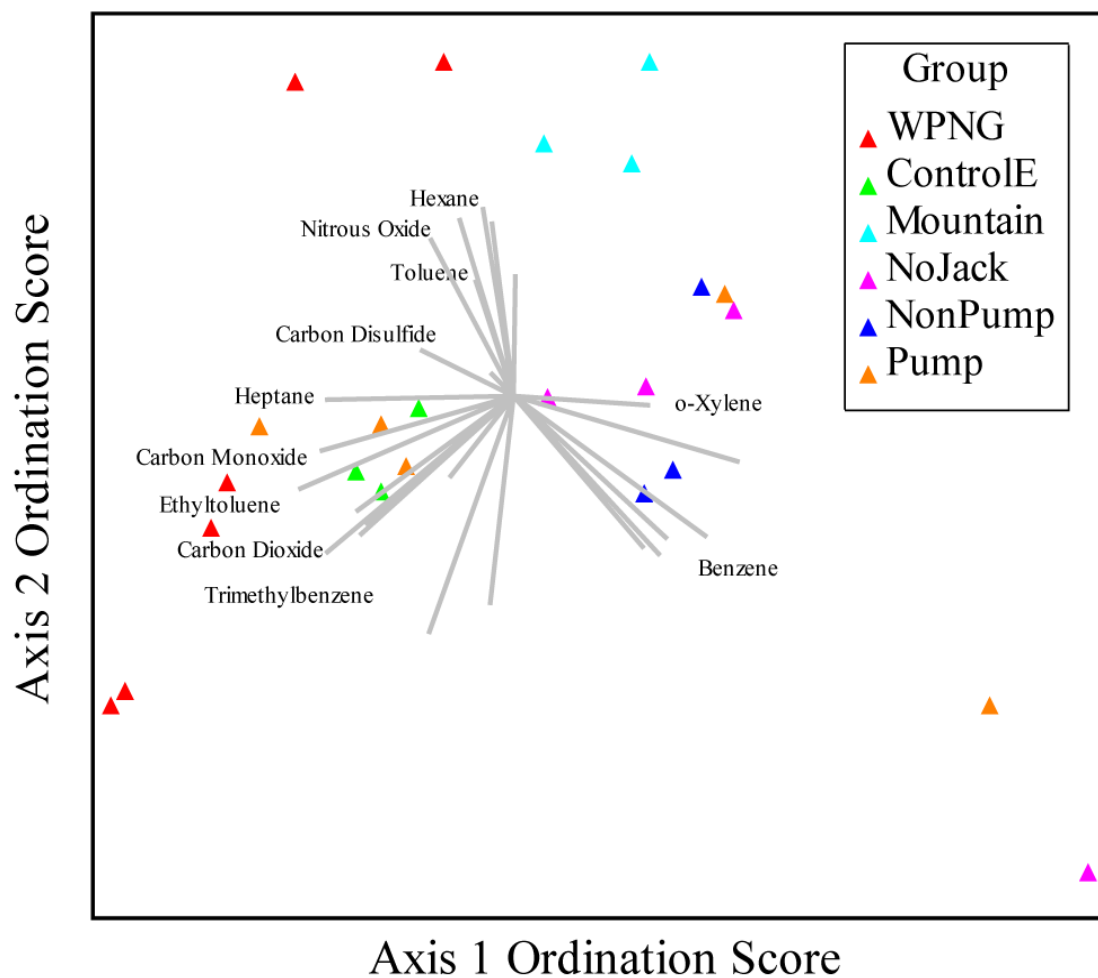
Figure 9. Continued.

There were 20 sites with a ratio of *i*-pentane to *n*-pentane at or below one (Figure 10), indicative of VOCs released from O&NG (Swarthout et al. 2013). Four sites, from groups Pump, Nojack, Nonpump and Mountain, had ratios above one, indicative of automobile emissions and fuel evaporation (e.g., Russo et al. 2010). PCA suggested some grouping based on suites of VOCs; For example, the four Mountain sites group together along Axis one (Figure 11; Table 3). A Monte Carlo test of significance of observed maximum indicator values determined seven significant VOCs (carbon dioxide, nitrous oxide, carbon monoxide, ethyltoluene, trimethylbenzene, *o*-xylene and hexane), that differentiated four of the six groups (Table 4). Presence of *o*-xylene and hexane seem to differentiate production sites from control sites. Average amount of natural gas produced in the month of sample collection was a significant predictive variable in the model ( $F = 18.87, p = 0.0015$ ). The amount of natural gas produced at a site was moderately correlated with Axis 1 ordination scores and explained over 65% of the variance ( $r^2 = 0.6538$ ) in the regression model (Figure 12).



*Figure 10.* Plot of *i*-pentane to *n*-pentane concentrations. Plot contains air samples collected at each of the 24 sites, including control groups (WPNG, ControlE, and Mountain) and production groups (No Jack, Non Pump, Pump). Ratios > 1 (above the line) are indicative of vehicle exhaust and ratios < 1 are indicative of fugitive emissions from oil and natural gas production.





*Figure 11.* Principal Component Analysis (PCA) ordination plot for Volatile Organic Compounds (VOCs). Sites were grouped by production or control group and color coded: red = WPNG, green = ControlE, light blue = Mountain, pink = NoJack, dark blue = NonPump, and orange = Pump. The plot indicates a suite of VOCs from each of the 25 sites. Only species with the highest indicator values are shown on the plot. Individual VOCs are indicated by the grey lines.

Table 3

*Site Details: Ordination Scores and Predictive Variables.*

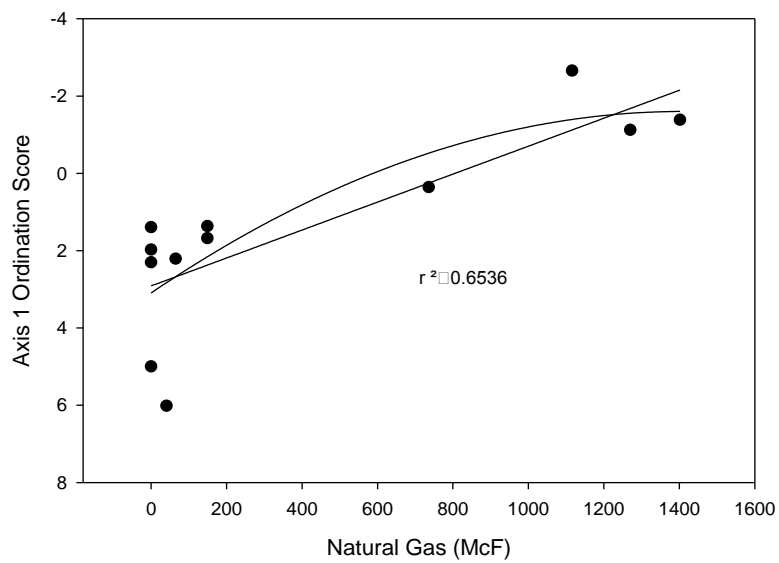
Site code	Axis 1	Axis 2	Wells	Roads	Oil	Pump code	Pump group	Gas	Month
s14	0.3549	-0.01	9	0	93	4	nojack	736	Oct
s17	2.3011	0.8737	6	0	0	4	nojack	0	Aug
s18	6.0172	-4.931	7	0	95	4	nojack	41	July
s25	1.3927	0.0848	1	0	0	4	nojack	0	Aug
s13	1.6771	-0.762	7	0	0	5	nonpump	149	Aug
s15	1.3654	-1.008	7	1	180	5	nonpump	149	Aug
s16	1.9708	1.1281	6	1	0	5	nonpump	0	Aug
s0	2.2061	1.0559	6	0	249	6	pump	65	Aug
s19	4.9991	-3.205	5	0	0	6	pump	0	July
s20	-1.127	-0.736	5	1	550	6	pump	1270	Aug
s21	-2.658	-0.317	4	0	439	6	pump	1116	Aug
s22	-1.386	-0.309	1	1	287	6	pump	1402	Sept

*Notes:* Category “Wells” represents the number of wells within 1km of the site. Category “Oil” is the amount of oil (BB) produced the month of data collection. “Gas” is the amount of natural gas (McF) produced the month of data collection. “Pump group” is the production group. “Month” is the month data were collected. “Roads” is a binary variable for presence of a major road within 1 km of the site (0 = no, 1 = yes). “Axis 1” and “Axis 2” are ordination scores from PCA.

Table 4

*Indicator Variables (IVs) from Observed Randomized Indicator Groups*

VOC	Max Group	(IV)	Mean	Std. Dev.	p*
Carbon dioxide	ControlE	17.3	17	0.21	0.0258
Nitrous oxide	ControlE	18.3	17.7	0.38	0.0424
Carbon monoxide	WPNG	43.7	24.7	8.24	0.0348
Ethyltoluene	WPNG	43.3	28.5	4.94	0.0087
Trimethylbenzene	WPNG	32.8	24.8	3.25	0.0149
o-Xylene	Nonpump	46.6	28.5	8.53	0.0357
Hexane	Pumping	47.1	30	8.86	0.0499



*Figure 12.* Plot of ordination scores vs. quantity of natural gas produced on site. Production sites include groups Nojack, Pump and Nonpump. Plot shows a linear correlation and  $r^2$  value as well as a possible nonlinear relationship (curved line).

There were two VOCs, acrolein and benzene, which exceeded Occupational Exposure Limits (OELs), specifically the time weighted average permissible exposure limit (TWA PEL; see Table 5). Maximum concentrations of both acrolein and benzene exceeded TWA PELs, but only acrolein average concentrations exceeded the TWA PEL in the Nojack group. All of the VOCs with available inhalation reference concentrations (RfC's) had exceedances in their average and maximum concentrations, but not for every treatment group (e.g., average carbon disulfide was only > 0 in the Pumping group). Benzene was chosen as the VOC for modeling, as it had exceedances in OELs and has biological relevance in the system. Parameters for model selection included variables previously listed (Table 6), as well as Time, because benzene was the only VOC with a time effect. If time was not a significant predictor it would still be included and controlled for in the model. For the GEE model, the "exchangeable" working correlation structure showed the lowest value of QIC (QIC = -735, QICu = -802) and smallest range of residual values (-0.5234 to 1.0) and thus was applied. Among the seven parameters used in model ranking, a univariate model including pump group, natural gas, hour, and direction proved to be the most adequate combination for prediction of benzene concentrations.

#### **Selected Model With Estimates**

$$\log(\hat{\mu}_i) = -1.1851 + 0.3101(\text{Nonpump}) - 1.1915(\text{Nojack}) + 0.1153(\text{NE}) - 0.0521(\text{SE}) - 0.0025(\text{NG}) + 0.2089(\text{HR1}) - 0.0058(\text{HR2}) - 0.6928(\text{HR3}) + 0.3476(\text{HR4}) + 0.5295(\text{HR5})$$

Table 5

Summary of Average and Maximum Volatile Organic Compound (VOC) Levels and Regulatory Limits.

CAS No.	Gas Name	Chemical Formula	DX4040		OEL's ppm <sub>1</sub>			EPA Reference <sub>4</sub>	Average Concentration ppm						True maximum Concentration ppm <sub>2</sub>					
			Range (ppm) <sub>1</sub>	Limit (ppm) <sub>2</sub>	TWA PEL	STEL	Ceiling		RfC mg/m <sup>3</sup> (ppb)	WPNG	Control E	Pumping	Mountain	Nonpump	Nojack	WPNG	Control E	Pumping	Mountain	Nonpump
75-07-0	Acetaldehyde	C <sub>2</sub> H <sub>4</sub> O	0 - 50	2	200			0.009	0.12	0.06	0.11	0.14	0.05	0.05	0.84	0.35	0.49	0.96	0.32	0.3
107-02-8	Acrolein	C <sub>3</sub> H <sub>4</sub> O	0 - 200	0.25	0.1			2x10 <sup>-5</sup>	0.02	0.03	0.08	0.08	0.08	<b>0.13</b>	<b>0.49</b>	<b>0.22</b>	<b>0.54</b>	<b>0.55</b>	<b>0.34</b>	<b>0.69</b>
71-43-2	Benzene	C <sub>6</sub> H <sub>6</sub>	0 - 50	0.13	1	5	25	0.03	0.00	0.03	0.16	0.00	0.09	0.27	0.14	0.34	<b>1.26</b>	0.06	0.68	<b>1.31</b>
106-99-0	Butadiene-1,3	C <sub>4</sub> H <sub>6</sub>	0 - 200	0.2	1	5		0.9	0.04	0.05	0.05	0.00	0.01	0.03	0.35	0.21	0.38	0	0.15	0.33
124-38-9	Carbon Dioxide	CO <sub>2</sub>	0 - 2000	<20	5000				373.33	373.80	358.62	342.04	354.37	358.46	434.84	395.67	411.14	413.94	375.22	389.93
75-15-0	Carbon Disulfide	CS <sub>2</sub>	0 - 100	0.17	20	30	30	0.7	0.00	0.00	0.01	0.00	0.00	0.00	0.16	0	0.71	0	0	0.15
630-08-0	Carbon Monoxide	CO	0 - 200	0.25	50	200			0.15	0.14	0.05	0.00	0.00	0.00	1.52	1.34	3.57	0	0.15	0
463-58-1	Carbonyl Sulfide	COS	0 - 50		5				0.03	0.02	0.03	0.01	0.03	0.02	0.07	0.03	0.05	0.03	0.04	0.05
110-82-7	Cyclohexane	C <sub>6</sub> H <sub>12</sub>	0 - 50	0.01	300			6	0.02	0.01	0.01	0.01	0.01	0.01	0.28	0.07	0.14	0.09	0.05	0.09
100-41-4	Ethyl Benzene	C <sub>8</sub> H <sub>10</sub>	0 - 100	0.08	100	125		1	0.01	0.03	0.03	0.01	0.04	0.08	1.42	0.69	0.42	0.18	0.3	0.67
620-14-4	Ethyl Toluene-3	C <sub>9</sub> H <sub>12</sub>	0 - 100						0.24	0.15	0.07	0.04	0.02	0.02	1.34	1.16	1.34	0.57	0.35	0.33
50-00-0	Formaldehyde	CH <sub>2</sub> O	0 - 50	0.09	0.75	2			0.20	0.23	0.16	0.14	0.15	0.12	0.68	0.56	0.43	0.48	0.34	0.31
142-82-5	Heptane-n	C <sub>7</sub> H <sub>16</sub>	0 - 50		500				0.07	0.02	0.04	0.04	0.02	0.01	0.57	0.18	0.43	0.26	0.08	0.13
110-54-3	Hexane-n	C <sub>6</sub> H <sub>14</sub>	0 - 100		500	100	510	0.2	0.02	0.00	0.01	0.02	0.00	0.00	0.47	0.07	0.3	0.49	0	0
78-78-4	Isopentane	C <sub>5</sub> H <sub>12</sub>	0 - 100		600				0.02	0.04	0.03	0.07	0.01	0.02	1.32	0.67	0.32	1.76	0.23	0.77
74-82-8	Methane	CH <sub>4</sub>	0 - 100	0.11					2.29	2.05	2.38	1.85	1.94	1.89	3	2.27	5.7	2.06	2.39	2.22
78-93-3	Methyl Ethyl Ketone	CH <sub>3</sub> COC <sub>2</sub> H <sub>5</sub>	0 - 200	0.14	200	300		5	0.18	0.12	0.28	0.16	0.25	0.29	0.72	0.74	0.95	1.01	0.87	1.09
109-66-0	Pentane-n	C <sub>5</sub> H <sub>12</sub>	0 - 100		1000				0.14	0.03	0.16	0.12	0.04	0.09	2.08	0.48	1.76	2.13	0.5	1.48
10024-97-2	Nitrous Oxide	N <sub>2</sub> O	0 - 100	0.02	25	5			0.23	0.25	0.21	0.23	0.23	0.22	0.34	0.3	0.31	0.32	0.32	0.34
108-88-3	Toluene	C <sub>7</sub> H <sub>8</sub> (C <sub>6</sub> H <sub>5</sub> CH <sub>3</sub> )	0 - 200	0.13	200		300	5	0.02	0.01	0.00	0.00	0.00	0.00	1.48	0.38	0.09	0	0	0.17
526-73-8	Trimethylbenzene (1,2,3)	C <sub>9</sub> H <sub>12</sub>	0 - 100	0.1	25			0.006	0.22	0.16	0.10	0.02	0.09	0.06	1.57	0.55	1.03	0.34	0.46	0.65
106-42-3	Xylene-m	C <sub>8</sub> H <sub>10</sub>	0 - 200	0.1	435	655	435	0.1	0.13	0.22	0.20	0.02	0.06	0.41	1.75	1.91	4.08	1.02	1.99	3.98
106-42-3	Xylene-o	C <sub>8</sub> H <sub>10</sub>	0 - 200	0.1	435	655	435	0.1	0.01	0.00	0.05	0.00	0.13	0.09	0.49	0	0.48	0.09	0.56	0.68
106-42-3	Xylene-p	C <sub>8</sub> H <sub>10</sub>	0 - 200	0.1	435	655	435	0.1	0.16	0.11	0.18	0.06	0.24	0.12	0.89	0.72	0.95	0.49	0.65	0.51

Notes: RfC exceedances are in purple, PEL exceedances are indicated by bold text

<sup>1</sup> Measurement Range

<sup>2</sup> Theoretical Lower Limit Detection based on 60 s measurement time, one component in nitrogen, detection limit defined as 3x stdev (noise)

<sup>3</sup> OEL's : Occupational Exposure Limits - (Integrated Risk Information System [IRIS] 2009; Occupational Safety and Health Administration [OSHA] 2009)

The TWA PEL is a Time Weighted Average Permissible Exposure Limit, which must not be exceeded in an 8-hour work shift of a 40-hour work week. STEL is a Short Term Exposure Limit, usually a 15- minute exposure that should not be exceeded at any time during a work day. A ceiling shall not exceed the 8-hour Time Weighted Average given for that substance in any 8-hour work shift of a 40-hour work-week.

<sup>4</sup> EPA Environmental Protection Agency (IRIS 2009)

<sup>5</sup> Maximum is the true (single) maximum observed value for each VOC in each group

Table 6

*Summary of Exchangeable Generalized Estimating Equation (GEE) Model Coefficients for Predicting Benzene Concentrations.*

Parameter	Level	Estimate	SE	2.50%	97.50%	Z	Pr >  Z	EC
Intercept		-1.1851	0.462	-2.0901	-0.28	-2.57	0.0103 *	0.3057
Pump Group	Nojack	0.3101	0.516	-0.7016	1.3217	0.6	0.548	1.3635
Pump Group	Nonpump	-1.1915	0.38	-1.9371	-0.4459	-3.13	0.0017 **	0.3037
Pump Group	Pumping							
Direction	NE	0.1153	0.16	-0.1977	0.4283	0.72	0.4702	1.1222
Direction	SE	-0.0521	0.109	-0.2659	0.1616	-0.48	0.6325	0.9492
Direction	W							
NG		-0.0025	3E-04	-0.0031	-0.0018	-7.22	<.0001 ***	0.9975
Hour	1	0.2089	0.363	-0.502	0.9198	0.58	0.5646	1.2323
Hour	2	-0.0058	0.36	-0.7107	0.6991	-0.02	0.9871	0.9942
Hour	3	-0.6928	0.37	-1.4187	0.0331	-1.87	0.0614	0.5001
Hour	4	0.3476	0.409	-0.4542	1.1494	0.85	0.3955	1.4156
Hour	5	0.5295	0.199	0.1396	0.9195	2.66	0.0078 **	1.698
Hour	6							

*Note:* Standard Error (SE), Confidence Intervals for the Estimate (2.50% and 97.50%), Z-scores and Exponentiated Coefficients (ECs) are also shown.

\* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$

## Discussion

The study findings show that flora and fauna are exposed to VOCs at different intensities on different O&NG sites. Monitoring and predicting exposure is important to private and public landowners on the PNG, oil field workers, policymakers and regulatory agencies. Concentrations of VOCs were not statistically higher in the NE and SE directions than in the W and winds clearly blew from all directions during data collection (Figure 8). Across each site are similar VOC patterns and directionality, which indicates that VOCs were found downwind. The likely reason wind direction was not a statistically significant factor was because the model compared wind direction within each group (e.g., Pump, Nonpump, Mountain etc.), not for each site, and there was quite

a bit of noise in the direction data. Wind direction is not consistent from month to month or even day to day and requires a measurement for each minute of data collection.

Volatile organic compounds have very complicated mixing chemistries and are difficult to estimate and track throughout the day. Warneke et al. (2014) found that plumes of VOCs were difficult to measure due to variable wind speed and direction as well as incomplete mixing close to the emission source.

The current study did not detect a consistent decrease in concentrations of VOCs throughout the day (see Appendix A). Regression analysis was used to compare Pumping sites' hourly, VOC concentrations (i.e., from 8:00 a.m. to 2:00 p.m. or from 10:00 a.m. to 4:00 p.m.), which showed trends amongst the five production sites (Figure 9). The five Pumping sites increased in VOC concentration, throughout the day, which is not something we anticipated. VOCs were expected to decrease with increasing temperatures, traffic and thus increasing NO<sub>x</sub>/VOC mixing ratios, although most studies are unable to show these trends (Gilman et al. 2013; Warneke et al. 2014). The only VOC with a decreasing trend was nitrous oxide, which is interesting considering Carslaw and Beevers (2013) found increasing trends in NO<sub>x</sub>, although it was unclear which nitrogen oxides were quantified. Oxidation of N<sub>2</sub>O by O<sub>3</sub> is common and yields molecular oxygen (O<sub>2</sub>) and either NO or dinitrogen dioxide (N<sub>2</sub>O<sub>2</sub>). The NO or N<sub>2</sub>O<sub>2</sub> is then oxidized (within a couple of hours) to form NO<sub>2</sub>. When NO<sub>2</sub> is hit by a photon of ionizing radiation from sunlight it reacts with O<sub>2</sub> to form ozone (Blaszczak et al. 1999). Although not measured in the currently study, the potential of ozone formation on sites is high.

## **Volatile Organic Compound (VOC) Sources and Trends**

There were 20 sites with a ratio of *i*-pentane to *n*-pentane at or below one (Figure 10), indicative of VOCs released from O&NG (Swarthout et al. 2013). It is interesting to note that the Mountain sites hovered along the *i*- to *n*- pentane line. One mountain site, with higher concentrations of both *i*- and *n*- pentane, was above the line and therefore more impacted by vehicle exhaust than O&NG emissions. This makes logical sense with the continuous flow of traffic through Estes Park and the lack of adjacent O&NG production. There were other sites near the line, indicating impact from traffic, including all the ControlE sites, a WPNG site, a Nojack site and a Pumping site. The Pumping site, site 19, was in the producing phase when selected for the study, but did not produce oil or natural gas the month of data collection. This could have reduced the Pumping group average concentrations and effected trends.

The VOCs identified on each site, also show patterns and trends. For the Mountain sites, we expected to detect some biogenic VOCs (BVOCs) such as aldehydes and some alkanes, released from the forested areas and lower levels of all other chemical groups. Average and maximum concentrations of acetaldehyde were in fact higher in Mountain than in other groups (Figure 5P; Table 5). Most chemicals, however, had concentrations significantly lower in the Mountain group such as the alkenes, alkanes, sulfides and oxygenated species. The mountain group had consistently lower VOCs than other groups across the board (Table 5).

Groups ControlE and WPNG had significantly higher concentrations of ethyltoluene and carbon dioxide than other groups (Figures 5G and 5W). It is not clear if these high concentrations were due to a combination of low wind, low atmospheric



mixing and high humidity (fog) or if cattle ranching played a role. Greenhouse gases (GHGs) were higher for the Pumping group in general. Methane, an alkane and a GHG, reached 5.7 ppm and carbon monoxide reached 7.1 ppm on Pumping sites (Figure 7). Methane is a potent greenhouse gas and the observed levels of ~6 ppm on Pumping sites indicate leaks. This implies that O&NG companies are losing money because of leaks, and investment in monitoring and maintenance are recommended. Average concentrations of methane ranged from 1.85 ppm (Mountain) to 2.38 ppm (Pumping; Figure 7). Even the background levels (Mountain, ControlE, WPNG) greatly exceed the global methane average of 0.782 ppm (U.S. EPA 2006). Overall, the Nojack group had higher average concentrations of VOCs than other groups, including significantly higher levels for benzene, ethylbenzene and acrolein (Figures 5F, 5I and 5Q).

Within the ordination plot (Figure 11), groups form based on VOC frequency and abundance. The key indicator species for WPNG appear to be carbon dioxide, nitrous oxide, carbon monoxide, ethyltoluene, trimethylbenzene, o-xylene, and hexane. The VOC with the highest relative abundance was carbon disulfide, which was found mostly on Pumping (max 40), and Nojack (max 25) sites. The species with the highest indicator scores were benzene (47), o-xylene (47), and hexane (47) followed closely by carbon monoxide (44), and ethyltoluene (43). The ordination plot seems to indicate that VOC frequency might be on Axis two (vertical axis), while VOC abundance is on Axis one (horizontal axis; Figure 11). Hexane had the highest IV of 47.1 and was mostly found in Mountain sites, which is why this group can be found clustered together at the top of the ordination plot (Table 4). Many of the sites clustered together (within their Pump group) according to their frequency and abundance scores. The Nojack and Pumping sites,

although a little more spread out, varied in the levels of O&NG produced on sites, and thus, have a different concentrations of VOCs.

With this new information, an ordination plot was created to explore the relationship between VOC ordination scores and the amount of natural gas produced at a site (Figure 12). Two of the pump groups clustered in the upper, right corner of the plot, indicating these sites have the highest, negative ordination scores and the highest production of natural gas. Regression analysis NonPump, NoJack and Pump sites (with little to no O&NG production) all cluster together according to their IN scores, while the Pump sites with more O&NG produced a different sweet of VOCs and/or at higher concentrations (Figure 12).

### **Generalized Estimating Equation (GEE)**

Based on the output from using GEE with the exchangeable working correlation structure, there was a significant group effect for the Nonpump group ( $p = 0.0017$ ) with estimated coefficient -1.1915. The data provide sufficiently compelling evidence to conclude mean concentrations of benzene differ between Nonpump, Pumping and Nojack groups, accounting for the effects of direction, hour, natural gas, and the autocorrelation associated with repeated observation of the same sites.

The Pump indicator in the model has three levels, with Nonpump and Nojack compared to Pumping. Therefore, the model suggests Nonpump sites are expected to have a mean benzene concentration that is ~0.3 times lower than Pumping sites and the Nojack group is 1.4 times higher than Pumping sites, all other variables held fixed. The model also shows significant differences associated with the hour (time) effect, with hours four and five having higher expected mean benzene concentrations than the sixth

(baseline) hour. This trend can also be seen in the Spaghetti Plot (see Appendix A). Empirical estimates of the standard errors and covariance were examined to determine model fit.

A binary variable could also be used with GEE to show TWA PEL exceedances, where exceeded = 1 and not exceeded = 0. In this instance, benzene time weighted average permissible exposure limit (TWA PEL) exceedances could be predicted using logistic regression with a binary distribution and link = "logit." The, z-scores, *p*-values, adjusted residuals, Receiver Operator Characteristic (ROC) curve and Hosmer and Lemeshow goodness of fit test could be used to estimate model fit.

We created this model to predict concentrations of benzene. For example, we might be interested in predicting the concentration of benzene on an older PNG site that is no longer actively pumping (Nonpump group), yet passively produced 40 Mcf of natural gas in a month. If sampled at 1:30 pm (Hr 6) NE of the wellhead, the predicted concentration is 0.41 ppm. This concentration is well below the TWA PEL of 1 ppm for benzene and given these parameters is likely not at risk of exceeding the limit. The predictors for the models were chosen before analysis to prevent overfitting the data. Some predictors, which were not used, but could be used to contribute to model predictive ability, include site operator, construction and infrastructure details. For example, only two sites out of 12 had exceedances of TWA PELs and these two sites were owned by the same company. In general, some companies may be doing a better job at detecting and preventing fugitive emissions than others. These details could be informative when predicting concentrations of benzene as well as exceedances of reference standards.

## Biological Relevance

Many of the VOCs found in this study are toxic to flora and fauna. Under the Clean Air Act, the EPA is required to set standards for pollutants considered harmful to human and environmental health. They have set National Ambient Air Quality Standards (NAAQS) have been set for six principal pollutants including carbon monoxide, lead, nitrogen dioxide, ozone, PM<sub>2.5</sub> and sulfur dioxide. Unfortunately, NAAQS do not exist for the VOCs examined in the current study, with the exception of carbon monoxide, which is 9 ppm measured over 8 hours, and 35 ppm measured over 1 hour. Generally, human and environmental health are not protected from VOCs unless in the workplace. For this reason, occupational exposure limits (OELs) were used in addition to the reference concentrations (RfCs) for inhalation exposure to determine if observed VOCs exceeded standards. In this particular ecosystem, the shortgrass steppe, there are many plants and animals in the immediate vicinity of sites.

All VOCs with available inhalation reference concentrations (RfCs) had exceedances in their average concentrations, but not for every treatment group (Table 5). For example, carbon disulfide was only > 0 in the Pumping group. There were two VOCs, acrolein and benzene, with Occupational Exposure Limit (OEL) exceedances, specifically the time weighted average permissible exposure limit (TWA PEL; see Table 5). The TWA PEL must not be exceeded in an 8-hour work shift of a 40-hour work week. Acrolein had concentrations exceeding the TWA PEL in all groups for maximum concentrations and in the Nojack group for average concentrations (Table 5). Acrolein is toxic to humans following inhalation exposure. Acute exposure can cause upper respiratory tract irritation; however, current information is not available or inadequate to

determine developmental, reproductive or carcinogenic effects in humans (U.S. EPA 2009).

Benzene is a carcinogenic compound causing leukemia (IRIS 2002). The Environmental Protection Agency (EPA) has estimated that a lifetime exposure of 1  $\mu\text{g}/\text{m}^3$  of benzene through inhalation leads to about six additional cases of leukemia per million inhabitants (assuming continuous inhalation for 70 years and indoor concentrations are the same as outdoor concentrations; U.S. EPA 2009). The RfC for benzene is 0.03 ppb. In our study, we found that the mean concentration of benzene was  $\sim 0.09$  ppb for all sites combined, which is three times the reference concentration (see Appendix A). Concentrations of Benzene were as high as 1.31 ppm (Nojack) and 1.26 ppm (Pumping).

Toluene concentrations were, on average, above 1 ppm on WPNG sites and 0.855 on Pumping sites (Table 5). The maximum concentrations were 2.39 ppm (WPNG) and 2.06 ppm (Pumping). Methyl ethyl ketone (MEK) was 0.719 on WPNG on average and had a maximum value of 1.47 ppm. The pump group also had relatively high concentrations of 1.09 ppm MEK. Toluene inhalation exposure can cause central nervous system dysfunction, while MEK causes developmental and musculoskeletal variations (IRIS 2003, 2005). Although the majority of VOCs do not exceed PELs, concentrations are still of potential concern if they deposit onto surrounding media. There were 20 sites with a ratio of *i*-pentane to *n*-pentane, at or below one, suggesting O&NG, and these sites have concentrations of VOCs at biologically relevant levels. All compounds found at these sites have the potential to deposit onto soil, water and in some cases, accumulate on the waxy cuticles or in the tissues of plants. This environment presents a complex

mixture of VOCs with multiple pathways of exposure. The data not only confirms that O&NG emissions are impacting the region, but also that this influence is present at all sites, including controls.

**CHAPTER III**  
**BENZENE, TOLUENE, ETHYLBENZENE AND XYLENE**  
**(BTEX) CONCENTRATIONS IN VEGETATION**

**Abstract**

Weld County, Colorado, has exponentially increased its oil and natural gas (O&NG) drilling and extraction in the last decade. Over 23,160 of Colorado's 54,194 active wells are located in Weld County (Colorado Oil and Gas Conservation Commission [COGCC] 2017). Volatile organic compounds (VOCs), such as benzene, toluene, ethylbenzene and the xylenes (BTEX), released from active and producing wells, have the ability to deposit (e.g., wet or dry) to surrounding plants. In May and June of 2014, *Bouteloua gracilis* and *Bouteloua dactyloides* leaves were collected from 20 O&NG production sites and BTEX were quantified in aboveground tissue. Sites were grouped according to production (date and amount): plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3). Deposition and accumulation of BTEX onto proximate flora significantly decreased with production age. Newer wells and sites with active pumpjacks had significant concentrations of benzene and toluene. BTEX were found on every site except one plugged and abandoned site. The average concentration of toluene and benzene on all sites combined were 2.32 ppbv and 13.18 ppbv, respectively. Benzene concentrations as high as 176 ppbv were detected. These concentrations are arguably biologically relevant as organisms within 100 m of ONG production are likely breathing and, if grazing, consuming toxic levels of BTEX. We recommend O&NG

production owners or operators increase monitoring for fugitive emissions and contain leaks to reduce deposition onto proximate vegetation.

### **Introduction**

Located in the Denver Julesburg (DJ) basin, the Niobrara shale play contains oil and natural gas resources trapped one to four thousand meters below surface. Slick, horizontal, hydraulic fracturing methods have allowed for a recent increase in unconventional oil and natural gas (O&NG) extraction from the play, but the environmental and human health effects are mostly unknown. There is little research examining the biotic impacts of energy development, specifically pollutant accumulation on proximate biota or cumulative ecosystem impacts (Souther et al. 2014).

Oil and natural gas production sites located on the Pawnee National Grassland (PNG) in northeastern Colorado may be negatively affecting shortgrass steppe flora and fauna. Volatile organic chemicals released from active and producing wells have the ability to deposit (e.g., wet or dry) to surrounding plants (Karl et al. 2010, Rodriguez et al. 2012), soils (Bloomfield et al. 2012) or waters (Hayes 2009, Jackson 2012, Swackhamer 2012), but empirical data are lacking. Pollutants commonly released at drilling and production sites include BTEX, naphthalene, formaldehyde and silica (Colborn et al. 2011). Without baseline data on proximate mediums including air, water and soil, as well as flora and fauna, regulation and management of pollutants are conducted blindly. Emissions from O&NG production, with long operational lifetimes of thirty plus years, have not been measured to date. The objective of this research is to quantify the deposition and accumulation of BTEX onto proximate flora (i.e., *Bouteloua gracilis* and *Bouteloua dactyloides*).



If BTEX are depositing onto flora, this could lead to restrictions and safety requirements for cattle, wildlife and humans in close proximity to an O&NG production site. Bamberger and Oswald (2012) found a high incidence of reproductive problems, upper respiratory issues, nosebleeds, diarrhea, vomiting, rashes, headaches and neurological problems in animals and humans near drilling sites. It has also been shown that benzene and toluene exposure increase lung lesions and pneumonia in calves, leading to death (Waldner and Clark 2009). Bechtel et al. (2009) found a decrease in the number of T-lymphocytes in beef calves exposed by inhalation to benzene levels of  $0.378 \mu\text{g} / \text{m}^3$  (0.118 ppbv) and toluene levels of  $0.713 \mu\text{g} / \text{m}^3$  (0.189 ppbv), which can weaken a calf's immune system. These studies examine the effects of inhalation exposure, but it is likely organisms are exposed to BTEX from multiple pathways, including oral exposure from eating grass and drinking water. Deposition and accumulation of BTEX were analyzed in the present study using supplemental information regarding pump activity, well production during month of collection and known toxicity levels for BTEX.

The United States Environmental Protection Agency (EPA) has not yet set standards for BTEX in or on grasses and food crops; however, there are BTEX standards for drinking water known as Maximum Contaminant Levels (MCLs). An MCL is the legal threshold limit on the amount of a substance that is allowed in public water systems under the Safe Drinking Water Act. The limit is usually expressed as a concentration in milligrams or micrograms per liter of water. To determine if deposition and accumulation of BTEX onto proximate flora is biologically significant if ingested by cattle, concentrations will be compared to the MCL for benzene in drinking water, which is 0.005 milligrams per liter (mg / L) or 5 parts per billion (ppbv) maximum. The health

effects of ingesting water containing benzene in excess of the MCL over many years could include anemia, decrease in blood platelets and increased risk of cancer (U.S. EPA 2009). If the ingestible levels of BTEX on grasses eaten by cattle and insects are near or surpass the MCLs for drinking water, this could have biological implications.

Concentrations will also be compared to the Reference Dose (Rfd) for Chronic Oral Exposure for each BTEX (U.S. EPA 2009).

The shortgrass prairie has three key grass species, two warm-season grasses, *Bouteloua gracilis* (blue grama) and *Bouteloua dactyloides* (buffalo grass), and one cool season grass, *Pascopyrum smithii* (western wheatgrass; Sims and Singh 1971). Blue grama and buffalo grass are the primary components of the shortgrass prairie and western wheatgrass is less abundant and found under moister conditions. *B. gracilis* is especially important in the shortgrass steppe because it is a nutritional and palatable grass for cattle. It becomes prolific in late June and remains nutritional into the winter, with sufficient protein levels for all cattle. By collecting and analyzing BTEX in *B. gracilis* and *B. dactyloides*, results could indicate that cattle and other fauna are potentially ingesting irregularly high doses of the carcinogens on a daily basis, although this is not going to be directly addressed in the current research. Our hypotheses are that deposition and accumulation of BTEX onto proximate flora will decrease with increased distance from the well (source of O&NG), will be greater downwind (NE and SE directions), will decrease with production age, will be greater on sites with active pumping and will be biologically significant.

## Methods

### Site Description

Physical and Social Setting: The PNG covers 193,060 acres (79,876 ha) and lies east of the Rocky Mountains at an elevation of 1,500 to 1,800 meters (Desalme et al. 2011). The three-decade averages of climatological variables (1981-2010) include an annual average temperature of 6.4 °C (43.6 °F) and mean annual precipitation of 42.62 cm (16.78 in; NOAA 2017a). In Colorado, temperature has increased by 1 °C since systematic measurements began in 1895 (Stohlgren et al. 2008). Recently, warming has been accelerated by human activities. Annual variation in temperature and precipitation has been significant and has fluctuated at irregular intervals between warm-dry years and cool-wet years (Stohlgren et al. 2008). The PNG is classified as a shortgrass prairie region, also known as a shortgrass steppe. The shortgrass steppe is distinguished by the height of its dominant grasses (blue grama and buffalo grass) and less than 50% of the ground in the PNG is covered by vegetation (Hazlett 1998). Soils on the site consist of 90% stoneham fine sandy loam from 0 to 13 cm, clay loam from 13 to 20 cm and loam from 20 to 36 cm, with 0 to 6% slopes (U.S. Department of Agriculture [USDA] 2014a). In some regions erosive forces have worn away loam to reveal shale, sandstone and siltstone (i.e., blowouts; Crabb 1981). The stratigraphy of the region includes carboniferous to tertiary sedimentary rocks. Layers of cretaceous sediment include the Laramie formation, Fox Hills sandstone, Pierre shale, Niobrara formation, Benton shale and Dakota Group (Crabb 1981). These shales contain commercial quantities of oil. The PNG is used extensively for irrigated agriculture and livestock and is a patchwork of private and government land. Communities found within or proximate to the grassland

are directly affected by the management of the grasslands and by those who use the lands for recreation, resource extraction and grazing.

### **Site Selection**

The PNG is divided into east and west landmasses, which cumulatively have hundreds of O&NG production sites and facilities, but only ~60 production sites and ~20 processing facilities are on parcels owned and leased by the Bureau of Land Management (BLM). Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3). Five sample sites were randomly selected from each group ( $n = 5$ ,  $N = 20$ ). Sites that were potentially dangerous or were not easily accessible from open, public roads were excluded. All sites in producing groups have been both vertically and horizontally drilled. During data analysis, researchers discovered that site 17 from PR3 was incorrectly grouped. The site had been re-fractured during that period, which is why it was placed in the PR3 group, but had actually been producing from a vertical well since 1990. For this reason, it was excluded from analysis.

### **Vegetation Tissue Collection**

In May and June of 2014, samples were collected from the 20 study sites. *Bouteloua gracilis* (blue grama) and *Bouteloua dactyloides* (buffalo grass) shoots were selected as the vegetation monitoring mediums as buffalo grass is the most prolific grass on the PNG in the spring and blue grama in the fall. Their abundance allows for replicate sampling at each study site. At each of the 20 sites, 100 m transects were laid in northeast, southeast and west directions from the pumpjack or stack, and two samples

were collected at distances of 25 m, 75 m, and 100 m in each direction. The leaves of *Bouteloua gracilis* and *Bouteloua dactyloides* were cut with stainless steel scissors and placed directly into sterile, labeled, glass vials with 120 mL of gas chromatography (GC) grade methanol added in the field. The septate lids were then sealed with a crimper onto the vials. Transported in a cooler and refrigerated at 4 °C until analysis. Samples were analyzed within 12 days of collection date. From the 20 sites a total of 360 vegetation samples were collected and analyzed using GC with a flame ionization detector (FID).

### **Gas Chromatography Flame Ionization Detector (GC FID) Analysis**

Vegetative samples were analyzed for BTEX using a modified version of the EPA's method 8260B, volatile organic compounds by gas chromatography mass spectrometry (GCMS) for solid waste matrices (Kelley 1997). Each sample was analyzed using a GCFID to identify types and amounts of BTEX. In an external standard calibration method, the absolute analyte response was plotted against the analyte concentration to create the calibration curve. A single calibration curve was created containing each analyte of interest (i.e., BTEX) and the coefficient of determination for the calibration was  $r^2 = 0.999$ . With detectors that have compound independent response, such as the FID, one can get fairly good estimates of the amount of an analyte based on a calibration curve. All calibration standards were prepared using GC grade methanol and ranged in concentration from 0.2 to 0.01 mg/L (ppm). Reference standards were the same as sample solutes, which eliminated the need for response factors. External standard concentrations of BTEX were similar to the components of the sample (~ 0.01 ppm). Concentrations in the ppb were completely undetectable with this analysis. Samples were well mixed (inverted 30 times) before extracting 1.0 microliter of the sample solution and

injecting into the Hewlett-Packard 5890 Series II GC (see Appendix B for instrument specifics). To reduce volume errors, all sample preparations and injections were performed by a single individual.

### **Statistical Analysis**

A Wilks' Lambda multivariate analysis of variance (MANOVA) was run on a model including response variables BTEX and predictors Direction, Distance and Production Group. When statistical significance was found in the model, univariate analysis of variance (ANOVA) tests with Tukey post-hoc tests were used to determine differences between groups. Tests and descriptive statistics were also used to determine if assumptions of normality, linearity and homogeneity of variance were met. The Levene's tests for homogeneity of variance were as follows: benzene  $p = 0.14$ , toluene  $p = 0.000186$ , ethylbenzene  $p = 0.9933$ , m,p-xylene  $p = 0.6327$  and o-xylene  $p = 0.6327$ . Lilliefors (Kolmogorov-Smirnov) normality test for each BTEX indicated that all BTEX were non-normal ( $p < 2.2e-16$ ). All BTEX were log transformed for statistical analysis and 0.01 was added to ensure all values were above zero for statistical analyses. Concentrations were converted back to their original values (mg/g) for descriptive statistics and discussion. Shapiro-Wilk test for normality indicated normal distribution post-transformation for all BTEX: benzene  $W = 0.541$ , toluene  $W = 0.579$ , ethylbenzene  $W = 0.199$ , m,p-xylene  $W = 0.0863$  and o-xylene  $W = 0.342$ . Data were linear and had appropriate post-transformation residual plots.

Whilst on location, researchers noticed that some sites had pumpjacks that were running very regularly and others that were not. It was not possible to establish exactly what time pumps ran throughout the day, but it was expected that sites seen pumping

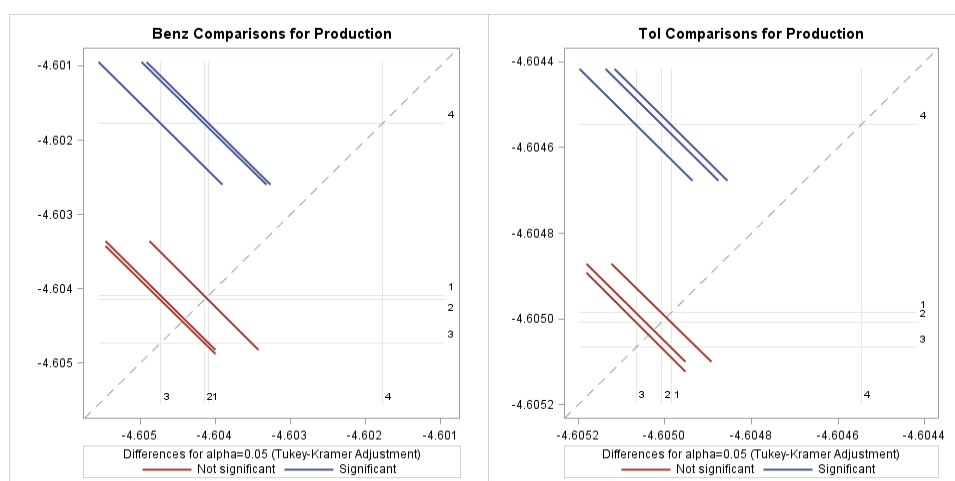
during the approximately six to eight hours on site, during data collection, would have higher deposition of BTEX. Pumpjacks mechanically lift liquid out of wells, moving a higher volume of O&NG than similarly aged, non-pumping sites. This “pumping” activity increased the risk of fugitive emissions. To determine if pumping was a significant factor, a separate analysis was conducted including only producing sites with pumpjacks, including sites 17, 6, 3, 2, 22, 21, 1, 19, 16, 15, 13 ( $N = 11$ ). Each site was then classified as 1 = pumping ( $n = 38$ ) or 0 = not pumping ( $n = 63$ ) based on whether the pumpjack was physically pumping during data collection. We also included factors Oil and Natural Gas (mean concentrations produced the month of data collection) as potential significant predictors in the model.

A Wilks' Lambda multivariate analysis of covariance (MANCOVA) was run on a model including response variables BTEX and predictors Direction, Distance, Production Group, Pumping, and covariates Oil and Natural Gas. When statistical significance was found in the model, univariate analysis of covariance (ANCOVA) tests with Tukey post-hoc tests were used to determine differences between groups. Data analyses were performed using R (v 1.0.44) and SAS (v 6.1.7601).

## Results

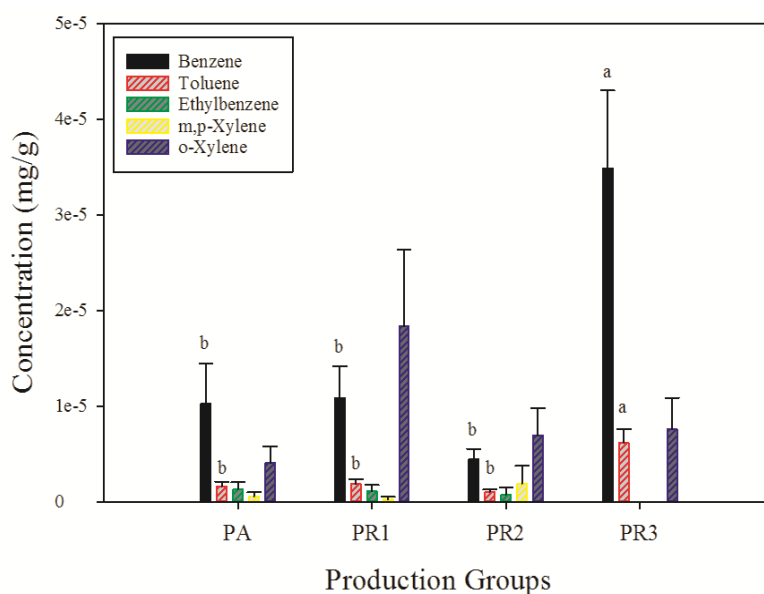
Results from the 19 sites indicate that BTEX were present in a majority of samples. Mean concentrations (mg BTEX /g veg) for all samples were: benzene  $1.5 \times 10^{-5}$ , toluene  $2.6 \times 10^{-6}$ , ethylbenzene  $7.9 \times 10^{-7}$ , o-xylene  $9.2 \times 10^{-6}$  and m,p-xylene  $6.9 \times 10^{-7}$ . MANOVA results using Wilks' Lambda statistic indicate a significant Pump Group effect ( $F(15, 420) = 3.25$ ,  $p < .0001$ ) when controlling for Distance and Direction. Univariate test statistic results show log transformed benzene ( $F(7) = 3.74$ ,  $p = 9 \times 10^{-4}$ ) and toluene

( $F(7) = 4.65, p < 0.0001$ ) concentrations were significantly higher in group PR3 than all other production groups (Figure 13). The PR1 group had the highest concentration of the xylenes, although not statistically significant (Figure 14; Table 7).



*Figure 13.* Significant effects of production group on benzene and toluene log transformed concentrations. Plots show LS-mean adjusted pairwise differences between groups, their significance levels and their individual confidence limits. The LS-means of each pair meet at their intercept (center of line). Blue line = significant difference between LS-mean, red line = similar LS-mean. Sites were grouped according to production (date and amount) including plugged and abandoned in the 1980s (PA = 2), producing since 1980-1990 (PR1 = 1), producing since 2000-2005 (PR2 = 3) and producing since 2006-2013 (PR3 = 4). Data were log transformed for analysis.





*Figure 14.* Mean concentrations of benzene, toluene, ethylbenzene, and xylenes (reverse log-transformed) across production groups. Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3). PR3 concentrations of benzene and toluene are significantly higher than all other groups. For models with  $p < .05$  in the ANOVA test, Student-Newman-Keuls post-hoc tests are shown. Means with the same letter are not significantly different.

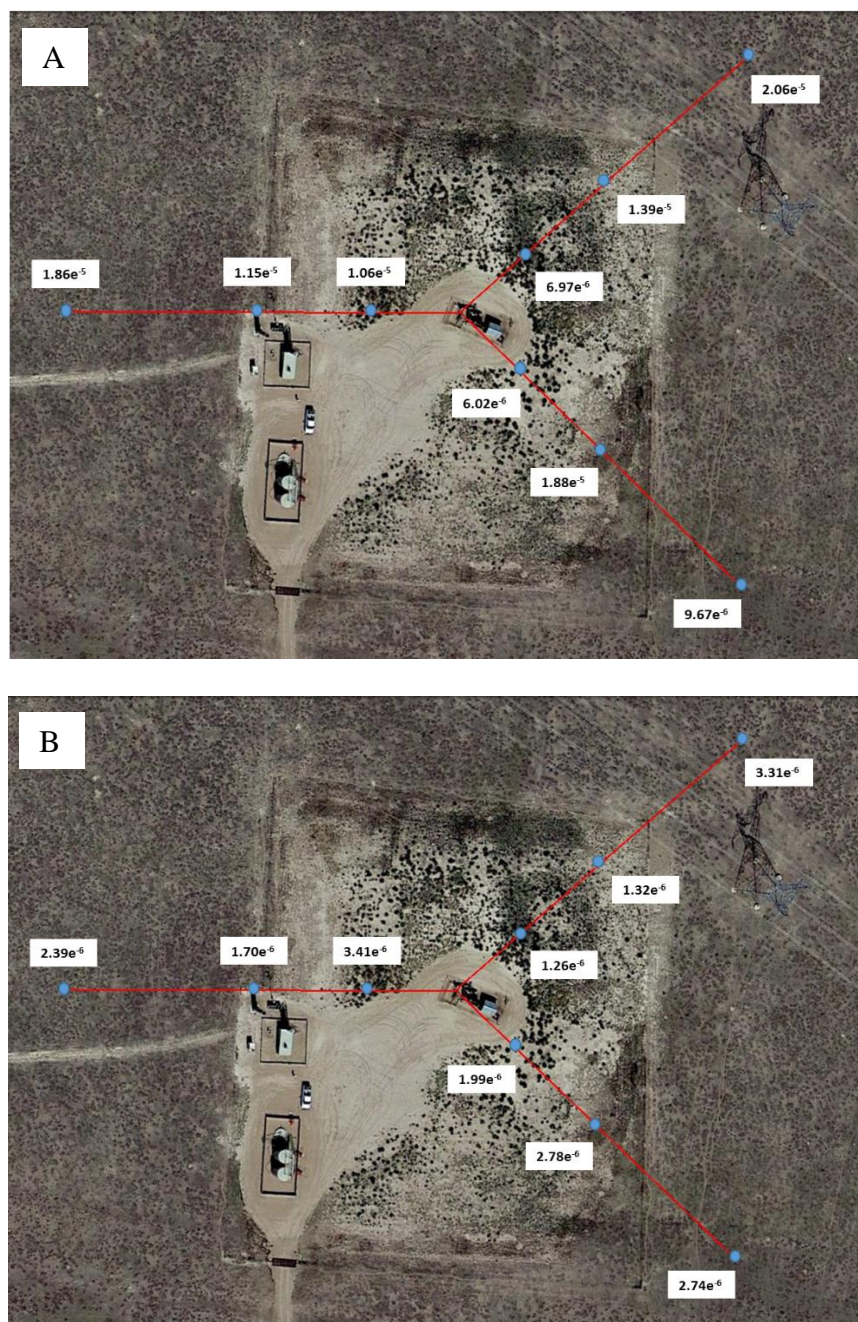
Table 7

*Mean Concentrations and Standard Errors for Benzene, Toluene, Ethylbenzene, and Xylenes (mg/g) Across Production Groups.*

VOC	PR1		PA		PR2		PR3	
	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error
Benzene	1.09E-05	3.33E-06	1.03E-05	4.21E-06	4.42E-06	1.10E-06	3.49E-05	8.10E-06
Toluene	1.84E-06	5.24E-07	1.62E-06	4.60E-07	1.03E-06	2.30E-07	6.17E-06	1.45E-06
Ethylbenzene	1.08E-06	6.62E-07	1.32E-06	7.18E-07	7.51E-07	7.51E-07	0.00E+00	0.00E+00
Xylene-o	1.17E-04	1.17E-04	5.93E-07	3.91E-07	1.89E-06	1.87E-06	0.00E+00	0.00E+00
Xylene-m,p	1.83E-05	8.06E-06	4.04E-06	1.74E-06	6.97E-06	2.79E-06	7.60E-06	3.27E-06

*Note:* Standard errors are reverse log-transformed. Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3).

Although deposition and accumulation of BTEX did decrease with distance from the source, this trend was not significant ( $F(10, 304) = 0.78, p = 0.644$ ). Deposition and accumulation of BTEX onto proximate flora was significantly greater in NE and SE directions from the wellhead as hypothesized ( $F(10, 304) = 0.63, p = 0.786$ ). Comparing within-site versus between-site standard deviation revealed the between-site standard deviation was greater than the within-site deviation. This implies that direction was a factor. We were not able to show that westerly winds were blowing BTEX in the same eastern direction every day. Any directional trends were lost when sites were combined for each BTEX (Figure 15 A-E).



*Figure 15.* Aerial view of transects, showing directional concentrations of benzene, toluene, ethylbenzene, and the xylenes. The numbers represent concentrations of A. benzene, B. toluene, C. ethylbenzene, D. m,p-xylene, and E. o-xylene (mg/g for all sites combined). The same aerial view is used to represent all sites.

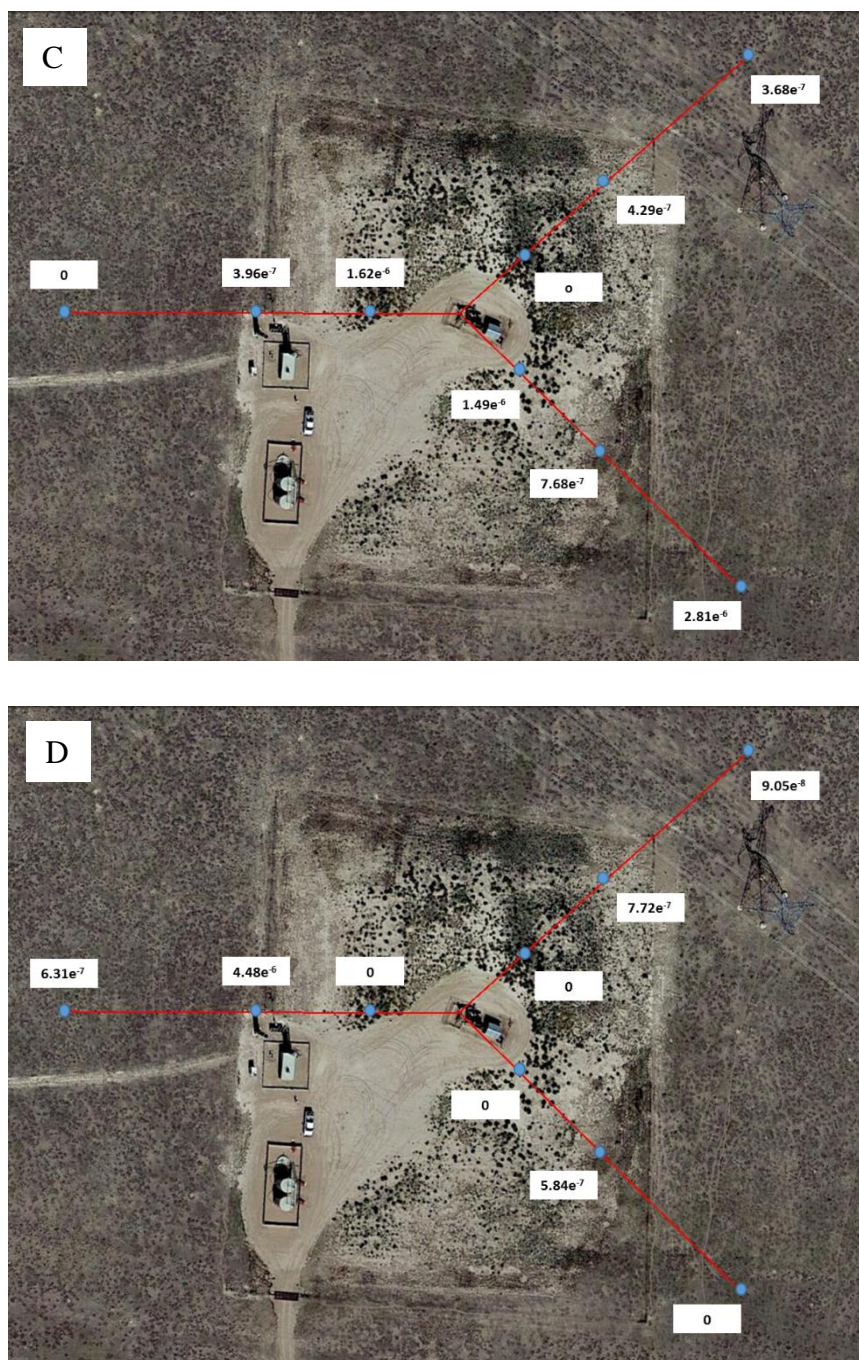


Figure 15. Continued.

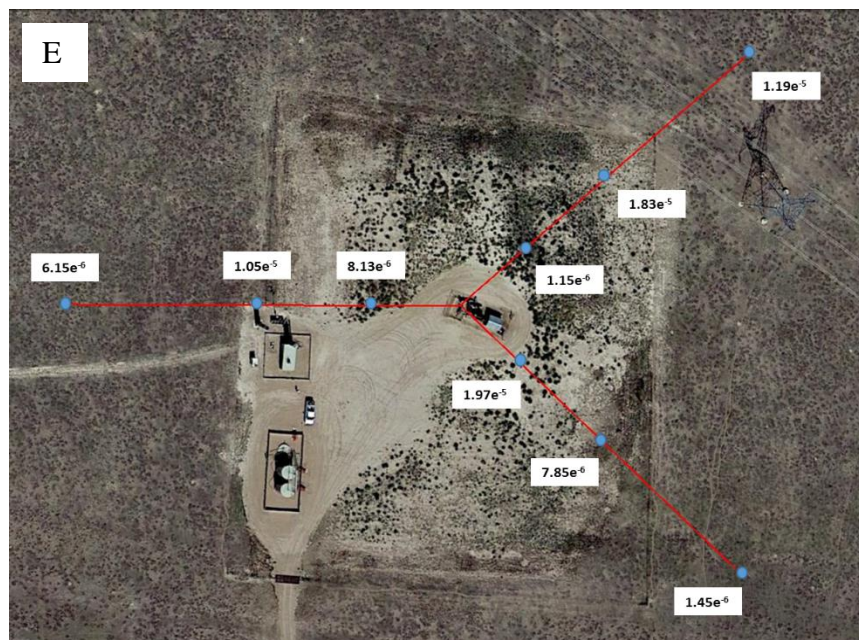
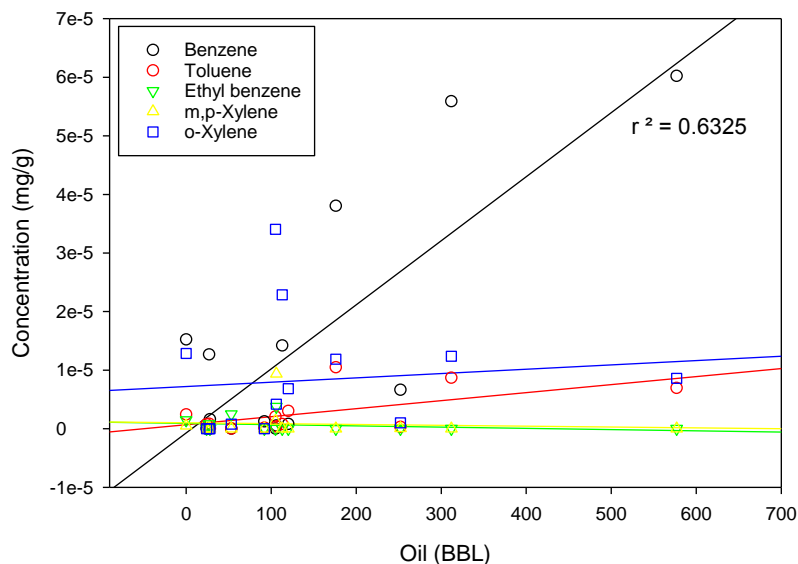
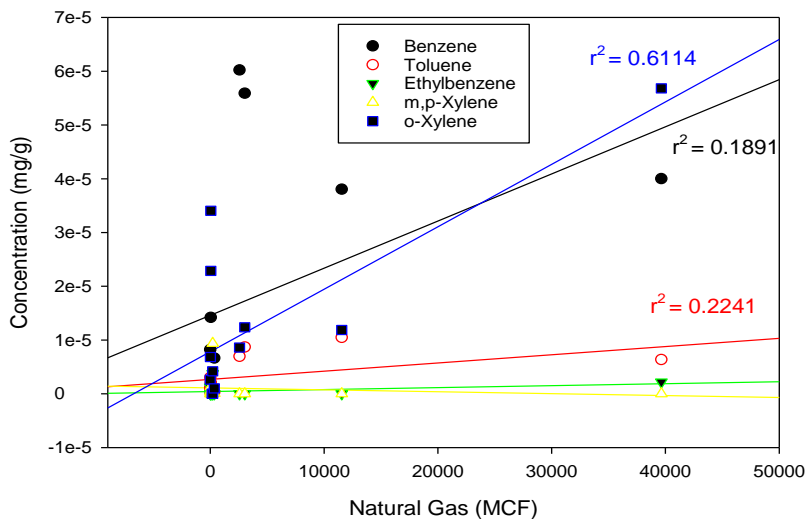


Figure 15. Continued.

For the analysis excluding PA sites, MANCOVA results indicated a significant Production Group effect ( $F(15, 411) = 2.03, p = 0.0127$ ) and significant covariate effects; the amount of oil produced the month of sample collection ( $F(5, 149) = 2.48, p = 0.0343$ ) and the amount of natural gas produced the month of sample collection ( $F(5, 149) = 4.2, p = 0.001$ ). As in the first model there were significant Pumping effects ( $F(5, 151) = 10.94, p < 0.0001$ ), but not Distance or Direction effects. The amount of oil produced the month of data collection (a covariate in the model) was a strong predictor for benzene ( $F(1) = 10.17, p = 0.0017$ ; Figure 16). The amount of natural gas produced the month of data collection (another covariate in the model) was also significant for benzene ( $F(1) = 5.99, p = 0.015$ ), toluene ( $F(1) = 9.32, p = 0.0027$ ) and o-xylene ( $F(1) = 7.4, p = 0.0073$ ) levels (Figure 17).

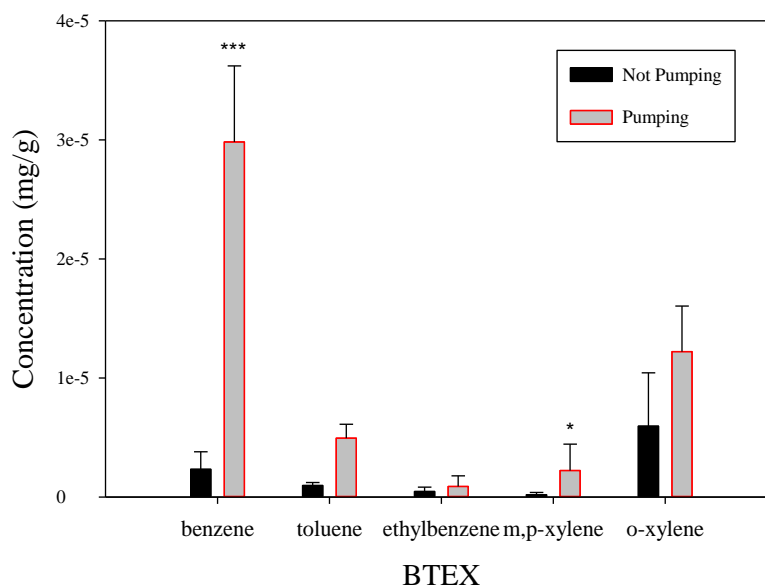


*Figure 16.* Correlation between concentrations of benzene, toluene, ethylbenzene, and xylenes (BTEX) and the amount of oil produced on a site. The quantity of oil produced the month of data collection represents the variable Oil. The  $r^2$  values are shown for BTEX with oil as a significant predictor in the statistical model ( $p < 0.05$ ).



*Figure 17.* Correlation between concentrations of benzene, toluene, ethylbenzene, and xylenes (BTEX) and the amount of natural gas produced on a site. The quantity of natural gas produced the month of data collection represents the variable Natural Gas. The  $r^2$  values are shown for BTEX with natural gas as a significant predictor in the statistical model ( $p < 0.05$ ).

Concentrations were higher for all BTEX on pumping versus non-pumping sites and were significantly higher for benzene ( $F(1) = 3.97, p = 0.001$ ) and m,p-Xylene ( $F(1) = 3.96, p = 0.0482$ ; Figure 18; Table 8). Average benzene concentrations exceeded the MCL for benzene in drinking water in PA, PR1 and PR3 groups and exceeded the RfD for Benzene in all Groups (Figure 19; Table 9).



*Figure 18.* Mean concentrations of benzene, toluene, ethylbenzene, and xylenes (BTEX) compared between pump activities. “Pumping” represents sites actively pumping during time of data collection and wells that were inactive during time of collection were termed “Not Pumping”. Error bars represent standard errors.

*Note:* \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$

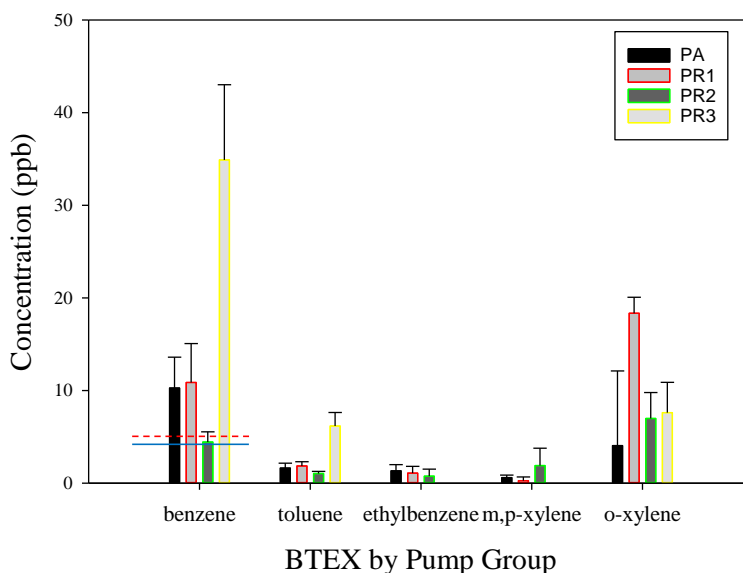
\*

Table 8

*Mean Concentrations (mg /g) and Standard Errors for Benzene, Toluene, Ethylbenzene and Xylenes Compared Between Pump Activities*

VOC	Not Pumping		Pumping	
	Mean	Std Error	Mean	Std Error
Benzene	2.33 E-6	1.47E-06	2.98E-05	6.38E-06
Ethylbenzene	4.63 E-7	3.67E-07	8.88E-07	8.88E-07
Toluene	9.70 E-7	2.51E-07	4.94E-06	1.17E-06
Xylene-m,p	1.90 E-7	1.90E-07	2.22E-06	2.22E-06
Xylene-o	5.95 Ee -6	4.48E-06	1.22E-05	3.83E-06

*Note:* “Pumping” represents sites actively pumping during time of data collection and wells that were inactive during time of collection were termed “Not Pumping”.



*Figure 19.* Relevant toxicity levels of benzene, toluene, ethylbenzene, and xylenes (BTEX) across the four production groups. Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3). Concentrations were converted from ppm to ppb for comparison with MCL. Red dashed line = MCL for benzene in drinking water (5 ppb), blue line = RfD oral dose for benzene (4 ppb d<sup>-1</sup>). Error bars represent standard errors.



Table 9

*Production Group Mean Observed Concentrations and Reference Concentrations for Benzene, Toluene, Ethylbenzene, and Xylenes.*

VOC	EPA Reference (ppb)		PR1 (ppb)		PA (ppb)		PR2 (ppb)		PR3 (ppb)		Total (ppb)
	RfD	MCL	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean
Benzene	4	5	10.86	3.33	10.26	4.21	4.43	1.11	34.91	8.10	15.12
Toluene	100	700	1.85	0.52	1.63	0.46	1.04	0.23	6.17	1.45	2.67
Ethylbenzene	80	1000	1.08	0.66	1.32	0.72	0.75	0.75	0.00	0.00	0.79
Xylene-m.p	200	10	0.27	0.27	0.59	0.39	1.89	1.88	0.00	0.00	0.69
Xylene-o	200	2288900.0	18.33	8.07	4.04	1.74	6.98	2.80	7.61	3.28	9.24

*Note:* RfD's and MCL's for BTEX and mean observed BTEX concentrations are in ppb per production group (PR1, PA, PR2, and PR3) and for all sites combined (Total). Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3). Concentrations were converted from mg/g to ppb for comparison to references. RfD is the reference dose for chronic oral exposure and the unit is ppb/day. Maximum Contaminant Levels (MCL) is the legal threshold limit on the amount of a substance that is allowed in public water systems under the Safe Drinking Water Act in ppb or µg per L of water.

## Discussion

Our hypotheses were that deposition and accumulation of BTEX onto proximate flora would decrease with increased distance from the well (source of O&NG), would be greater downwind (NE and SE directions), would decrease with production age, would be greater on sites with active pumping and would be biologically significant. As determined by the MANOVA, Distance and Direction were not significant predictors of BTEX concentrations in the model. The age of the well (i.e. Production group) did play a significant role in the concentrations of BTEX found on proximate flora, with the newer, PR3 wells having significantly greater concentrations of benzene and toluene, even when controlling for all other factors (Figure 14). When we examine the other three production group mean concentrations, we can see PR1 also had high concentrations of benzene and o-xylene, likely due to site 8, the high natural gas producing site (Table 7; Appendix B).

For the analysis excluding PA sites, MANCOVA results indicated a significant Production Group effect, significant covariate effects (Oil and Natural Gas) and

significant Pumping effects, controlling for Distance and Direction. The amount of oil a site produces is strongly correlated with concentration of benzene found on proximate flora, indicating that high oil producing sites may have higher fugitive emissions of benzene. This is not a surprise as crude oil emits benzene and emissions are frequent on O&NG sites. Pétron et al. (2014) determined O&NG operations on Colorado's front range release almost three times more methane and seven times more benzene into the air than previously estimated. Natural Gas was a significant predictor for benzene, toluene and o-xylene. The  $R^2$  values indicate there is only a strong correlation between increasing natural gas concentrations and o-xylene concentrations on vegetation, although we suspect the relationship between natural gas and benzene is either non-linear, semi-log or quadratic (Figure 17). Air emissions of xylene from petroleum fractions arise from loading operations, storage, and equipment leaks (U.S. EPA 1994b), therefore, these events should be closely monitored on high natural gas production sites. The newer sites (group PR3), with the highest amounts of natural gas produced, such as site 22, had the highest deposition of BTEX onto proximate vegetation.

Deposition and accumulation of BTEX onto proximate flora was significantly greater on sites that were actively pumping versus those that were not pumping the day of collection (Figure 18). Concentrations were significant for benzene and m,p-xylene with the average deposition of  $2.98e^{-5}$  mg /g and  $2.22e^{-6}$  mg /g (Table 8). It was difficult to know how often or what time a pumpjack was actively pumping, as this information was not readily available and varied from site to site. Whilst on location, some pumpjacks would run for about an hour every four hours, some seemed to run continuously, while others, although listed as a producing site on the Colorado Oil and Gas Conservation

Commission (COGCC) website, did not appear to pump at all. On one of our sites, a bird built its nest in the arm of the pumpjack and so it clearly had not moved for weeks, maybe even months. Actively pumping sites are bringing O&NG to the surface where it is either separated on site or is piped to another facility. During this production process, fugitive emissions of BTEX are escaping and are depositing onto proximate flora.

Transport and storage of BTEX throughout *Bouteloua* tissues is, to my knowledge, undocumented. BTEX could potentially diffuse across the cuticle, and be transported to other shoots (e.g., stems, flowers) and/or roots (Li, Li, and Chen 2016). It is unclear whether *Bouteloua* are receiving BTEX strictly from wet or dry depositions. Soil permits were retracted for “archeological purposes” the week of data collection, therefore researchers were unable to collect and compare soil samples with vegetation samples.

The highest concentration of any BTEX, across all samples, was for o-xylene with a maximum concentration of 279.97 ppbv. The specific well that had the spike in o-xylene was near a methane collection and processing facility. The sample with the spike was farthest from the well (e.g., 100 m) and closed to the methane processing facility. This type of interference would impact trends in directionality and distance. It is nearly impossible to find a site on the PNG what is not within close proximity to other production sites, and thus depending on transport mechanisms, BTEX and other VOCs could travel a kilometer before depositing (Rodriguez et al. 2012). Concentrations of each BTEX were found on vegetation everywhere on the PNG, as seen in PA background levels (Table 9).

Without collecting and determining emissions at the same time as vegetation collection, it is difficult to conclude that deposition onto proximate flora did not include emissions from other nearby or regional sources. In general, the study sites were very low producers, with the exception of sites 8 and 22, which had high production of natural gas (see Appendix B). It would have been beneficial to study many, high producing sites such as site 8, but the research was limited by site availability on the PNG, permitting and funding.

We found concentrations of BTEX in vegetation on every site except one, PA site 10 (see Appendix B). The average concentration of toluene on all sites combined, including the PA sites, was 2.67 ppb, which is far below the MCL of 1000 ppb, but beyond the RfD for toluene (Table 9). Benzene is a carcinogenic compound causing leukemia. The average concentration for benzene on all sites combined, including the PA sites, was 15.12 ppb, which exceeds the RfD and MCL for benzene (Table 9).

Concentrations of benzene on the vegetation were as high as 176 ppb. This is arguably a biologically relevant concentration based on previous human impact research (McKenzie et al. 2012; Colborn et al., 2014; Thompson et al. 2014; Bolden et al. 2015), cattle research (Bechtel et al., 2009; Waldner and Clark, 2009) and a combination of the two (Bamberger and Oswald 2012).

It is likely that BTEX are depositing onto soils, plants and bodies of water, but further quantitative research is required. Each of these is a medium of exposure to proximate flora and fauna. Organisms, including cattle, pronghorn deer, prairie dogs, and insects within 100m of ONG production are likely breathing these BTEX and could also be consuming them at toxic levels.

**CHAPTER IV**  
**MACRO AND MICRO MINERAL CONCENTRATIONS**  
**IN VEGETATION**

**Abstract**

Minerals, including heavy metals, are released during the production phase of Oil and Natural Gas (O&NG) development and have the ability to deposit, via wet or dry deposition, onto water, soils and vegetation near the emission source. A field study conducted on the Pawnee National Grassland shortgrass steppe investigated relationships between O&NG production, mineral deposition onto proximate flora, the concentration of these minerals in plant shoots, and their effect on foraging quality. Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3). In May and June of 2014, *Bouteloua gracilis* (blue grama) and *Bouteloua dactyloides* (buffalo grass) shoots were collected from nineteen O&NG production (O&NG) sites and minerals were quantified in plant tissues using X-ray fluorescence (XRF) analysis. MANOVA results indicated that O&NG production Group (PA, PR1, PR2, PR3) and Distance from the wellhead (25 m, 50 m, 100 m) were significant factors in the model, controlling for Direction. There were also strong interaction effects for some nutrients, making separation of Distance and Group effects difficult. Of the macro minerals, K, P and S were significantly higher on vegetation found at 25 m and 50 m than 100 m. Ca was highest on PA sites, while P and K were highest on PR1 and PR2 sites. Concentrations of micro minerals were in the

following order Fe > Cl > Pb > Br > Mn > Sr > Ba > Zn > Cu > Se > Ni > Hg > Cr. Se (5.67 ppm), S (0.33%) and K (1.21%) concentrations were potentially above maximum tolerable concentrations for cattle (based on 2 kg daily intake). All other nutrient concentrations are potentially appropriate for grazing cattle, depending on specific cattle and grazing characteristics. Toxic elements Br (54 ppm) and Sr (46 ppm) were present in samples far below maximum tolerable levels, while concentrations of Hg (1.54 ppm) and Pb (83 ppm) were beyond daily maximum tolerable levels for cattle when considering a 2 kg DM diet. We also compared nutrient levels to data collected by Fresquez et al. (1991) and concentrations of micro minerals were comparable to *Bouteloua* grown in sludge treated soils, indicating a substantial impact from O&NG production. This impact has had a lasting effect on vegetation as seen with Pb levels on PA sites reclaimed over 30 years ago.

### **Introduction**

Human activities such as construction, energy production, waste disposal, vehicle exhaust, and coal and fuel combustion cause an increase in mineral (heavy metal) accumulation in the environment (Chen et al. 2005; Chambers et al. 2009). Dust particles containing these metals are ejected into the atmosphere, can be deposited onto proximate water, vegetation and soils (Sakagami et al., 1982; Wilhelm 2001; Fatoba et al. 2016) and can subsequently enter organismal tissues.

Generally, macro minerals are found in plant shoots and roots in concentrations greater than 1000 ppm or mg / kg of dry plant tissue. Micro minerals on the other hand are found in lower concentrations, sometimes less than 1.0 ppm and include the heavy metals. In vegetation, an increase in one micro mineral can have antagonistic impact on

other micro minerals. For example, if iron is in excess in substrate or tissue, this can cause deficiency of magnesium and boron, which can negatively affect vegetative growth and reproduction in plants (Marschner 1995). High zinc (Zn) levels can cause deficiency of nickel, which maintains the function of the urease enzyme in plants. If vegetation growing near an O&NG production site accumulates Zn, this can cause a decrease in nickel levels, which can result in a toxic accumulation of urea, causing marginal necrosis of blades (Marschner 1995). Toxicity levels specific to *Bouteloua gracilis* and *Bouteloua dactyloides* are unknown, although we can compare *Bouteloua* mineral concentrations to those found in other studies (; Moxon et al. 1951; Nelson et al. 1970; Fresquez et al. 1991; Mayland et al. 2006; Schiebout 2012) to address whether O&NG is increasing mineral content in proximate vegetation. We can also determine if mineral concentrations are adequate to meet nutritional needs of cattle and if concentrations are below maximum tolerable levels.

It has been shown that O&NG production can also have an antagonistic effect on vegetation by depleting the soils of nitrogen, iron and phosphorous, causing erosion and impacting the quality of surface waters (McBroom et al. 2012). Production activities such as topsoil removal, road building and land clearing result in ecosystem effects such as sedimentation, habitat fragmentation, loss of seed banks and soil nutrients as well as shifts in community composition. The well pads typically cover a 1.2–2.7 ha area and are placed atop crushed stone or wooden mats to support heavy equipment and thick liners to contain spills (Drohan and Brittingham 2012). When sites are reclaimed on the PNG, including plugged and abandoned sites, topsoil is spread, fertilizer is added and native

species seed is spread. This brings some nutrients back into the system and allows the steppe vegetation to remediate itself over time.

*Bouteloua gracilis* is the most common C<sub>4</sub> grass on the Pawnee National Grassland shortgrass steppe in Northeastern Colorado and is important for cattle grazing and rehabilitation of degraded soils. To our knowledge, there have been no studies concerning mineral uptake or deficiencies in native species of grasses on the shortgrass steppe caused by O&NG production and development. In the current study, sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3). The objectives of this study were to compare across four O&NG production Groups (PA, PR1, PR2, PR3) and three distances (25 m, 50 m, 100 m) from the wellhead (1) macro and micro mineral concentrations in vegetation (*Bouteloua gracilis* and *Bouteloua dactyloides*), (2) compare values to maximum tolerable levels and general dietary guidelines for cattle and (3) compare concentrations of minerals to those in *Bouteloua gracilis* grown in sludge treated soils. We expected higher mineral concentration near the source (wellhead) and lower concentrations with increasing distance from the source, a trend observed in other related metal deposition studies (Jaradat et al. 2005, Galal and Shehata, 2015). Higher concentrations were also expected on newer producing sites (PR sites) when compared to older sites, including PR1 and the plugged and abandoned sites (PA). We expected to find mean concentrations of minerals (including heavy metals) above general dietary guidelines for cattle and to find concentrations similar to those found in vegetation supplemented with sewage sludge.



## Methods

### Site Description

The Pawnee National Grassland (PNG) located in Northeastern Colorado is a shortgrass steppe divided into two, large east and west landmasses. Sites were randomly selected from eastern PNG National Forest Service land ( $n = 63$ ) for permitting purposes. We grouped sites according to status (PA or PR) and production date (spud date) to chronologically examine mineral deposition. Group 1 included PA sites = Plugged and abandoned in the 1980s ( $n = 5$ ), Group 2 were the PR1 sites = Producing since 1980-1990 ( $n = 4$ ), Group 3 were PR2 = Producing since 2000-2005 ( $n = 5$ ), and Group 4 were PR3 = Producing since 2006-2013 ( $n = 5$ ). Five sample sites were randomly chosen from each group ( $N = 19$ ). The three-decade averages (1981-2010) of climatological variables for New Raymer (the nearest city to study sites) include an annual average temperature of 6.4 °C (43.6 °F) and mean annual precipitation of 42.62 cm (16.78 in; NOAA 2017a).

### Vegetation Tissue Collection

In May and June of 2014, samples were collected from the 19 study sites. A mix of *Bouteloua gracilis* (blue grama) and *Bouteloua dactyloides* (buffalo grass) shoots were selected as the vegetation monitoring mediums as buffalo grass is the most prolific grass on the PNG in the spring and blue grama in the fall. Their abundance allows for replicate sampling at each study site. At each of the 20 sites, 100 m transects were laid in northeast, southeast and west directions from the wellhead, and two samples were collected at distances of 25 m, 75 m, and 100 m in each direction. The leaves of *Bouteloua gracilis* and *Bouteloua dactyloides* were cut with stainless steel scissors and placed directly into sterile, labeled, zip-locked bags. Samples were transported in a cooler

and refrigerated at 4 °C until analysis. Samples were analyzed within 12 days of collection date. From the 19 sites a total of  $N = 342$  vegetation samples were collected and analyzed. Samples were thoroughly rinsed with ultra-pure water (minimum resistivity of 18.2 MΩ cm) using Milli-Q plus Millipore system water (Molsheim, France). They were then dried in an oven overnight at 80 °C, ground and prepared for physical and chemical analysis. The samples contained only living blades of grass and any dead blades of grass or other types of tissue (stems and spikelets) were removed. Soil properties impact nutrient and heavy metal availability. Therefore, macro and micro nutrient levels of *Bouteloua* may differ among distinct soils. Unfortunately, permits for soil samples were retracted due to archeological restrictions and thus a comparison of soil minerals to vegetation minerals was not possible.

### **X-Ray Fluorescence (XRF) Analysis**

In the spring of 2016, we developed a methodology with the capacity to analyze NIST peach leaf standards within the 5% margin of error established by Stosnach and Gross (2013). We placed 50-70 mg of dried plant into 10 mL test tubes with 5 mL of concentrated nitric acid. The samples were placed in a digester block under a hood with a watch glass and were heated to ~ 100 °C for 1.5 hr. Solution volume was reduced to ~2 mL and solution color transitioned from cloudy and green to clear and yellow during digestion. The solutions were allowed to cool before capping the test tubes and allowing the digest to sit overnight. The following day, each acid digest was transferred to a volumetric flask with the use of a glass funnel. The test tubes and funnel were washed thoroughly with ultra-pure mili-Q water and the washings were added to the volumetric flask. Final dilution volume was reached with ultra-pure mili-Q water and 10 μL of 1,000

ppm gallium standard was added to each 10 mL volumetric flask. The solutions were vortexed for 5 minutes then 10  $\mu$ L of solution was immediately transferred to the center of a polyacrylic XRF disc. The discs were dried in a desiccator overnight. The next day, the x-ray beam in the XRF was warmed and a gain correction was performed. Samples were analyzed with the following parameters: 1000 second live time, liquid quantification, Ga standard element, 1  $\mu$ g mL<sup>-1</sup> standard concentration and 10  $\mu$ g sample solution per plate.

This methodology produced acceptable accuracy and precision. Percent recovery was calculated for method development with peach leaf standard, however *Bouteloua* species were not available in the NIST database and thus percent recovery was not calculated for this experiment. The method was used to identify four macro minerals [potassium (K), sulfur (S), calcium (Ca), and phosphorous (P)] and fourteen micro minerals (heavy metals indicated by asterisks) [arsenic (As\*), barium (Ba\*), bromine (Br), chlorine (Cl), chromium (Cr\*), cobalt (Co\*), copper (Cu\*), iron (Fe\*), lead (Pb\*), manganese (Mn), mercury (Hg\*), nickel (Ni\*), selenium (Se\*), strontium (Sr), and zinc (Zn\*)] were evaluated. Heavy metals are not well defined (Bhat and Khan 2011) and so we will refer to any toxic metal without any known beneficial effects as heavy metals.

### **Statistical Analysis**

The data set includes 19 response variables (minerals), four of which are macro minerals measured in percentages of elements in leaf tissue (P, S, K, Ca) and the remaining micronutrients (Mn, Fe, Ni, Cu, Zn, As, Br, Pb, Hg, Se, Sr, Ba, Cl, Cr, Co) were measured in  $\mu$ g mg<sup>-1</sup> of leaf tissue. Factors included in the model were Distance and O&NG group (controlling for direction). All replicate samples were average before

statistical analysis. A Henze-Zirkler's Multivariate Normality Test indicated data were not multivariate normal, thus, data were log-transformed and assumptions were re-evaluated. Replicates on each plot were averaged and a multivariate analysis of variance (MANOVA) test followed by univariate analysis of variance ANOVAs (with log-transformed outcomes) were used to compare Group and Distance least square means (LSmeans), adjusted for multiple comparisons with Tukey-Kramer test for each nutrient. Data analyses were performed using R (v 1.0.44) and SAS (v 6.1.7601).

### **External Comparisons**

The National Research Council (NRC) publishes equations to estimate phosphorus and calcium requirements, very specific to the age, weight, sex and gestation period of beef cattle (National Research Council [NRC] 2016). For the other important macro and micro minerals, less is known about specific dietary requirements. Therefore, the NRC can only provide maximum tolerable levels and general dietary guidelines for some of these minerals. The maximum tolerable concentration is defined as the dietary concentration that (when fed for a limited period) will not impair animal performance and will not produce unsafe residues in human food derived from the animal (NRC 2016). Mineral levels provided by the NRC were compared to macro and micro mineral levels in the current study. Mineral levels were also compared to a previous study conducted by Fresquez et al. (1991) to determine if concentrations near O&NG production sites were similar to those in *Bouteloua gracilis* treated with sludge.

### **Results**

The MANOVA test determined effects on nutrient concentrations were significant for Distance ( $F(34, 266) = 12.66$ ,  $p < 0.0001$ ), Group ( $F(51, 397) = 2.28$ ,  $p < 0.0001$ ) and

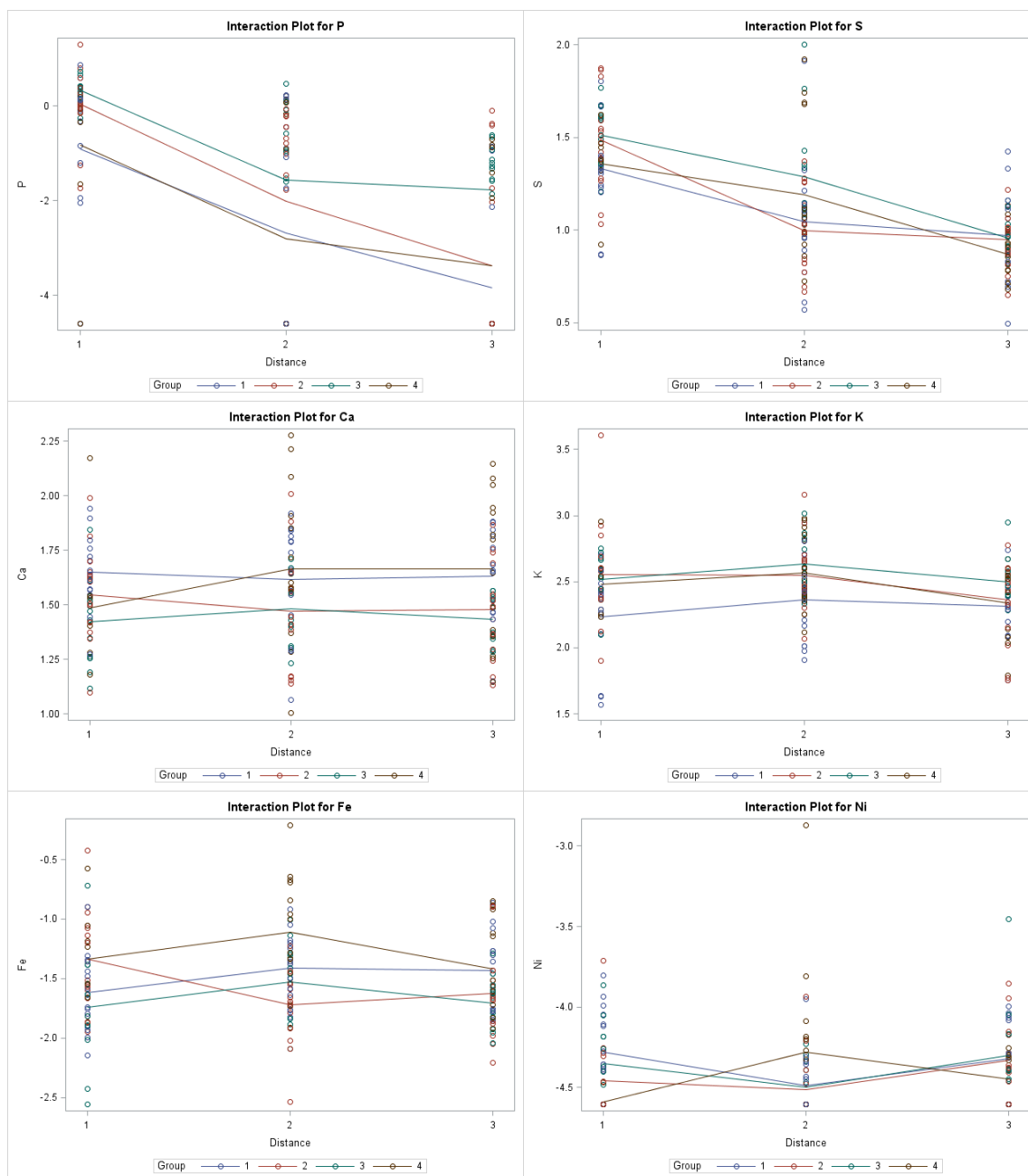
interaction effects ( $F(102, 765) = 1.39, p < 0.0103$ ). ANOVA results indicate significant interaction effects between Group and Distance Factors for Fe, Ni, Cl and Br (Table 10; Figure 20). Significant effects of Distance (10 minerals) and Group (9 minerals) were interpreted independently for all other minerals. Of the macro minerals, K, P, and S were significantly higher in vegetation at 25 m and 50 m than 100 m (Figure 21). Ca was highest on PA sites, while P and K were highest on PR1 and PR2 sites (Figure 22). There were also significant Distance (Pb, Se, Ba, Cr, Hg, Br, Cu) and Group (Mn, Fe, Se, Ba, Zn, Br) effects among the micronutrients (Table 10). Significant Distance effects in micro minerals were as follows: Cu was significantly higher at 100 m than 25 m, Br was higher at 100 m than 50 m, Cr was higher at 100 m than 25 m, Hg was highest at 25 m, and Pb, Sr and Ba were all higher at 50 m and 100 m than 25 m (Figure 21). Significant Group effects in micro minerals were as follows: Mn, Fe and Ba were all had the highest concentrations on the PR3 sites, whilst Br was highest on PR1 sites and Sr was highest on PA sites (Figure 22). Concentrations of micro minerals were in the following order  $Fe > Cl > Pb > Br > Mn > Sr > Ba > Zn > Cu > Se > Ni > Hg > Cr$  (Table 11, Figures 23 and 24). Frequency of micro minerals can also be informative. Cobalt was a very infrequent nutrient only identified in one sample on a PR1 site, while As, Cd and Al were non-detected in samples. Hg was found in 116 samples, most at 25 m from the wellhead.

Table 10

*Nutrient Analysis of Variance (ANOVA) Results for Production Group and Distance.*

	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F		Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
K	Model	11.000	2.369	0.215	2.710	0.003 **	P	Model	11	275.218	25.020	8.51	<.0001 ***
	Distance	2.000	0.612	0.306	3.850	0.023		Distance	2	196.945	98.472	33.48	<.0001 ***
	Distance*Group	6.000	0.352	0.059	0.740	0.618		Distance*Group	6	8.226	1.371	0.47	0.8325
	Group	3.000	1.362	0.454	5.720	0.001 **		Group	3	50.309	16.770	5.7	0.001 ***
Ca	Model	11.000	1.295	0.118	1.940	0.038 *	S	Model	11	7.467	0.679	11.17	<.0001 ***
	Distance	2.000	0.031	0.016	0.260	0.775		Distance	2	5.946	2.973	48.94	<.0001 ***
	Distance*Group	6.000	0.273	0.046	0.750	0.610		Distance*Group	6	0.701	0.117	1.92	0.0808
	Group	3.000	0.902	0.301	4.960	0.003 **		Group	3	0.423	0.141	2.32	0.0774
Mn	Model	11.000	4.291	0.390	1.650	0.090	Pb	Model	11.000	5.422	0.493	5.180	<.0001 ***
	Distance	2.000	1.209	0.604	2.560	0.081		Distance	2.000	4.551	2.276	23.930	<.0001 ***
	Distance*Group	6.000	0.743	0.124	0.520	0.789		Distance*Group	6.000	1.181	0.197	2.070	0.060
	Group	3.000	2.879	0.960	4.070	0.008 **		Group	3.000	0.015	0.005	0.050	0.984
Fe	Model	11.000	5.568	0.506	3.780	<.0001 ***	Se	Model	11.000	2.318	0.211	3.800	<.0001 ***
	Distance	2.000	0.303	0.151	1.130	0.326		Distance	2.000	1.580	0.790	14.250	<.0001 ***
	Distance*Group	6.000	2.345	0.391	2.920	0.010		Distance*Group	6.000	0.260	0.043	0.780	0.587
	Group	3.000	2.611	0.870	6.500	0.000		Group	3.000	0.525	0.175	3.160	0.027 *
Ni	Model	11.000	1.504	0.137	2.060	0.027 *	Ba	Model	11.000	43.916	3.992	7.310	<.0001 ***
	Distance	2.000	0.248	0.124	1.870	0.158		Distance	2.000	24.708	12.354	22.610	<.0001 ***
	Distance*Group	6.000	1.164	0.194	2.920	0.010 *		Distance*Group	6.000	1.731	0.288	0.530	0.787
	Group	3.000	0.174	0.058	0.870	0.457		Group	3.000	11.670	3.890	7.120	0.000 ***
Cu	Model	11.000	2.399	0.218	1.560	0.118	Cl	Model	11.000	61.972	5.634	2.930	0.002 **
	Distance	2.000	1.039	0.520	3.710	0.027 *		Distance	2.000	3.355	1.678	0.870	0.421
	Distance*Group	6.000	0.427	0.071	0.510	0.802		Distance*Group	6.000	50.825	8.471	4.400	0.000 ***
	Group	3.000	0.783	0.261	1.860	0.138		Group	3.000	4.439	1.480	0.770	0.513
Zn	Model	11.000	3.889	0.354	2.210	0.017 *	Cr	Model	11.000	0.288	0.026	1.280	0.239
	Distance	2.000	0.348	0.174	1.090	0.340		Distance	2.000	0.195	0.098	4.800	0.010 *
	Distance*Group	6.000	1.229	0.205	1.280	0.271		Distance*Group	6.000	0.037	0.006	0.300	0.936
	Group	3.000	2.433	0.811	5.060	0.002 **		Group	3.000	0.035	0.012	0.570	0.639
Se	Model	11.000	4.796	0.436	1.830	0.054	Hg	Model	11.000	3.022	0.275	6.180	<.0001 ***
	Distance	2.000	0.763	0.382	1.600	0.205		Distance	2.000	2.213	1.106	24.880	<.0001 ***
	Distance*Group	6.000	2.480	0.413	1.730	0.117		Distance*Group	6.000	0.198	0.033	0.740	0.616
	Group	3.000	1.788	0.596	2.500	0.062		Group	3.000	0.250	0.083	1.880	0.136
Br	Model	11.000	38.145	3.468	3.280	0.001 **							
	Distance	2.000	7.236	3.618	3.420	0.035 *							
	Distance*Group	6.000	15.684	2.614	2.470	0.026 *							
	Group	3.000	12.743	4.248	4.020	0.009 **							

Notes: \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$



*Figure 20.* Interaction plots of minerals. Prior to data collection and analysis, sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3). In the figure, Distance 1 = 25 m, 2 = 50 m, 3 = 100 m and groups are coded by color and coded 1-4 (group 1 in blue = PA, 2 in red = PR1, 3 in green = PR2, 4 in brown = PR3). Data were log transformed prior to analysis.

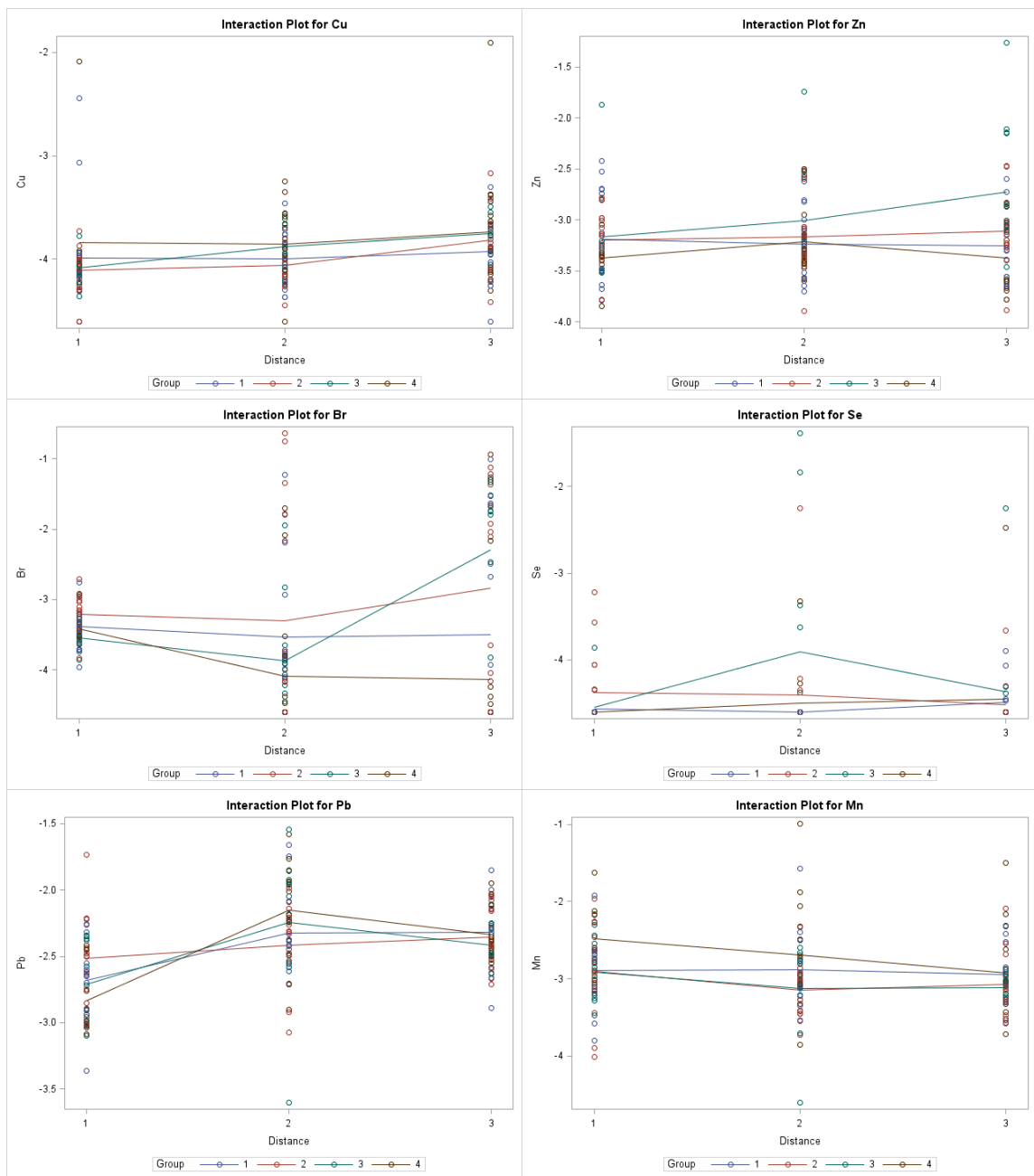


Figure 20. Continued.



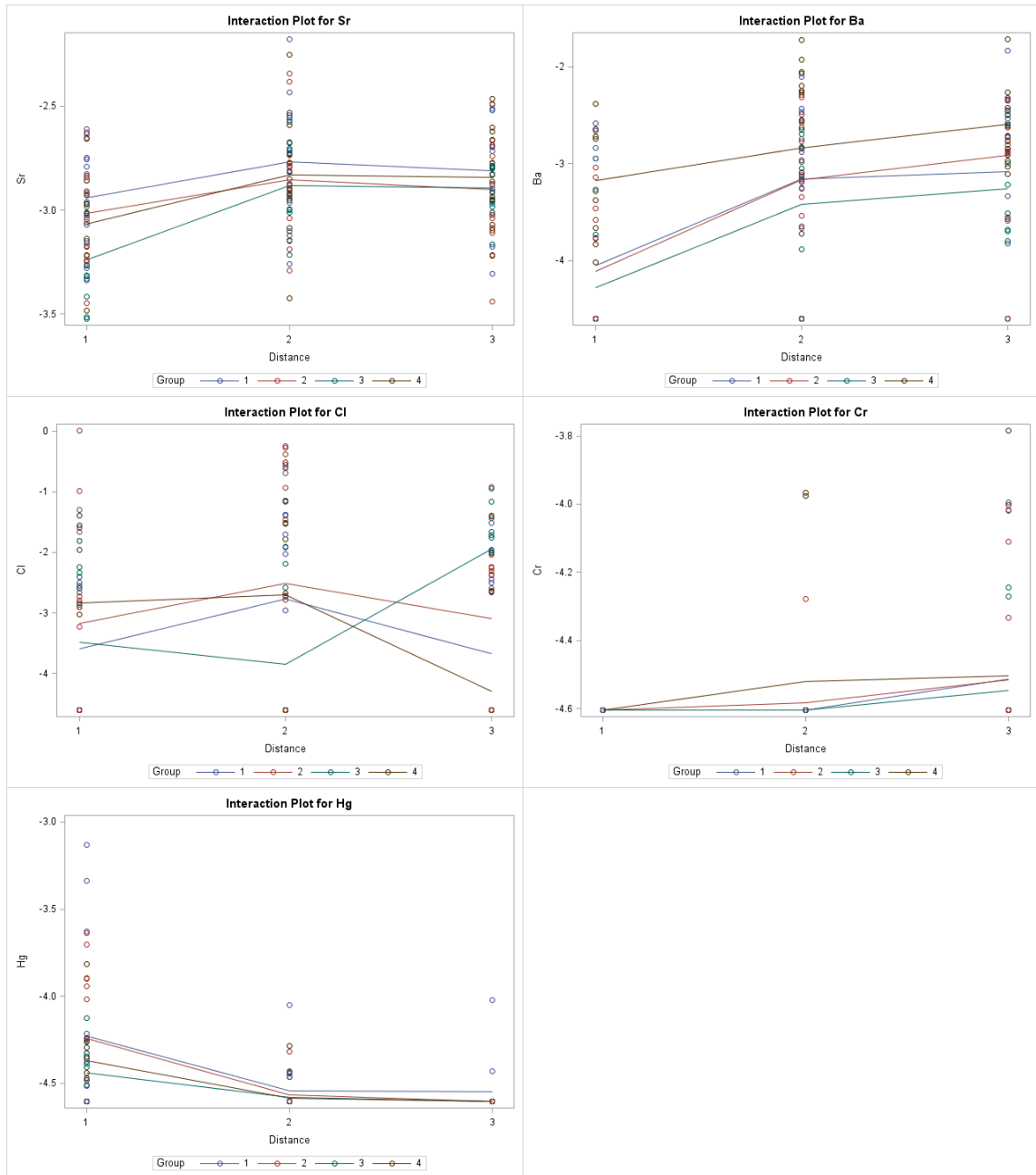
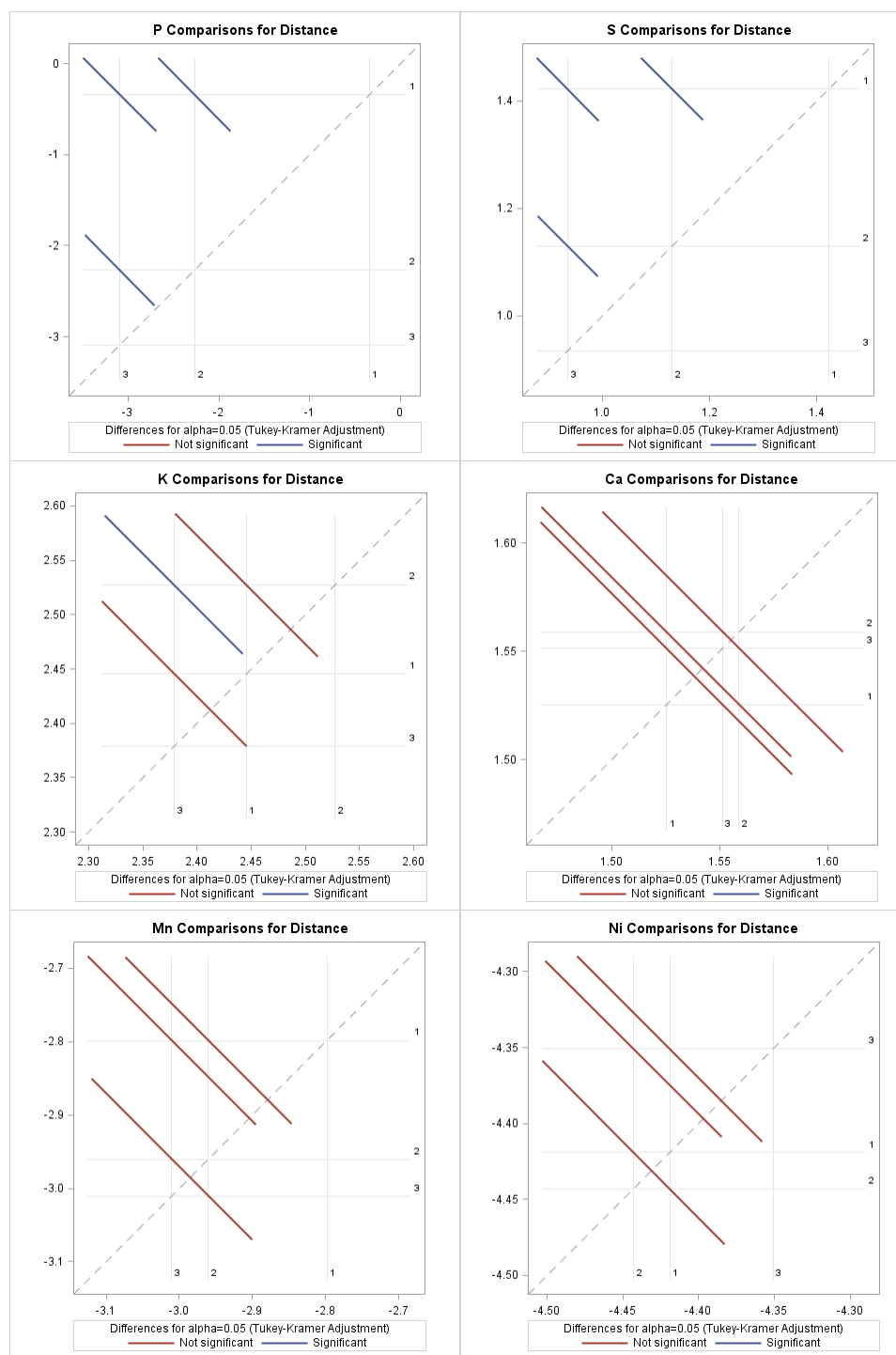


Figure 20. Continued.



*Figure 21.* Significant effects of distance (from the wellhead) on nutrients. Each plot shows the LS-means adjusted pairwise differences for distances, their significance levels and their individual confidence limits. The LS-means of each pair (on axes) meet at their intercept (center of line). The blue line indicates LS-means are significantly different between distances and the red line indicates groups have similar LS-means. Distances: 1 = 20 m, 2 = 50 m, 3 = 100 m. Data were log transformed for analysis.

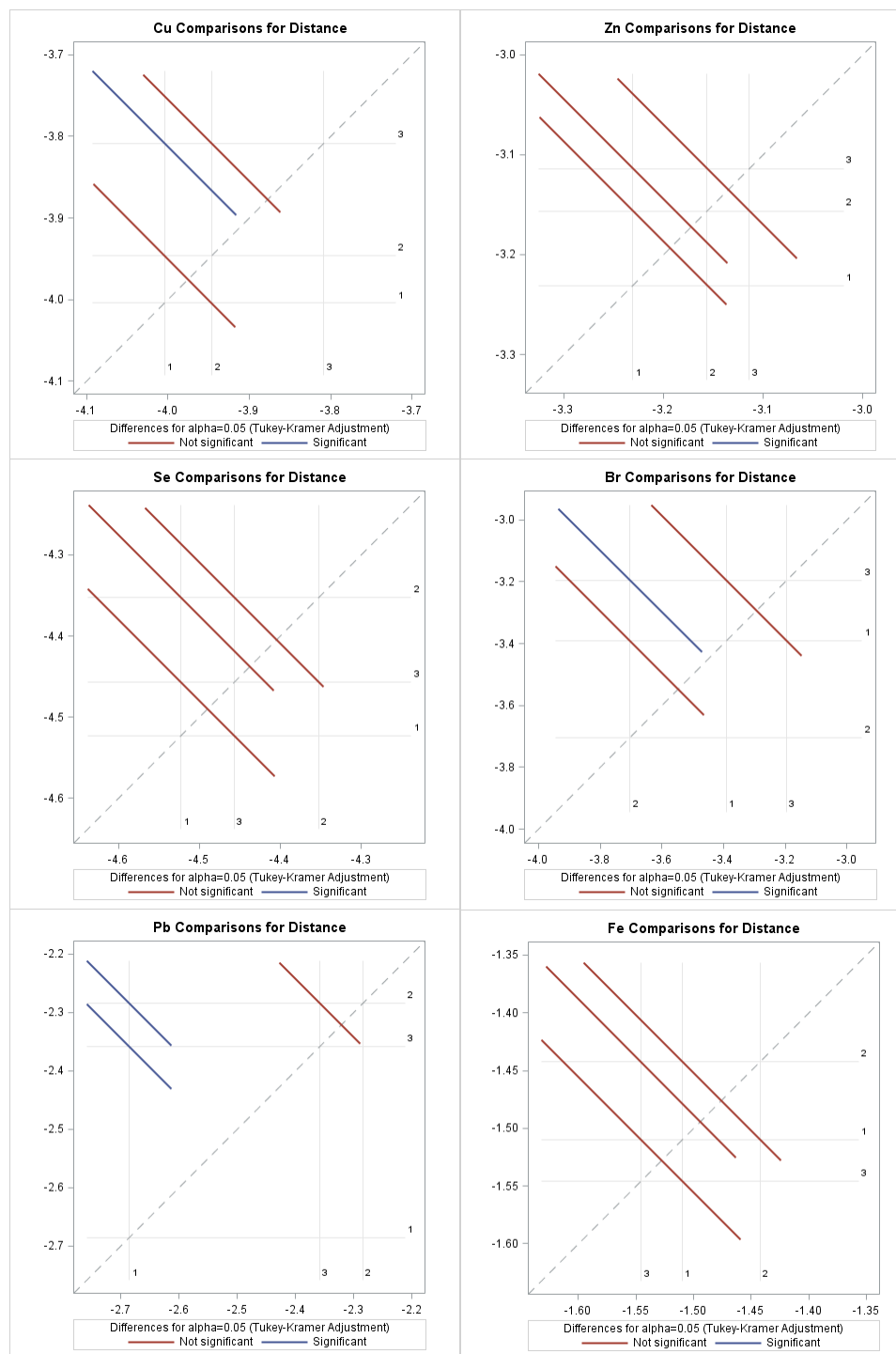
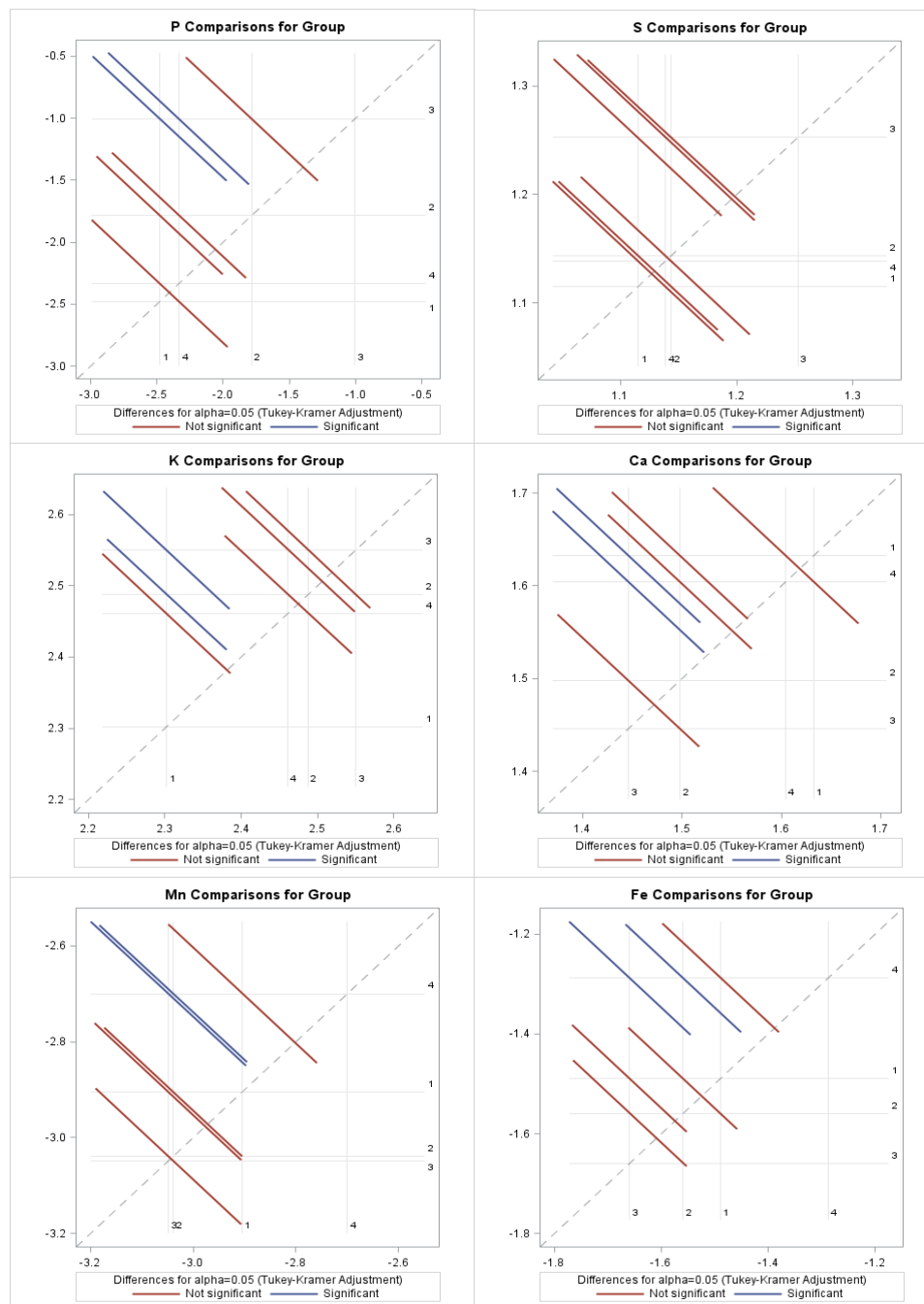


Figure 21. Continued.



*Figure 22.* Significant effects of production group on nutrients. Each plot shows nutrient LS-means adjusted pairwise differences between groups, their significance levels and their individual confidence limits. The LS-means of each pair meet at their intercept (center of line). Blue line = significant difference between LS-mean, red line = similar LS-mean. Sites were grouped according to production (date and amount) including plugged and abandoned in the 1980s (PA = 2), producing since 1980-1990 (PR1 = 1), producing since 2000-2005 (PR2 = 3) and producing since 2006-2013 (PR3 = 4). Data were log transformed for analysis.

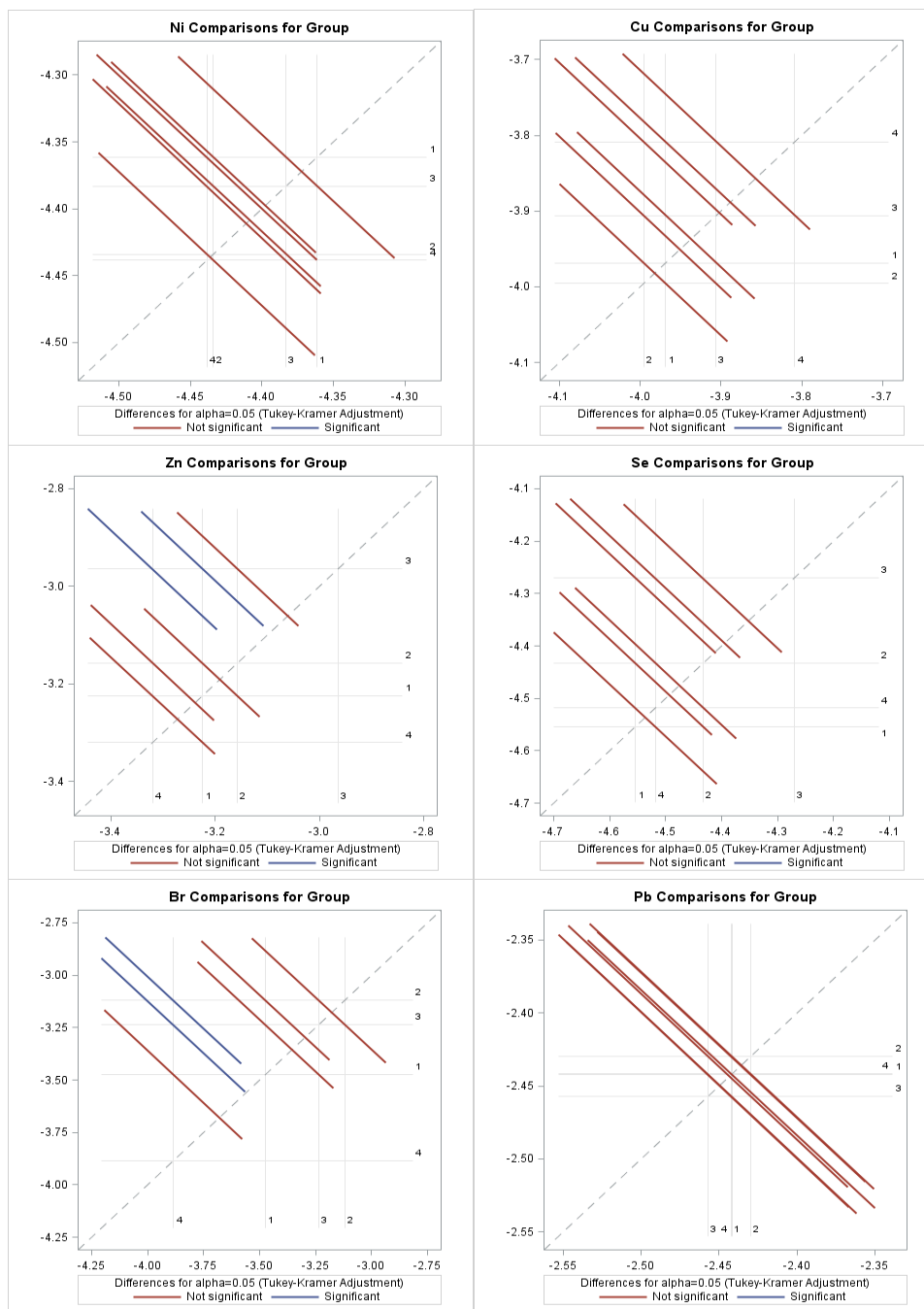


Figure 22. Continued.

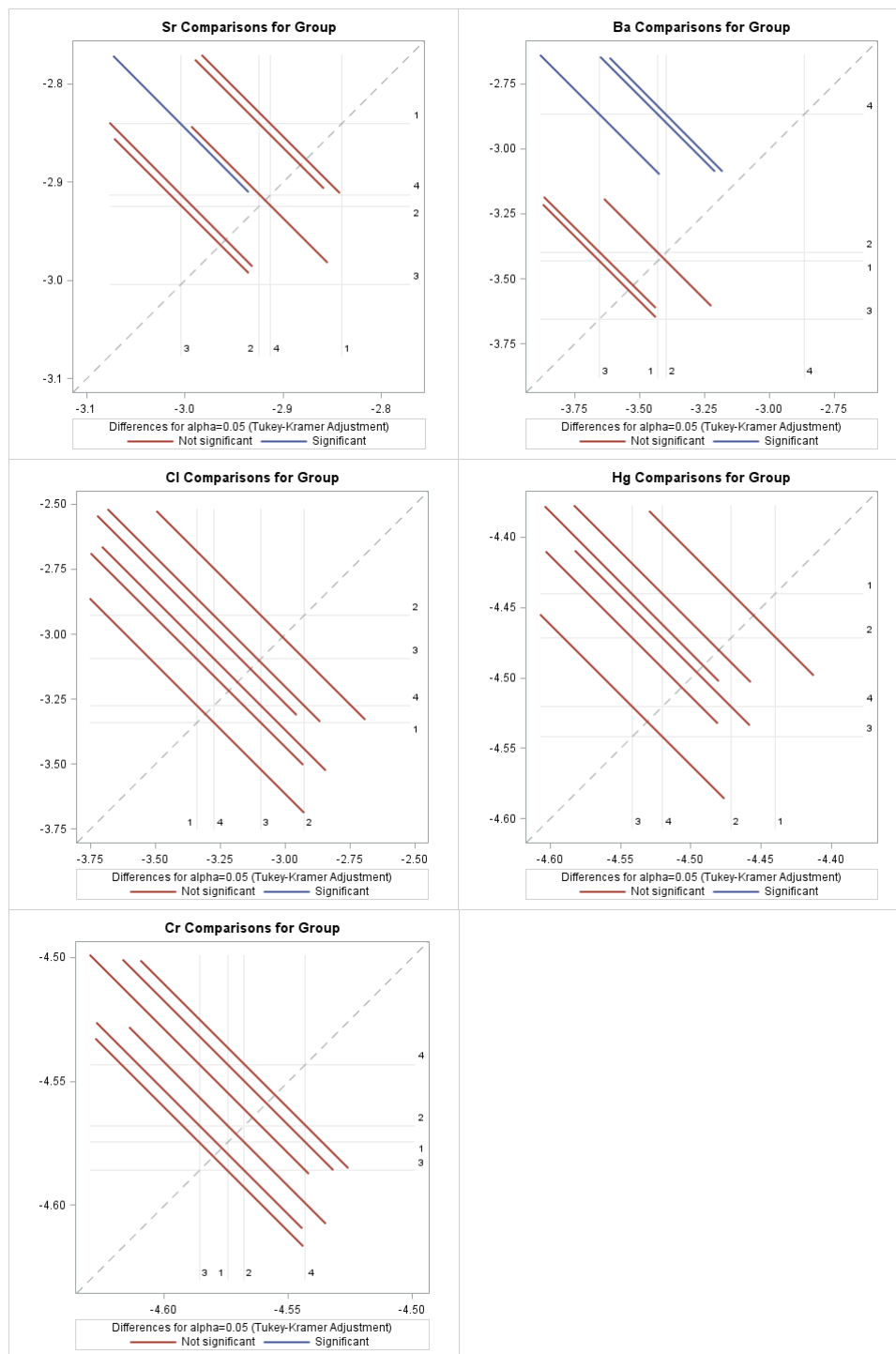


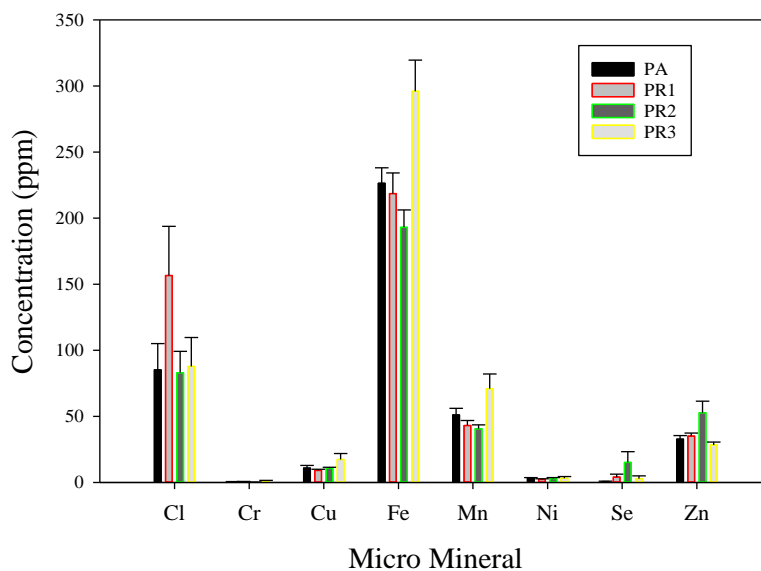
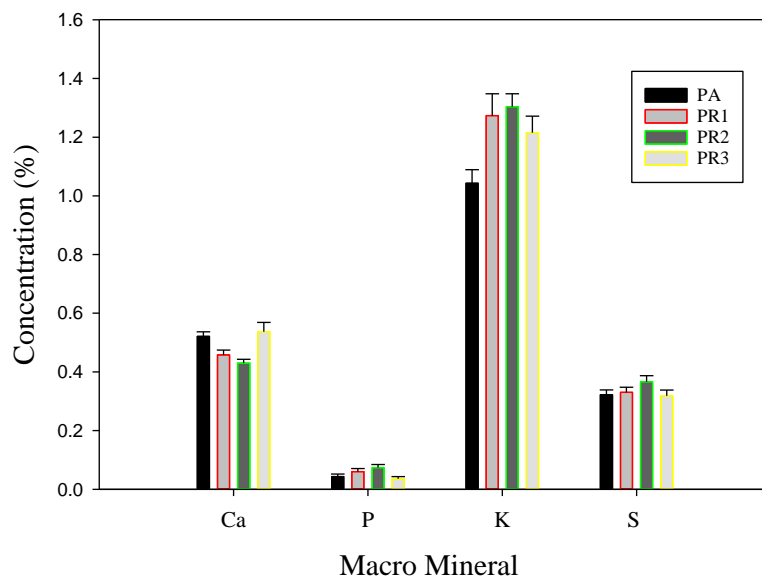
Figure 22. Continued.

Table 11

*Heat Map of Mineral Means for Production Group\*Distance.*

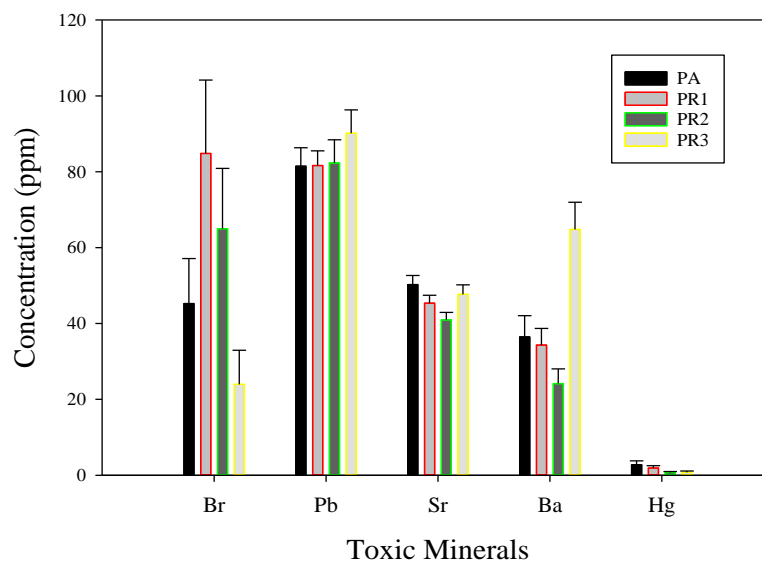
Mineral	Unit	Site means Group*Distance											
		PA_25	PA_50	PA_100	PR1_25	PR1_50	PR1_100	PR2_25	PR2_50	PR2_100	PR3_25	PR3_50	PR3_100
P	%	0.08	0.03	0.01	0.13	0.04	0.02	0.14	0.05	0.03	0.07	0.04	0.01
S	%	0.39	0.30	0.27	0.45	0.28	0.26	0.46	0.38	0.26	0.39	0.35	0.24
K	%	0.98	1.11	1.04	1.39	1.32	1.11	1.26	1.42	1.24	1.22	1.35	1.07
Ca	%	0.53	0.52	0.52	0.48	0.45	0.45	0.42	0.45	0.42	0.46	0.56	0.56
Mn	ppm	51.81	54.47	46.07	52.46	36.23	40.37	47.73	39.10	35.18	84.23	78.23	55.52
Fe	ppm	198.13	241.30	241.87	276.51	177.70	201.66	185.88	218.23	175.29	274.42	353.03	247.37
Ni	ppm	4.33	1.48	3.70	1.98	1.21	3.49	3.26	1.26	4.33	0.19	5.86	1.85
Cu	ppm	12.46	9.20	11.01	6.73	7.44	13.08	7.00	11.06	13.90	20.40	12.47	20.76
Zn	ppm	35.57	31.44	31.12	32.20	35.01	37.82	38.07	46.91	73.30	24.95	32.54	25.78
Se	ppm	0.48	0.00	1.59	4.08	6.84	1.28	0.93	36.11	8.41	0.00	1.99	5.31
Br	ppm	25.35	44.77	68.75	31.72	102.64	120.15	19.34	20.26	155.46	23.57	21.49	26.83
Pb	ppm	61.64	92.41	91.78	75.34	82.67	86.92	59.00	107.73	80.29	50.73	112.78	88.42
Sr	ppm	44.01	55.22	51.73	40.22	49.45	46.37	30.00	47.09	45.77	37.93	51.18	49.48
Ba	ppm	15.31	45.09	50.92	10.03	41.08	51.84	7.04	30.88	34.54	39.20	73.62	70.06
Cl	ppm	53.95	152.26	43.83	120.97	266.86	82.05	44.14	30.08	174.96	89.74	158.05	11.21
Cr	ppm	0.00	0.00	1.26	0.00	0.26	1.17	0.00	0.00	0.69	0.00	1.18	1.50
Hg	ppm	6.55	0.74	0.75	5.27	0.46	0.00	1.95	0.25	0.00	3.07	0.25	0.00

*Note:* Colors range from orange to blue, with the lowest concentrations in bright orange and the highest concentrations in bright blue. Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3).



*Figure 23.* Nutrient means across oil and natural gas production groups. Sites were grouped according to production (date and amount) including plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3).





*Figure 24.* Toxic element means across oil and natural gas production groups. Sites were grouped according to production (date and amount) including plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3).

When concentrations of macro and micro minerals were compared to the cattle grazing maximum tolerable conditions, Se (5.67 ppm), S (0.33%) and K (1.21%) concentrations were potentially above maximum tolerable concentrations for cattle at all stages (based on 2 kg daily intake) and the Se mean concentration (15.15 ppm) for the PR1 group was exceptionally high (Table 12). All other nutrients concentrations in vegetation were at appropriate mineral levels for grazing cattle, depending of course on very specific cattle and grazing details. Determining whether mineral requirements meet potential daily intake requires specific information such as cow weight, stage of growth and amount of dry matter (DM) consumed daily. Concentrations of vegetation minerals in Table 12 are only comparable if cattle eat 1 kg of vegetation daily, and thus the potential mineral intake per day is doubled if the cattle eat 2 kg. Take for example a 450 kg cow consuming 2 kg DM per day. If this DM were from *Bouteloua* found within 100

m of an O&NG production site, then  $\sim 9.74 \text{ g d}^{-1}$  Ca would be consumed by the calf. The requirement for normal maintenance and growth is  $0.0154 \times \text{SBW}/0.5$ , where SBW is shrunk body weight, which is  $\sim 13 \text{ g d}^{-1}$  Ca. The maximum tolerable level is  $0.02 \times 2,000$  g or  $40 \text{ g d}^{-1}$  and thus the cow would have to eat more than 4 kg of the vegetation to receive a toxic dose. For Fe, the cow needs  $\sim 50$  ppm each day and if consuming 2 kg of *Bouteloua* would receive  $\sim 467 \text{ ppm d}^{-1}$ , more than meeting the organism's needs, yet below toxicity levels of 500 ppm (albeit close).

Table 12

*Daily Mineral Requirements and Max Tolerable Concentration for Cattle, Available Mineral Content in Vegetation on Production Sites and Mineral Content in Fresquez et al. (1991) Vegetation*

Mineral	Unit DM	Daily mineral requirements <sup>a</sup>				Available Mineral Content <sup>b</sup>					Potential mineral intake per day <sup>c</sup>					Fresquez (1991) Vegetation <sup>d</sup>														
		Growing & Finishing	Gestation	Lactation	Max. Tol. Level	Group Means					Distance Means			Group Means					No Treatment Means				Sludge 90 (ug ha <sup>-1</sup> ) Means							
						Total	PA	PR1	PR2	PR3	25	50	100	Total	PA	PR1	PR2	PR3	25	50	100	Total	S1	S2	S3	Total	S1	S2	S3	
K	%	0.60	0.60	0.70	2.00	1.21	1.04	1.27	1.30	1.22	1.21	1.29	1.11	2.42	2.09	2.55	2.61	2.43	2.42	2.59	2.22	1.23	1.24	0.66	1.78	2.27	2.58	1.82	2.40	
S	%	0.15	0.15	0.15	0.3-0.5	0.33	0.32	0.33	0.37	0.32	0.43	0.32	0.26	0.67	0.64	0.66	0.73	0.64	0.85	0.65	0.51	-	-	-	-	-	-	-	-	
Cl	ppm	-	-	-	-	103.15	85.18	156.63	83.06	87.72	77.43	158.22	75.13	206.29	170.37	313.25	166.12	175.44	154.85	316.44	150.26	-	-	-	-	-	-	-	-	
Cr	ppm	-	-	-	1000.00	0.53	0.38	0.48	0.23	1.05	0.00	0.38	1.17	1.07	0.76	0.95	0.46	2.09	0.00	0.76	2.34	-	-	-	-	-	-	-	-	
Cu	ppm	10.00	10.00	10.00	40.00	11.99	10.88	9.08	10.66	17.32	10.70	9.99	14.76	23.97	21.77	18.17	21.31	34.65	21.40	19.98	29.51	-	-	-	-	-	-	-	-	
Fe	ppm	50.00	50.00	50.00	500.00	233.56	226.41	218.63	193.13	296.05	230.91	249.11	217.33	467.11	452.83	437.25	386.26	592.11	461.83	498.22	434.66	794.00	842.00	874.00	666.00	300.00	308.00	262.00	330.00	
Mn	ppm	20.00	40.00	40.00	1000.00	51.41	51.00	43.02	40.67	70.94	56.21	52.69	44.51	102.81	102.00	86.04	81.33	141.87	112.43	105.37	89.03	56.00	62.00	44.00	62.00	161.33	120.00	266.00	98.00	
Ni	ppm	-	-	-	50.00	2.86	3.14	2.23	2.95	3.12	2.71	2.52	3.30	5.72	6.29	4.46	5.91	6.23	5.41	5.03	6.61	-	-	-	-	-	-	-	-	
Se	ppm	0.10	0.10	0.10	5.00	5.67	0.65	4.07	15.15	2.82	1.59	9.93	3.98	11.34	1.30	8.13	30.30	5.63	3.18	19.85	7.97	-	-	-	-	-	-	-	-	
Zn	ppm	30.00	60.00	60.00	500.00	37.22	32.78	35.01	52.76	28.34	33.46	35.93	40.97	74.45	65.57	70.02	105.52	56.68	66.92	71.85	81.94	49.33	52.00	44.00	52.00	94.00	146.00	94.00	42.00	
Toxic																														
Br	ppm					200.00	54.76	45.24	84.84	65.02	23.96	25.53	48.71	91.43	109.53	90.48	169.68	130.04	47.92	51.06	97.42	182.86	-	-	-	-	-	-	-	-
Pb	ppm					30.00	83.90	81.48	81.64	82.34	90.14	63.37	98.43	87.00	167.81	162.97	163.28	164.68	180.29	126.74	196.86	174.01	1.00	1.00	1.00	1.00	1.27	1.80	1.00	1.00
Sr	ppm					2000.00	46.06	50.25	45.35	40.95	47.67	38.54	50.93	48.34	92.11	100.51	90.70	81.91	95.34	77.07	101.86	96.67	-	-	-	-	-	-	-	-
Ba	ppm					-	39.94	36.46	34.32	24.15	64.83	15.56	48.55	52.50	79.88	72.93	68.63	48.30	129.66	31.12	97.10	104.99	-	-	-	-	-	-	-	-
Hg	ppm					2.00	1.54	2.77	1.91	0.73	0.76	4.50	0.43	0.18	3.09	5.54	3.82	1.47	1.53	9.01	0.87	0.36	-	-	-	-	-	-	-	-

Notes: A. Includes potential mineral intake for cattle per day given a 2 kg DM. Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3).

<sup>a</sup> Source: NRC (2016) Percent required depends on weight of organism.

<sup>b</sup> Mean concentrations of minerals and toxic elements across production groups

<sup>c</sup> Daily intake calculated for vegetation on O&NG sites assuming 2 kg DM

<sup>d</sup> *B. gracilis* mineral concentrations after 0 and 90 (µg ha<sup>-1</sup>) sewage sludge treatment (Fresquez et al. 1991).

Mineral	Unit	Calcium and Phosphorus requirements and maximum tolerable concentrations for cattle <sup>a</sup>						Available Mineral Content in O&NG vegetation <sup>c</sup>						Fresquez (1991) vegetation <sup>d</sup>												
		Daily mineral requirements						Group Means			Distance Means			No Trt. Means				Sludge Trt. Means								
		Maintenance	Growing & Finishing		Gestation <sup>b</sup>		Lactation	Max Tolerable Level	Total	PA	PR1	PR2	PR3	25	50	100	Total	S1	S2	S3	Total	S1	S2	S3		
Ca	g/d	0.0154 x SBW/0.5	NPG x 0.071/0.5		CBW x (13.7/90)/0.5		Yn x 1.23/0.5		0.02 x DMI		4.87	5.21	4.58	4.30	5.37	4.76	4.96	4.87	5.13	6.10	5.40	3.90	4.80	5.40	4.10	4.90
P	g/d	0.016 x SBW/0.68	NPG x 0.039/0.68		CBW x (7.6/90)/0.68		Yn x 0.95/0.68		0.007 x DMI		0.53	0.43	0.60	0.74	0.36	1.09	0.40	0.15	1.53	1.50	1.20	1.90	2.87	2.60	2.40	3.60

Notes: B. Includes special calculations for P and Ca.

<sup>a</sup> SBW shrunk body weight using 450 kg; NPG is net protein requirement for gain (i.e., retained protein), g/d; Yn is milk yield, kg/d; CBW is calf birth weight, kg; and DMI is dry matter intake 2,000, g/d. The digestibility for Ca is 50% and for P it is 68%.

<sup>b</sup> Last 90 days of pregnancy <sup>c</sup> based on 2,000 g/d DM

It is also important to consider the relationships between minerals. For example, calcium should always be included in diets at a greater concentration than phosphorus and ratios of 7:1 are generally tolerated by cattle (NRC 2016). If ratios fall below 1:1, animal performance may be affected (Dowe et al. 1957, Alfaro et al., 1988). This is true even if phosphorus is below the maximum tolerable concentration listed in Table 12. Our mean ratio of Ca to P was ~ 10:1. Concentrations of P and Ca are required during lactation depend on the amount of milk produced and fetal weight, thus requirements must be calculated on an individual basis (NRC 2016). Geisert et al. (2010) determined that P requirements were around 0.10 to 0.17% DM or 7 to 14 g/d<sup>-1</sup>. Our concentrations of Ca were similar to those reported by Fresquez et al. (1991); however, P concentrations were much lower (Table 12). It appears all other concentrations of minerals measured in the current study are comparable to *Bouteloua* grown in the control soils of Fresquez et al. (1991) with the exception of Pb, which was much higher. Toxic elements Br (54 ppm) and Sr (46 ppm) were present in samples far below maximum tolerable levels, while concentrations of Hg (1.54 ppm) and Pb (83 ppm) were beyond daily maximum tolerable levels for cattle when considering a 2 kg DM.

### **Discussion**

We expected to find higher mineral content on newer producing sites, such as PR3 and PR4 and to find a significant distance effect with higher concentrations near the source (25 m). We did find statistical differences between Group and Distance Factors although results were variable and some minerals had significant interaction effects. Concentrations of macro minerals K, P, and S were significantly higher closer to the well and were highest on PR1 and PR2 sites. Calcium was highest on PA sites and the mean

concentration (0.52%) seemed to be comparable to concentrations found by others including Fresquez et al. (1991; 0.54-0.61%), Nelson et al. (1970; 0.18-0.4%) and Moxon et al. (1951; 0.40%). Samples collected by Schiebout (2012) on PNG native reference sites, without O&NG production, showed mean concentrations of ~ 4%, a magnitude higher than concentrations found on our O&NG sites.

It has been shown that Ca, Mn and especially P concentrations are lower in plants on reclaimed pastures than on native sites (Mayland et al. 2006) and we have noticed a similar trend. Phosphorous levels on O&NG sites (0.05%) were low when compared to other studies Fresquez et al. (1991; 0.15-0.26%), Nelson et al. (1970; 0.03-0.22%), Moxon et al. (1951; 0.11%) and Schiebout (2012; ~ 0.79%). This was especially true when comparing Schiebout's native sites on the PNG to O&NG production sites. All sites had concentrations less than 0.79% at all distances. This indicates P concentrations are deficient specifically due to impact by O&NG production and associated activities and not due to low background concentrations in the area. During initial construction, a typical PNG wellpad impacts 0.049 km<sup>2</sup> and after reclamation may be reduced to less than 0.006 km<sup>2</sup> (Baynard et al. 2017). When you include buffered roads, the impact increases to 12.63 km<sup>2</sup>, which is a substantial area. Vegetation mineral levels seem to indicate lasting impacts on vegetation and possibly soils on producing sites (PR) as well as reclaimed (PA) sites.

Calcium and P work together to form bones, which is why the ratio of Ca: P is important, especially for growing calves. Our mean ratio is concerning at 10:1 as P levels are possibly inadequate for growth and bone formation; however effects of the ratio on performance has been overemphasized (NRC 2016). P is also required by ruminal

microorganisms for cellular metabolism and is required for the maintenance of acid-base osmotic balance (NRC 2016). Requirements for P are very different depending on life stage of the cow. For example, during lactation, in excess of maintenance (3.9 g P/ 100 g protein gain), 0.95 g P/kg is required per kg of milk produced (Ellenberger et al. 1950). There are also additional fetal requirements and so physiological status of an animal (pregnant vs. non-pregnant) and other environmental factors and stressors should be considered when determining daily mineral requirements. In grazing livestock, P deficiency is the most prevalent mineral deficiency worldwide (McDowell 2003). When determining P requirements and deficiency, it is likely more valuable to test fecal matter than the vegetation concentrations or to test them in conjunction with one another.

Micro minerals met dietary guidelines for most nutrients although they exceeded for Se in all PR groups and could possibly be exceeded for Fe. Beef cattle only need 0.1 ppm of Se/kg DM and excess amounts are stored in cattle muscle (Lawler et al. 2004). Concentrations of Se ranging from 5 to 40 ppm Se/kg result in chronic toxicosis (alkali disease) and can cause diarrhea, ataxia and death from respiratory failure (NRC 2016). Mean concentrations of Se in the current study ranged from 0.65 ppm in the PA group to 15.15 ppm in the PR2 group. In management, the best solution and even preventative measure for Se toxicity is to simply rotate pastures for foraging (McDowell 2003) and move water sources away from O&NG production sites.

Cattle need approximately 50 ppm of Fe in their daily diet, although requirements are lower for older cattle due to efficient red blood cell Fe turnover (Underwood 1977). Fe toxicity can cause a decrease in feed intake, diarrhea, and hypothermia, but it does not appear to occur in the current study (~ 470 ppm assuming 2 kg daily intake) and is not of

immediate threat to the livelihood of cattle. The major concern with Fe toxicity is that concentrations as low as 250 ppm can cause an antagonistic Cu depletion in cattle (Bremner et al 1987), although Cu concentrations in the current study appear adequate (~12 ppm DM).

When we designed the experiment, we specifically chose to quantify concentrations of nutrients inside the plant, indicating vegetation health, although specific toxicity and deficiency levels were not available. With XRF analysis, we were not able to distinguish between compounds or isotopes, thus the bioavailability of each nutrient is assumed to be equal or lower than the available concentration. This study indicates concentrations at or below daily requirements could possibly indicate mineral deficiency. Micro minerals are available in two forms, organic (bound to sulfates, carbonates or oxides) and inorganic (bound to amino acids or protein complexes), the latter increase bioavailability, yet cost significantly more (Alltech 2017). If the mean concentration of any micro mineral is drastically lower than required levels for cattle, regardless of form (organic or inorganic) or bioavailability, supplementation would be required. Although supplementing forage diets is an option, it should be avoided unless absolutely necessary to prevent environmental problems associated with excess nutrient runoff from cattle waste.

Species of concern in the current study include Zn, with concentrations significantly lower near the wells (25 m). Zinc is an essential component of carbohydrate, protein, lipid and nucleic acid metabolism (Casey and Krebs. 1986). Concentrations of only 30 ppm DM satisfy requirements for cattle, but cattle can consume concentrations 200 times the recommended concentration without adverse effects (NRC 2016). A large

proportion of Zn in forage is in the plant cell wall, but it is not known how this affects absorption (Whitehead et al. 1985). Deficiencies can affect vulnerable populations more than others, including lactating cattle, which require much more Zn to produce milk containing 3 to 5 mg/L.

Results were compared to another study conducted by Ramírez et al. (2004) examining seasonal and annual means of macro and trace minerals in *B. gracilis*. We found that PNG macro minerals were similar, although P was slightly lower, and micro minerals in general were substantially higher than their mean annual averages (Cu = 6 ppm, Fe = 108 ppm, Mn = 39 ppm, Zn = 49 ppm, Ca = 0.1%, P = 0.08% and K = 0.5%). Ramírez et al. (2004) also found mineral concentrations were significantly higher in fall and spring versus summer, and we did not consider this variable. Our samples were collected from May- June and thus seasonal variation might play a role in nutrient levels. Deficiencies as well as toxic accumulation of macro and micro minerals could also partially be due to nutrient loading and translocation in plant tissue (Singh et al. 2010). Our samples excluded roots and other parts of the shoot (e.g., inflorescence, stem), whereas Fresquez et al. (1991) measured all shoots and did not distinguish between tissues.

We expected to find toxic minerals above maximum toxicity concentrations for cattle and similar to those found in vegetation supplemented with sewage sludge. We did find heavy metals such as Pb and Hg exceeded toxicity concentrations for cattle on all sites (PA, PR1, PR2, and PR3) and mean levels of Pb were 80 times higher than sewage treated grasses. Diets deficient in a particular essential mineral can enhance the accumulation and toxicity of minerals. Ca, Fe, and Zn deficiency, for instance, enhance



susceptibility to Pb toxicity in cattle, whereas increased Ca and Fe reduce Pb toxicity (Goyer 1997; Alonso et al. 2004). Lead is a common cause of cattle poisoning, causing anemia, sterility and fetal death. The O&NG extraction process concentrates Pb (including Lead-210), Hg and other naturally occurring radionuclides and brings them to the surface of the environment and into contact with plants, soils and animals (Schmidt 1998; Chambers et al. 2009). Lead 210 ( $^{210}\text{Pb}$ ) can accumulate in tubes, valves and tanks on O&NG production sites and facilities (Gray 1990, 1993; Hartog et al. 1998, 2002). Natural gas pipelines are especially well documented for their iron sulfide and iron oxide deposits on the proximate environment (Baldwin 1998; Godoy et al. 2005). Other compounds found on O&NG production sites include zinc sulfide, metallic lead, lead oxides, lead sulfide, barium sulfate (barite), and calcium carbonate (calcite; Hartog et al. 1998; Schmidt 1998).

There are many opportunities during O&NG production phases (e.g., drilling, fracturing, and development) and transportation, for dust particles containing minerals and heavy metals, to land on and stick to plant cuticles and surrounding soils. These minerals could then be transported into plant tissues. Various studies in grasslands of arid or semi-arid zones have shown that there is a low risk of heavy metal accumulation in vegetation (Lane 1988; Fresquez et al. 1991; Gaskin et al. 2003); however, our concentrations indicate otherwise. Arid regions typically have alkaline pH soils, which limit the mobility and bioavailability of heavy metals in soils and other mixed substrates, ensuring concentrations remain and even accumulate for decades (Han et al. 2001). Metal uptake and availability in vegetation largely depends on plant physiology (McBride et al. 2013). The availability of minerals for absorption and utilization into biological systems

is of the utmost importance, yet absorbability is highly variable depending soils, moisture and vegetative species (Suttle 2010). Bioavailability and solubility of heavy metals in *Bouteloua gracilis* and *Bouteloua dactyloides* are largely unstudied, and thus further research is required to fully understand the availability of nutrients and toxic metals to livestock and other grazers on the shortgrass steppe.

Some heavy metals, such as cadmium and arsenic, were not detected, possibly due to limitations of XRF analysis. Atomic adsorption (AA) techniques are superior to XRF when sensitivity and low detection limits (ppb) are desired. AA can also detect lighter macronutrients such as sodium, which could not be quantified using XRF. Magnesium and Iodine quantification using XRF are also not well established or reliable and were not quantified in the current study (Mir-Marqués et al. 2014).

Although we cannot determine concentrations of minerals depositing onto vegetation near O&NG production, we can confidently report mineral concentrations of clean grass, free from soils and dust particles. Micro mineral deficiencies (P and Zn) as well as toxic elements (Hg, Pb, and possibly Ba) were identified on all plots including sites that have been plugged and abandoned for over 30 years. It could be that O&NG construction and production are negatively impacting the quality and productivity of vegetation and soils on the shortgrass steppe and that contaminants remains for decades after site reclamation. As these O&NG sites are frequently visited by grazing cattle (Figure 25) and other organisms on the PNG, further analysis of soils, vegetation and mineral bioavailability are required to assess the full extent of O&NG impact.



*Figure 25.* Cattle grazing near condensate tanks on an oil and natural gas production site.

## CHAPTER V

### SHIFTS IN SHORTGRASS STEPPE VEGETATION

#### Abstract

With new technological advances in slick horizontal fracturing, we are seeing an unprecedented increase in the frequency and magnitude of O&NG production, causing a novel impact on native flora and fauna. The objective of this study was to characterize proximate vegetation cover, diversity and functionality during well production and following abandonment. It was hypothesized that vegetation composition would differ significantly with distance from the wellhead, would differ with time since well completion (beginning of site restoration), and that reclamation would be successful by the BLM standards, but would reveal shifts in community structure. Study sites were randomly selected from Pawnee National Grassland (PNG) public lands in Northeastern Colorado. Sites ( $N = 20$ ) were grouped according to status (PA or PR) and production date (spud date) to chronologically examine restoration success: PA = Plugged and abandoned in the 1980s ( $n = 4$ ), PR1 = Producing since 1980-1990 ( $n = 6$ ), PR2 = Producing since 2000-2005 ( $n = 5$ ), PR3 = Producing since 2006-2013 ( $n = 5$ ). Cover and species data were taken between July and September of 2014 and 2015. Vegetation communities were measured in 2 m x 10 m plots at 25 m, 50 m and 100 m in northeast, southeast and west directions from each wellhead (total of 180 plots). Restoration quality was determined using three of nine parameters recommended by the Society for Ecological Restoration, including species diversity, presence of indigenous species and

presence of functional groups. Indices included richness (S), evenness (E), Shannon's diversity (H), Simpson's diversity (D), Functional Diversity  $FD_Q$  (FD), and Functional Redundancy (FR). In general, PA 100 m sites were distinctly different from PR sites, including PR1 sites, which were reclaimed for > 30 yrs. As expected, at 20 m and 50 m, sites had substantially more bare ground and introduced species than at 100 m. PR3 sites had the highest percentage of bare ground, which was as high as 10% at 100 m. Total, there were 16.5% non-natives on all plots combined and 2% of species sampled were invasive. Satisfactory reclamation was achieved at 50 m on PR1 and PR2 sites as vegetation was at 80% total cover when compared to 100 m. PA sites were the highest in diversity indices E, H, D and FD, and PR3 were the lowest in S, E, H, D and FD. Thus, it seems recovery over time is possible. We did not find high functional redundancy on our O&NG sites; instead, we found high species diversity and high functional diversity on PA sites. On patches of land disturbed by O&NG, C3 grasses and introduced forb abundances remain intact for longer periods of time. These disturbed communities with greater spatial heterogeneity than *Bouteloua gracilis* dominated sites could persist with the abandonment of sites. We proposed that novel intensities of O&NG disturbances along with other synergistic disturbances promoted species and functional shifts in vegetation. Long-term experiments in natural field settings are required to understand these complex systems and to develop management and restoration strategies.

### **Introduction**

In Northeastern Colorado, energy development fragments and disturbs shortgrass steppe habitat. The expansion of Oil and Natural Gas (O&NG) production across the grasslands exacerbate degradation, fragmentation and habitat loss (Nasen et al. 2011,

Slonecker et al. 2012; Jones et al. 2015) and has the potential to change landscape dynamics, ecosystem functionality, vegetation communities (Smith et al. 1988; Simmers and Galatowitsch 2010) soil structures (Rowell and Florence 1993) and wildlife populations (Naugle 2011). Impacted populations include birds (Ingelfinger and Anderson 2004; Aldridge and Boyce 2007; Gilbert and Chalfoun 2011; Hamilton et al. 2011; Kalyn Bogard and Davis 2014; Yoo and Koper 2017) and ungulates (Sawyer et al. 2006; Sawyer et al. 2009; Beckmann et al. 2012).

On April 23, 2013 there were 63 active O&NG operations and 19 production facilities on the Pawnee National Grasslands (PNG) public lands, along with dozens of roads and multiple natural gas pipelines (e.g., Lilli Field and Badger pipelines) being constructed (U.S. Department of Agriculture [USDA] 2013) and an unknown number of active sites on adjacent private parcels. Today approximately 179,650 acres of the PNG (93%) are available for oil and gas production, storage, and transportation (USDA 2014a). Currently, the Eastern portion of the PNG has a combined producing and non-producing O&NG footprint of 0.84% from 746 wells (Baynard et al. 2017). The PNG not only has a series of complex private and public landscapes but also has a complex socio-political system with a multitude of stakeholders including private landowners, oil and gas companies, farmers, ranchers, tourists, researchers and governmental bodies. This has made it difficult to quantify the environmental costs of energy development as we lack long-term ecological studies and baseline data. The impacts of O&NG production on water systems, vegetation communities, air quality and grazing are largely unknown. The current research analyzes effects of O&NG development on PNG vegetation

communities by characterizing proximate vegetation cover during well production and following abandonment.

One major concern for government agencies is that they have not been able to quantify habitat loss, fragmentation and degradation pre and post O&NG reclamation. The Great Plains have experienced extensive habitat loss and conversion, exceeding habitat protection by a ratio of 8:1 (Hoekstra et al. 2004). There has also been high interannual variability in precipitation correlated with decreased plant productivity (Knapp et al. 2006; Yang et al. 2008; Miranda et al. 2009; Cherwin and Knapp 2012). It is currently unknown if adequate grazing lands have been reduced, potentially leading to overgrazing of native grasses and indirect effects such as increased invasive species pressure and soil erosion. Indirect habitat losses may be substantially larger than direct habitat losses, due to shifts in distribution of grazers (Sawyer et al. 2006).

Also in question is the effectiveness of reclamation and monitoring of plugged and abandoned wells. The well pads typically cover a 1.2-2.7 ha area and are placed atop crushed stone or wooden mats to support heavy equipment and thick liners to contain spills (Drohan and Brittingham 2012). These sites seem uninhabitable, but aggressive generalists find a way to survive and reproduce on site until reclamation, which at the time of data collection included spreading topsoil, adding fertilizer and seeding with native species (U.S. Department of Agriculture [USDA] 1997). The goal of the United States Forest Service (USFS), under the Revised Final Environmental Impact Statement, is to encourage and facilitate orderly exploration, development, and production of minerals and reclamation of disturbed areas in an environmentally sound manner (USDA 1997). The statement defined satisfactory reclamation as meeting plan requirements and

having desired vegetation at 80% potential cover (i.e., compared to adjacent, undisturbed areas). The process usually takes 3 to 5 years, but depending on precipitation and development size, vegetation might not be able to rebound to the desired vegetative cover of 80% (compared to adjacent undisturbed lands) within that timeframe.

At the community level there is increased risk to potential invasion of noxious weeds and invasive plant species on O&NG sites (Larson, Anderson, and Newton 2001; Manier et al. 2014). On average, 1.5-3.1 ha of vegetation is cleared for the development of a single shale well (Entrekin et al. 2011). Few grassland landscapes of the North American prairie remain adequate in area and distribution to sustain sufficient diversity, functionality and biota native to the landscape (Samson et al. 2004). The resilience of vegetative communities after an O&NG disturbance depends on time, space, life history characteristics, reclamation strategies and cumulative stressors (Minnick and Alward 2015).

Ecosystem functions sustain key ecosystem services and traditional diversity measures, on their own, might not adequately capture ecosystem stability and functionality (Mori et al. 2013). It has been shown that grazing on the shortgrass steppe limits species richness and induces a high uniform cover of dominant *Bouteloua gracilis* and *Bouteloua dactyloides* C<sub>4</sub> grasses (Adler and Lauenroth 2000). It has also been suggested that functional redundancy instead of species redundancy or species diversity is correlated with the community stability (Kang et al. 2015). On the shortgrass steppe there are many species of differing photosynthetic pathways, durations and growth habits that belong to various families. These species coexist to form a complex community



network of interspecific interactions, contrary to the concept of functional redundancy, which assumes complex communities are inherently unstable (May 1972).

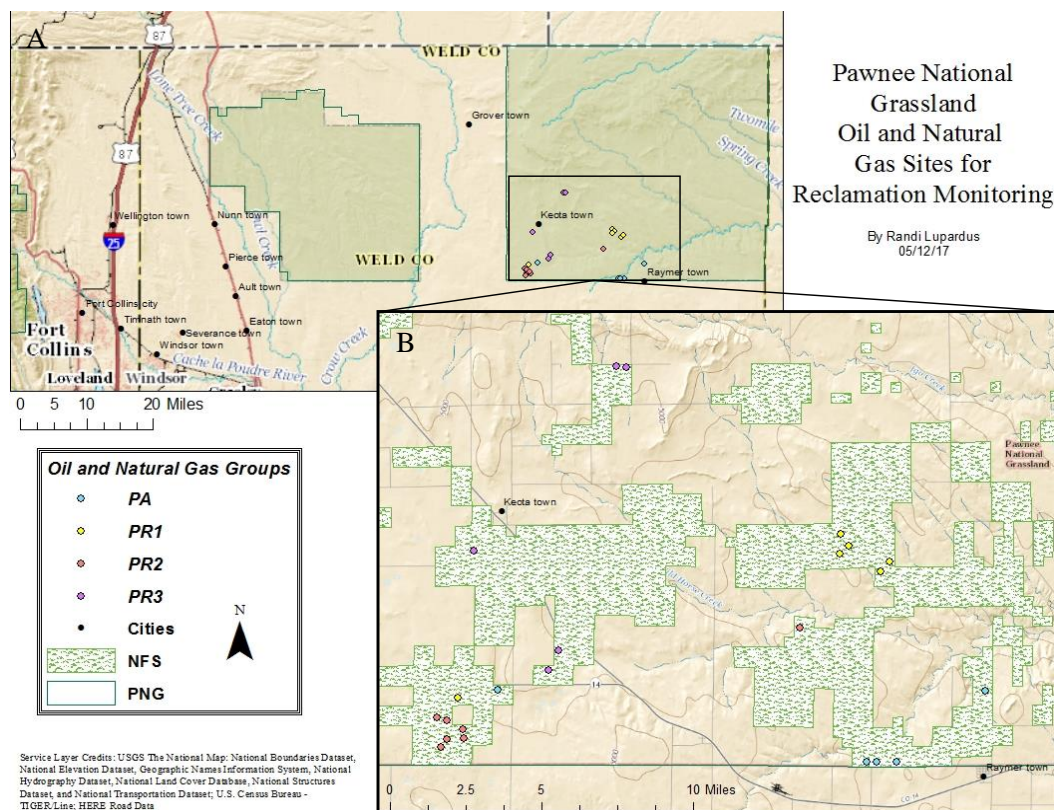
We must examine the specific functional diversity and resilience of this system. On the PNG, it is possible that with the synergistic effects of increased O&NG development, occurring on a short time scale with novel frequency and magnitude combined with an assortment of old (e.g., cattle grazing, fire) and new (e.g., climate change) stressors, the plant communities might shift in structure and function. The objective of this study was to characterize proximate vegetation cover, diversity and functionality during well production and following abandonment. It was hypothesized that vegetation composition would differ significantly with distance from the wellhead, would differ with time since well completion (beginning of site restoration), and that reclamation would be successful by the BLM standards, but would still indicate shifts in community structure. We propose that novel O&NG disturbances along with other synergistic disturbances are promoting species and functional shifts in vegetation.

## **Methods**

### **Physical and Social Setting**

The PNG is 78,100 ha of land that lies within an approximate 50 by 100 km checkerboard of private and public lands (Figure 26). It is discontinuously distributed as a result of private land acquisition that began during the Dust Bowl era of the 1930s. This distribution resulted in a diverse set of land users and land managers, and contributed to the importance and relevance of disturbance on the shortgrass steppe. Communities found within or proximate to the grassland are directly affected by the management of the

grasslands and by those who use the lands for recreation, resource extraction and grazing purposes.



*Figure 26.* Pawnee National Grassland oil and natural gas sites for reclamation monitoring. PA = Plugged and abandoned in the 1980s ( $n = 4$ ), PR1 = Producing since 1980-1990 ( $n = 6$ ), PR2 = Producing since 2000-2005 ( $n = 5$ ), PR3 = Producing since 2006-2013 ( $n = 5$ ). A. Northeastern Colorado, B. zoom of study sites.

The climate is typical of mid-continental semiarid temperate zones but is somewhat drier because of a strong rain shadow effect of the Rocky Mountains to the west. Annual precipitation, and its seasonal distribution, profoundly influences this semiarid grassland. Precipitation-induced changes cascade through the ecosystem, causing fluctuations in vegetative structure, the abundance and species composition of biotic communities, and ecosystem functions such as net primary productivity, nitrogen

mineralization and trace gas fluxes. The three-decade (1981-2010) averages of climatological variables include an annual average temperature of 6.4 °C (43.6 °F) and mean annual precipitation of 42.62 cm (16.78 in; NOAA 2017a).

Many studies have shown that warm season grasses benefit from reduced nutrients, increased stress conditions and grazing disturbances (Coffin and Lauenroth 1988; Paschke et al. 2000; Cherwin et al. 2009). Long-lived C<sub>4</sub> grasses such as blue grama (*Bouteloua gracilis*) dominate under the characteristically dry conditions of the shortgrass steppe by efficiently accessing available water. Blue grama is especially important in the shortgrass steppe because it is a nutritional and palatable grass for cattle. Other important plants include buffalo grass (*Buchloe dactyloides*), prickly pear cactus (*Opuntia polyacantha.*), rabbitbrush (*Chrysothamnus nauseosa*), saltbush (*Atriplex canescens*) and western wheatgrass (*Agropyron smithii*; Sims and Singh, 1971). The shortgrass steppe stores most biomass and resources belowground, so aboveground disturbances do not drastically alter the vegetative community.

Natural disturbances such as fire and grazing impact the spatial heterogeneity of grassland vegetation communities (Adler and Lauenroth 2000; Peco et al. 2006; Augustine and Milchunas 2009). Large herbivore grazing was an important part of the evolutionary history of the shortgrass steppe and still is today. Herbivory in this system is considered to be a vital, routine disturbance affecting morphologically distinct groups of plant species (Stahlheber and D'Antonio 2013). Disturbances related to soil erosion and deposition occur over longer time and larger spatial scales and are often linked to regional shifts in precipitation and climate. Fire, grazing and drought are complementary pressures selecting for a similar set of characteristics, antagonistic to competition, on the

shortgrass steppe (Bergelson 1990; Sala et al. 1996). In this system, a true disturbance would be a lack of disturbance, or possibly a disturbance of novel magnitude.

### **Study Sites**

The PNG is divided into east and west landmasses (Figure 24), with a combined 389 private and public O&NG sites on the eastern portion (Baynard et al. 2017). Site selection was narrowed down to only those on National Forest Service land ( $n = 63$ ) for permitting purposes. Sites were grouped according to status (PA or PR) and production date (spud date) to chronologically examine restoration success: PA = Plugged and abandoned in the 1980s ( $n = 4$ ), PR1 = Producing since 1980-1990 ( $n = 6$ ), PR2 = Producing since 2000-2005 ( $n = 5$ ), PR3 = Producing since 2006-2013 ( $n = 5$ ). Five sample sites were randomly chosen from each group ( $N = 20$ ). One site from the PR1 group was closed due to soil contamination and was excluded from the study and another site in the PR1 group was added, but was categorized incorrectly (due to a re-fracturing event) and was thus moved to the PR2 group post data collection. Sites that were potentially dangerous or were not easily accessible from open, public roads were excluded. Site codes and historical data are listed in Table 13.

Table 13

*Site Locations and Historical Use of Land.*

	Site	Date of Appraisal <sup>a</sup>	Grazing <sup>b</sup>	Structure <sup>b</sup>	Crops <sup>b</sup>
PR1	2	10-10-38			
	3	05-19-44	On site	Fence not on site	
	6	05-19-44	On site	Fence not on site	
PA	8	Unknown			
	7	01-28-39			Unknown
	9	12-15-39	On site		
	10	09-14-38	Unknown		Unknown
	11	09-01-38			On site
PR2	12	09-01-38			On site
	24	Unknown			
	13	10-7-38			
	14	12-16-38			
	15	10-07-38			
	16	05-24-38			
PR3	17	05-24-38			
	19	11-29-39		Building unknown	Unknown
	18	05-31-39	Unknown		Unknown
	20	05-15-40	Unknown	Building unknown	Unknown
	21	05-15-40	Unknown	Building unknown	Unknown
	22	12-16-38			On site

<sup>a</sup> Date of appraisal is the day the BLM purchased the land and mineral rights for the site

<sup>b</sup> Presence of grazing, structures or crops on site at time of purchase

Note: PA = Plugged and abandoned in the 1980s ( $n = 4$ ), PR1 = Producing since 1980-1990 ( $n = 6$ ), PR2 = Producing since 2000-2005 ( $n = 5$ ), PR3 = Producing since 2006-2013 ( $n = 5$ ).

## Vegetation Measurement

Monitoring parameters included estimating percent vegetation cover by species and percent total vegetation cover (i.e., sum of all species) in plots; data were taken between July and September of 2014 and 2015. Three transects ran 100 meters in northeast, southeast and west directions from the wellhead. Vegetation communities were measured in 2 m x 10 m plots at 25 m, 50 m and 100 m along each transect (total of 180 plots). Plot distances were chosen to represent least disturbed or mostly reclaimed area

(100 m), intermediately disturbed or interim reclamation area (50 m) and highest disturbance area, typically within a barbed fence (25 m).

### **Vegetation Indices and Definitions**

Reclamation was considered satisfactory when cover on the disturbed area reached 80% total cover of adjacent, undisturbed land cover (USDA 1997). Restoration quality was determined using parameters recommended by the Society for Ecological Restoration, including species composition and ecosystem functionality for long term stability (McDonald et al. 2016: Table 3). Percent cover and bare ground were determined for each plot and diversity was determined using indices of richness (S), evenness (E), Shannon's diversity (H) and Simpson's diversity (D). Species were given three letter acronyms (and were categorized into functional groups including family (Phylogenetic), duration (annual/ biennial/ perennial), growth habit (grass, forb, vine, subshrub, shrub, tree), photosynthetic pathway (C3, C4, CAM), as well as into descriptive categories status (native vs. introduced) and invasive (non-invasive vs. invasive; USDA 2014; see Appendix D). Functional diversity, using Rao's Quadratic Entropy (FD<sub>Q</sub>; Rao 1982; Botta-Dukát 2005) was estimated using PC ORD and functional redundancy (inverse of Rao's Q) was estimated using inversely proportional functional diversity (Pillar et al. 2013). Community structure and functional stability were interpreted as no effect of O&NG if PR Groups (1-3) had similar vegetation cover, species diversity, functional redundancy (decreased functional diversity) and introduced species when compared to the PA Group.

## Statistical Analysis

We examined variation in plant species composition among plots with a Nonmetric Multidimensional Scaling (NMS) ordination of the species x plot matrix based on plant cover. We used the Sørensen distance measure as implemented in PCORD (v 7.0; MjM Software Design, Glenden Beach, OR, U.S.). NMS ordination is free from assumptions regarding multivariate normality, and allows for unbalanced designs and a large number of species. Ordinations were rotated to load the strongest environmental variable onto a single axis. Linear relationships between ordination scores and environmental (including functional) variables (bare ground, S, E, H, D, FD, photosynthetic pathway, native, invasive and family) were depicted as joint plots. Species with two or less occurrences ( $n = 27$ ) were deleted to improve interpretability. We conducted multivariate analyses of Distance x Group (i.e., PA, PR1, PR2, PR3) effects on basal cover of 137 species using a Multi-Response Permutation Procedure (MRPP). After finding a significant result to the omnibus test (type 3), follow up regression contrasts compared specific level effects of Distance and Group factors on status, bare ground and invasive. Logistic regression models were used to predict presence or absence of invasive and introduced species while a normal general linear model (glm) was used to predict percent bare ground, (Table 14).

Frequency values for family, duration, growth habit and photosynthetic pathway were calculated for each Distance and O&NG group, controlling for Direction, then were converted to abundance scores. As abundance scores were multivariate normal, a Multivariate Analysis of Variance (MANOVA) test determined Distance and Group effects and univariate analysis of Variance (ANOVA) tests determined Distance and

Group effects, subsequent to significant MANOVA. Indicator Species Analysis (ISA) with a Monte Carlo test of significance determined characteristic species for the four O&NG Groups. Dufrene and Legendre's (1997) ISA method provides a simple, intuitive solution to the problem of evaluating species associated with groups of sample units. It combines information on the concentration of species abundance in a particular group and the consistency of occurrence of a species in a particular group, then produces indicator values (IVs) for each species in each group. These IVs for each species in each group are tested for statistical significance using a Monte Carlo technique.

To address diversity of sites, a Multivariate Analysis of Variance (MANOVA) test determined Distance x Group effects on species richness (S), evenness (E), Shannon diversity (H) and Simpson's diversity (D). MANOVA was selected over MMRP due to model fit and met assumptions. Discriminate analysis of variance (ANOVA) tests determined Distance and Group effects on functional diversity (FD<sub>Q</sub>) and its inverse, functional redundancy (FR). Traits included in FD<sub>Q</sub> included species family, duration, growth habit and photosynthetic pathway. We also conducted univariate analyses of Distance and Group effects on diversity indices (i.e., S, E, H and D), subsequent to significant MANOVA. Data analyses were performed using PCORD (v 7.0), R (v 1.0.44) and SAS (v 6.1.7601).

Table 14

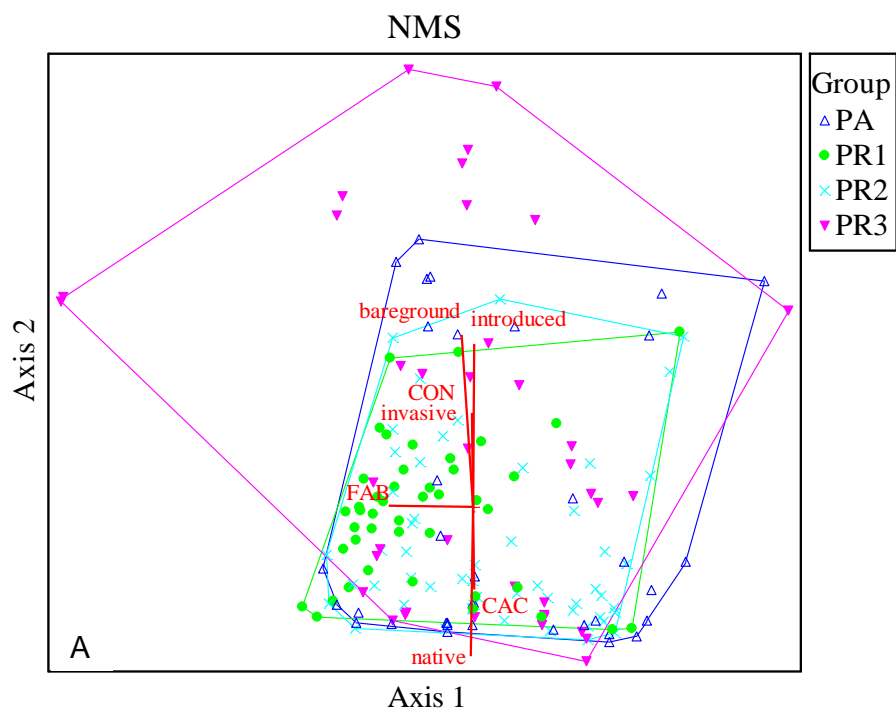
*Family Used in Logistic Regression According to Response Variable Distribution.*

Family	Link	Response Variable
Normal	none	Bare ground
Binomial	logit	Invasive
Binomial	logit	Status



## Results

For the remainder of the article, Families will be referred to by their three-letter abbreviation and species by their six-letter alpha codes (see Appendix D). The NMS ordination yielded a three-dimensional solution with a final stress of 14.85, and axes one, two and three cumulatively explained 66% of the variation in the original species matrix (Figure 27A- 27C). The first axis accounted for 18.2% of the variance and was correlated with two species, BOUDAC a warm season C<sub>4</sub> perennial clonal grass ( $r = -0.533$ ) and a C<sub>4</sub>, perennial, bunchgrass BOUGRA ( $r = 0.549$ ; Figures 27F and 27G). The scores were also moderately correlated to family FAB, positively correlated with axis one, similarly to BOUDAC. This axis likely accounted for small scale differences between patches of BOUGRA and BOUDAC based on environmental differences such as soil texture or functional differences such as propagule pressures (BOUDAC is stoloniferous) on PA and PR1 sites. These two species, BOUDAC and BOUGRA, also corresponded with axis two scores explaining 17.2% variance. Species positively correlated with axis two included an introduced, perennial C<sub>3</sub> grass AGRCRI ( $r = 0.395$ ) and a broadleaf, taprooted, annual forb with rough unpalatable cocklebur, XANSTR ( $r = 0.444$ ; Figures 27E and 27H). Species negatively correlated with axis two included BOUGRA ( $r = -0.615$ ) and BOUDAC ( $r = -0.491$ ), this time clustered together based on large-scale similarities. The only environmental variable that strongly and positively loaded onto axis two was percent bare ground ( $r = 0.689$ ). Species differences are likely due to differences in plant cover. BOUDAC and BOUGRA are very successful in competitive environments with low percent bare ground and grazing, whereas introduced species such as XANSTR and AGRCRI need space and few competitors (high percent bare ground).



*Figure 27.* Nonmetric Multidimensional Scaling (NMS) ordination of the plots in species space. Although there was a 3D solution, each figure shows 2D NMS results for two of the three rotated axes. Sites were grouped by status (PA or PR) and production date (spud date): PA= Plugged and abandoned in the 1980s (blue triangles), PR1= Producing since 1980-1990 (solid green circles), PR2= Producing since 2000-2005 (blue X), PR3= Producing since 2006-2013 (solid pink triangles). Lines represent joint plots ( $r^2$  cutoff = 0.40) of environmental variables: bare ground cover, introduced species, invasive species, Families Convolvaceae (CON), Fabaceae (FAB), and Cactaceae (CAC). Polygons enclose the subset of plots belonging to each production group. A = Axis 1 and 2, B = Axis 1 and 3, C = Axis 2 and 3, D = only includes species PASSMI, E = XANSTR only, F = BOUDAC only, G = BOUGRA only, H = AGRCRI only. Species codes are in Appendix D.

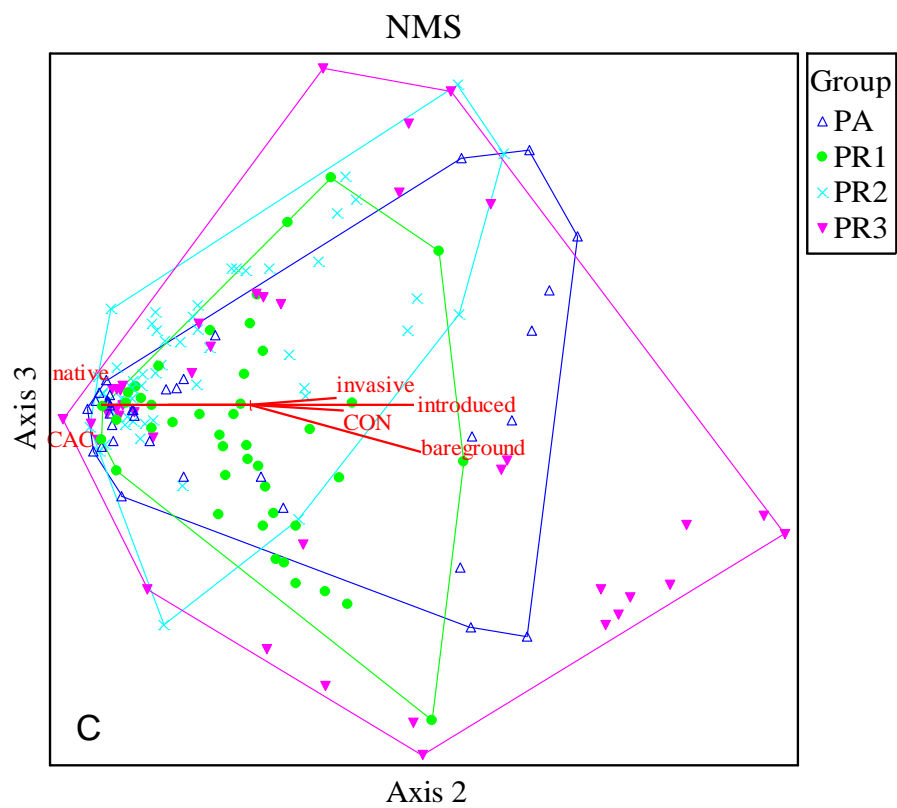
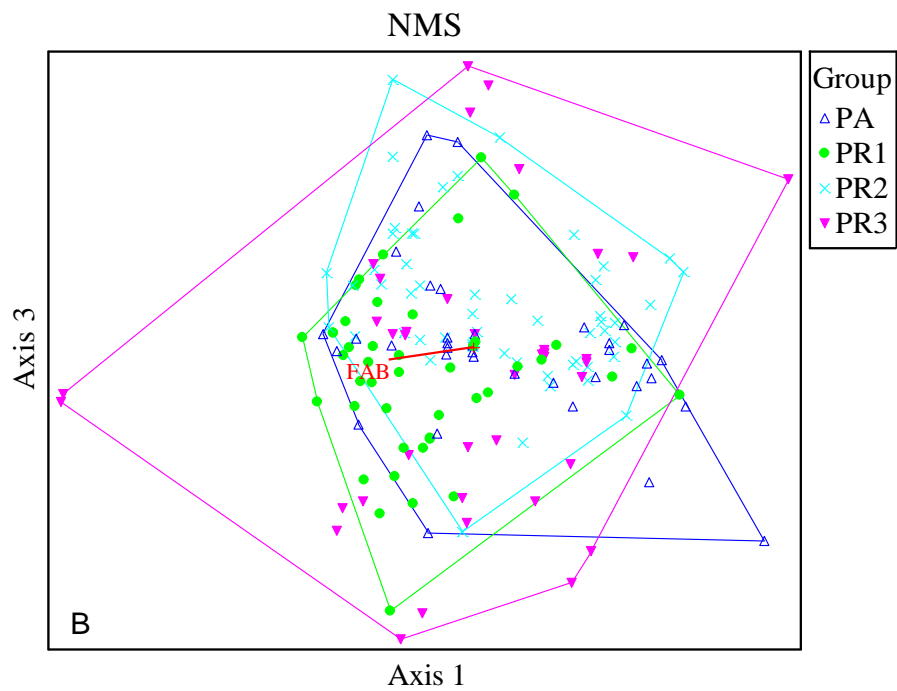


Figure 27. Continued.

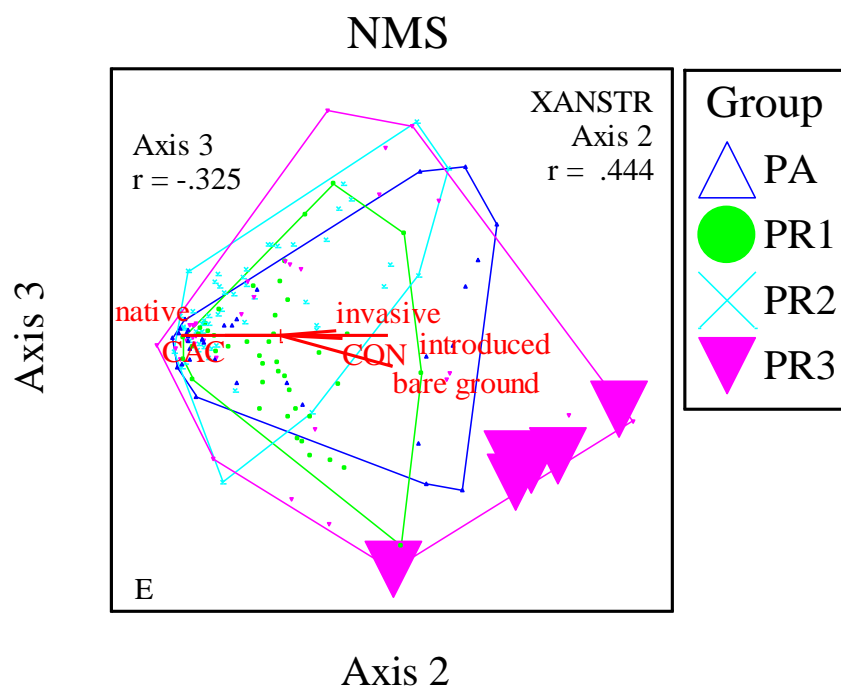
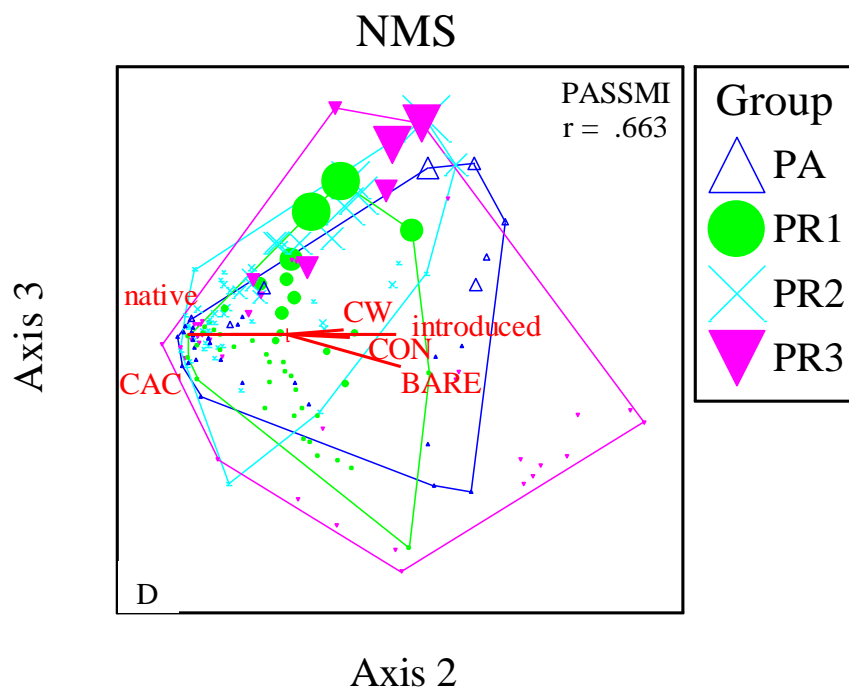


Figure 27. Continued.

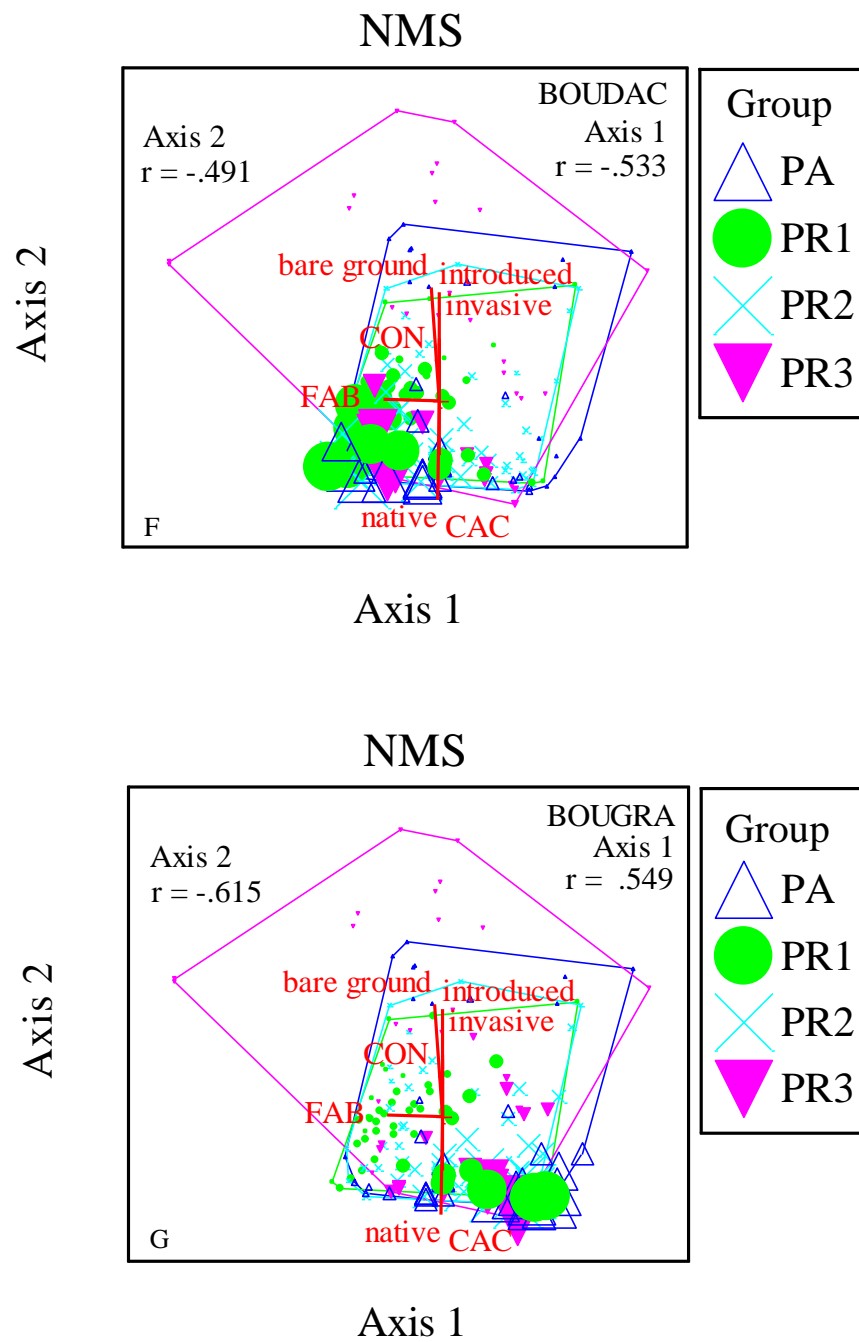


Figure 27. Continued.

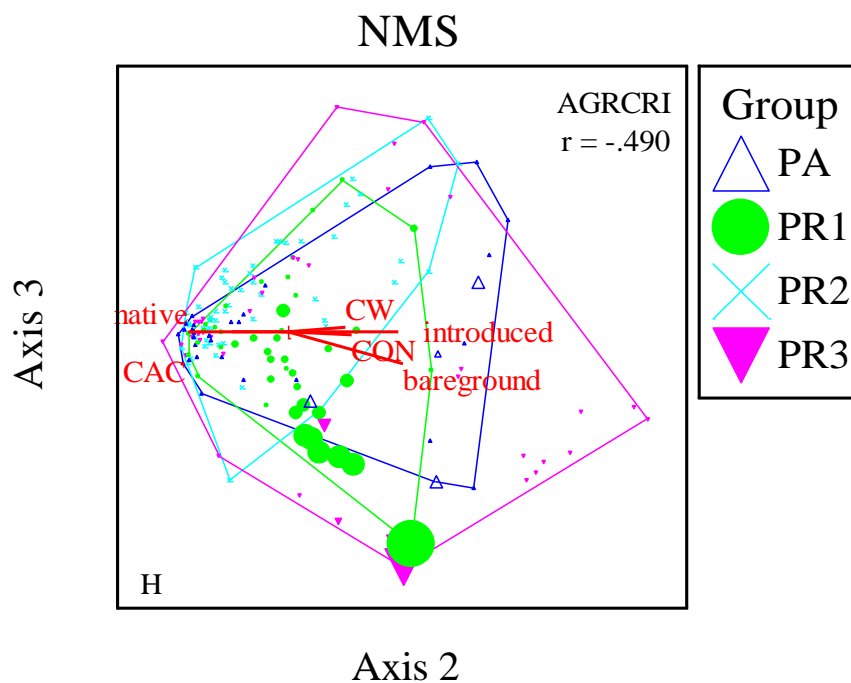


Figure 27. Continued.

Specific Families also correlated with axis two including CON ( $r = 0.51$ ) and AMA ( $r = 0.412$ ) on the positive end and CAC ( $r = -0.477$ ) on the negative end. Axis two likely represents the status of species ranging from native species strongly negatively correlated with axis two, to introduced species strongly positively correlated with axis two.

Axis three was most strongly, positively correlated with the C<sub>3</sub>, perennial, rhizomatous grass PASSMI ( $r = 0.663$ ; Figure 27D). Negatively correlated species included AGRCRI ( $r = -0.49$ ) as well as XANSTR ( $r = -0.325$ ). It is not surprising that there was a correlation between decreasing native species on the negative end of axis one ( $r = -0.556$ ) and increasing introduced species on the positive end ( $r = 0.591$ ) along with bare ground ( $r = 0.343$ ) and invasive species ( $r = 0.490$ ). Thus, axis three likely

represents a gradient of disturbance with a combination of environmental and functional characteristics at play. On the increasing axis, bare ground would have originally created an opportunity for invasion by introduced species (AGRCRI, XANSTR) and generalist species from families such as Convolvulaceae (bindweed family), well known for its aggressive, annual vines. On the other end of the spectrum is a much less disturbed environment with native species on lands that have been recovering since the Dust Bowl. The increase in PASSMI is likely due to the age of the site and time since disturbance, with native C<sub>3</sub> grasses taking over the older, more recovered sites.

The MMRP results indicated species composition varied significantly across O&NG production groups, although the effects were small ( $A$  values  $< 0.1$ ; Table 15). All groups had statistically different composition of vegetation, with the exception of PR1 versus PR2. Indicator Species Analysis indicated that there were 40 significant ( $p \leq 0.05$ ) indicator species (Table 16). Of these 40 significant species, one species, PSOTEN had 84% relative abundance in the PA group and an indicator value (IV) of 58 (see Appendix D). The second highest IV (38.9) was for AGRCRI, also found on PA groups. It is interesting to note that PR1 sites did not contain similar species (AGRCRI, PASSMI, PSOTEN) to PA sites. The PR1 sites underwent interim reclamation using similar reclamation strategies as PA sites over 35 years ago, although infrastructure (pumpjack, fence, and tanks) had been removed and roads were allowed to naturally revegetate on PA sites.

Table 15

*Multi-Response Permutation Procedure Results: Pairwise Comparisons of Vegetation Cover Between Production Groups.*

Groups Compared	T	A	<i>p</i>
PR2 vs. PA	-11.30	0.04271	2.7E-07 *
PR2 vs. PR3	-12.53	0.05693	2.9E-07 *
PR3 vs. PR1	-2.48	0.01076	0.02659
PA vs PR3	-15.43	0.07435	1E-08 *
PA vs. PR1	-8.90	0.04108	5.94E-06 *
PR3 vs. PR1	-4.96	0.02991	0.002387 *

*Note: P values corrected for multiple comparisons using Bonferoni adjustment ( $p \leq 0.008$ )*



Table 16

*Indicator Species Analysis for Significant Species in Production Groups.*

Species	Max Group	IV	Mean	Std. Dev.	P *
AGRCRI	PA	38.9	12.4	3.11	0.0001
BOUDAC	PA	30.4	24.8	2.31	0.0226
CHELEP	PA	8.9	3.2	1.6	0.0105
HESCOM	PA	21.3	12.4	3.68	0.0249
MELOFF	PA	20	3.8	1.67	0.0001
PASSMI	PA	23.9	18.9	3.53	0.009
PSOTEN	PA	57.7	17.6	4.43	0.0001
SCHPAN	PA	8.6	4.1	1.94	0.0311
ARTFIL	PR1	8.3	2.4	1.45	0.0063
ARTFRI	PR1	21.6	13	3	0.0141
CHOTEN	PR1	5.6	2.1	1.36	0.0395
CONARV	PR1	9.7	4.9	2.14	0.0333
ECHVIR	PR1	9.5	2.9	1.51	0.0045
ERINAU	PR1	17.4	7.6	2.29	0.0024
HETVIL	PR1	25.2	8.3	2.29	0.0001
LUPARG	PR1	5.6	2.1	1.34	0.0379
QUILOB	PR1	5.6	2.1	1.36	0.0418
SOLROS	PR1	8.8	3.5	1.71	0.0181
SPOAIR	PR1	12.9	3.7	1.88	0.002
AGRSTO	PR2	11	5	2.25	0.0054
ARIPUR	PR2	12.3	5.5	2.15	0.0109
CALBER	PR2	8.7	4.1	2	0.0216
ERILON	PR2	34	7.3	2.4	0.0001
ERYASP	PR2	20.4	9.5	2.99	0.0037
FESOC	PR2	26.1	13	2.97	0.0016
HORJUB	PR2	15.6	7.4	2.82	0.0122
LUPPUS	PR2	9.3	3	1.52	0.0091
PANVIR	PR2	7.4	2.8	1.6	0.018
PLAPAT	PR2	24.2	17.6	2.76	0.0258
RATCOL	PR2	11.3	6.2	2.21	0.0303
SOPNUT	PR2	23.5	13.5	2.44	0.0022
SPHCOC	PR2	29.7	22.4	2.38	0.0087
THLARV	PR2	13.6	7.9	2.13	0.0223
AMAPOW	PR3	11.1	2.9	1.48	0.0013
AMASPI	PR3	20.8	4.4	1.86	0.0001
ASTMOL	PR3	6.7	2.4	1.48	0.0375
DIGSAN	PR3	12.6	4.2	1.98	0.003
SALTRA	PR3	11.9	6.9	2.58	0.0478
XANSTR	PR3	13.3	3.2	1.58	0.0006

Notes: See Appendix D for species codes. Max group = group with maximum species IV (indicator value). Each species can indicate only one group. P\* species with  $p \leq 0.05$  for a group. Site codes: plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3).

We conducted regression analysis with contrasts estimating Group and Distance effects on vegetation traits (status, percent bare ground, and invasive; Tables 17 and 18). The linear contrasts indicated statistical effects of Group and Distance for variables bare ground and status, but not for invasive. We anticipated that PA sites at 100 m would have a lower abundance of introduced species than PR sites. Only PR1 20 m, PR1 50 m, PR3 20 m, and PR3 50 m contained significantly higher abundances of introduced species. Of the 2079 individuals identified, 343 were introduced (~ 20%).

Table 17

*Estimates of Production Group and Distance Effects on Vegetation Traits*

Trait	Parameter	DF	Estimate	SE	Chi-Square	Pr > ChiSq
<u>Invasive</u>	Intercept 0	1	3.10	0.74	17.63	<.0001
	Intercept 1	1	6.87	1.23	31.02	<.0001
	Distance	1	0.02	0.02	0.81	0.37
	Distance*Group	1	0.01	0.01	0.75	0.39
	Pump	1	-0.21	0.28	0.58	0.45
<u>Status</u>	Intercept	1	0.15	0.03	31.84	<.0001
	Distance 20	1	-0.02	0.04	0.21	0.65
	Distance 50	1	-0.03	0.04	0.63	0.43
	Group*Distance PR1 20	1	0.20	0.06	12.15	0.00 *
	Group*Distance PR1 50	1	0.14	0.06	6.23	0.01 *
	Group*Distance PR2 20	1	0.07	0.05	1.84	0.18
	Group*Distance PR2 50	1	0.04	0.05	0.56	0.45
	Group*Distance PR3 20	1	0.19	0.06	9.67	0.00 *
	Group*Distance PR3 50	1	0.17	0.06	9.01	0.00 *
	Group PR1	1	-0.08	0.04	3.53	0.06
	Group PR2	1	-0.03	0.04	0.89	0.34
	Group PR3	1	-0.01	0.04	0.05	0.83
	<u>Bare Ground</u>	Intercept	1	4.18	1.25	11.20
Distance 20		1	2.48	1.82	1.87	0.17
Distance 50		1	0.82	1.80	0.21	0.65
Group*Distance PR1 20		1	11.66	2.65	19.31	<.0001 *
Group*Distance PR1 50		1	-1.16	2.66	0.19	0.66
Group*Distance PR2 20		1	6.30	2.42	6.79	0.01 *
Group*Distance PR2 50		1	-2.41	2.41	1.00	0.32
Group*Distance PR3 20		1	10.76	2.87	14.06	0.00 *
Group*Distance PR3 50		1	19.17	2.61	53.92	<.0001 *
Group PR1		1	2.49	1.88	1.75	0.19
Group PR2		1	0.22	1.71	0.02	0.90
Group PR3		1	8.40	1.79	21.91	<.0001 *

*Notes:* Traits include status, percent bare ground and invasive. Contrasts are not shown for models in which factors were not significant. The negative Estimates (E's) indicate sites were less likely than PA 100 m, to contain introduced species and positive E's indicate sites were more likely to contain introduced species. \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$

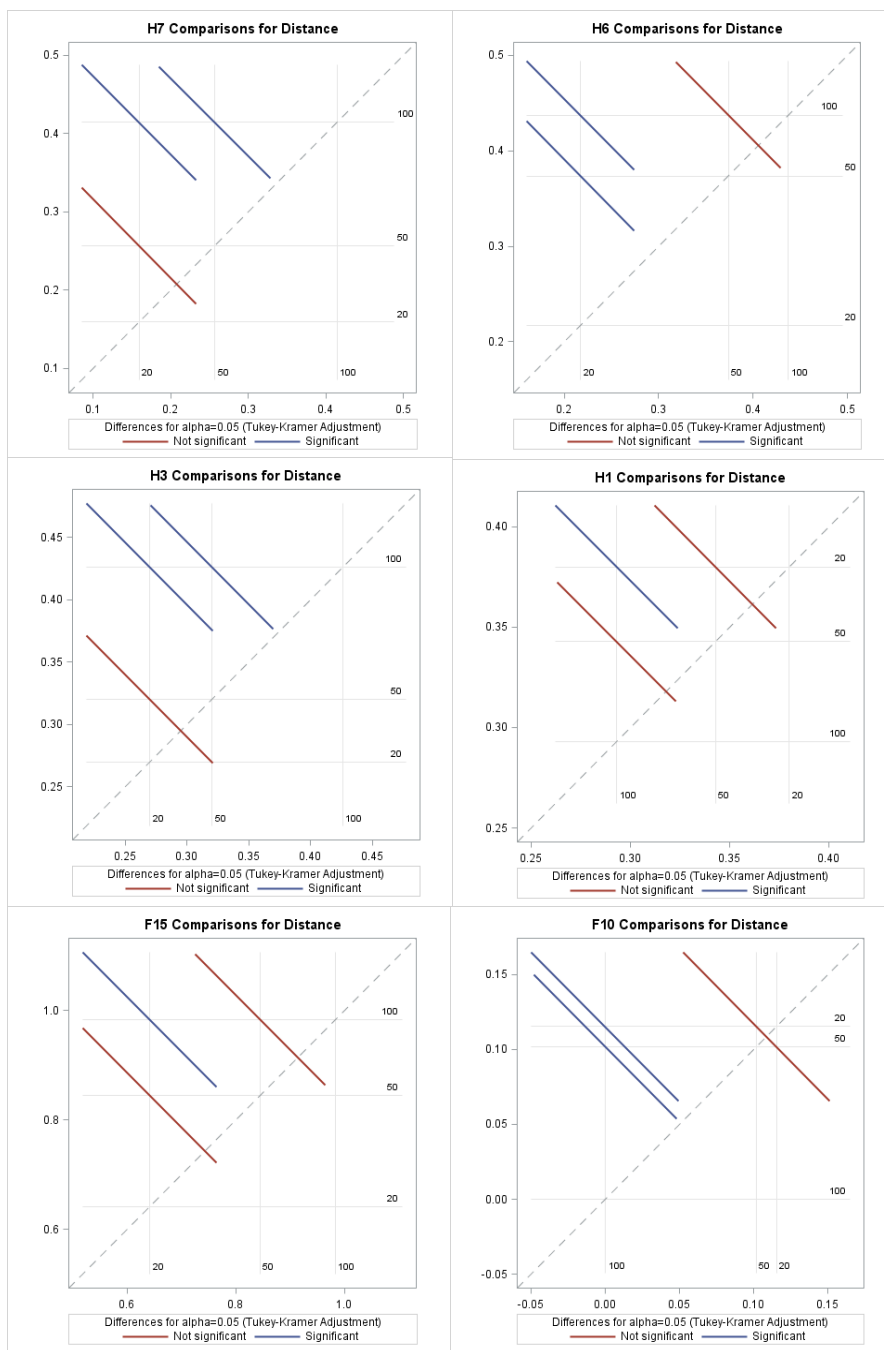
Table 18

*Regression Models and Fit Statistics*

Model Description		Model Fit Statistics					
Family (distribution)	Response Variable	-2 Log Likelihood	AIC	AICC	BIC	Chi-Sq	Chi-Sq/DF
Normal	Bare Ground	7522.51	7546.51	7546.66	7614.21	1265.80	0.61
Binomial (logit link)	Invasive	394.92	404.92	404.95	433.12		
Binomial (logit link)	Status	1806.03	1830.03	1830.18	1897.71	2079.00	1.01

Group PR3 had the highest abundance of bare ground  $E = (8.39)$  regardless of distance (Table 17; see Appendix D). Negative values of  $E$  indicate a lower percentage of bare ground. Percent bare ground was relatively similar in the PA group from 100 m to 20 m. In general, plant cover slowly replaces bare ground, but in this system, bare ground can still frequently be found on PA sites “recovered” for  $> 35$  years (see Appendix D). Multivariate Analysis of Variance (MANOVA) test determined effects on species relative abundance of functional traits (family, duration, growth habit and photosynthetic pathway) were significant for Group (Wilks' Lambda = 0.10,  $F(123,369) = 3.37$ ,  $p < 0.0001$ ) and Distance (Wilks' Lambda = 0.42,  $F(82, 246) = 1.6$ ,  $p = 0.0033$ ), but not for Group\*Distance interaction (Wilks' Lambda = 0.17,  $F(246, 740) = 1.04$ ,  $p = 0.36$ ).

Univariate ANOVAs, with Tukey-Kramer Least Squares Means Adjustments for Multiple Comparisons, determined significant Group and Distance effects. The ANOVAs indicated statistical effects of Distance and Group were significant for all variables (Figures 28 and 29). Sites 100 m from the wellpad had higher abundances of perennials and lower abundances of annuals (Figure 28). They also had a higher abundance of species from the family LIN, a higher abundance of forb/herb/subshrubs, shrubs and  $C_3$  grasses (Figure 28). Closest to the wellhead (25 m) we found a higher abundance of forb/herbs, a lower abundance of shrubs and a lower abundance of species from the LIN and MAL Families.



*Figure 28.* Significant effects of distance (from the wellhead) on duration, family, growth habit, and photosynthetic pathway. Each plot shows LS-means adjusted pairwise differences between groups, their significance levels and their individual confidence limits. The LS-means of each pair meet at their intercept (center of line). A blue line indicates means are significantly different between distances. A red line indicates groups have similar LS-means. Axis ticks indicate mean diversity values. Distance 1 = 20 m, 2 = 50 m, 3 = 100 m.

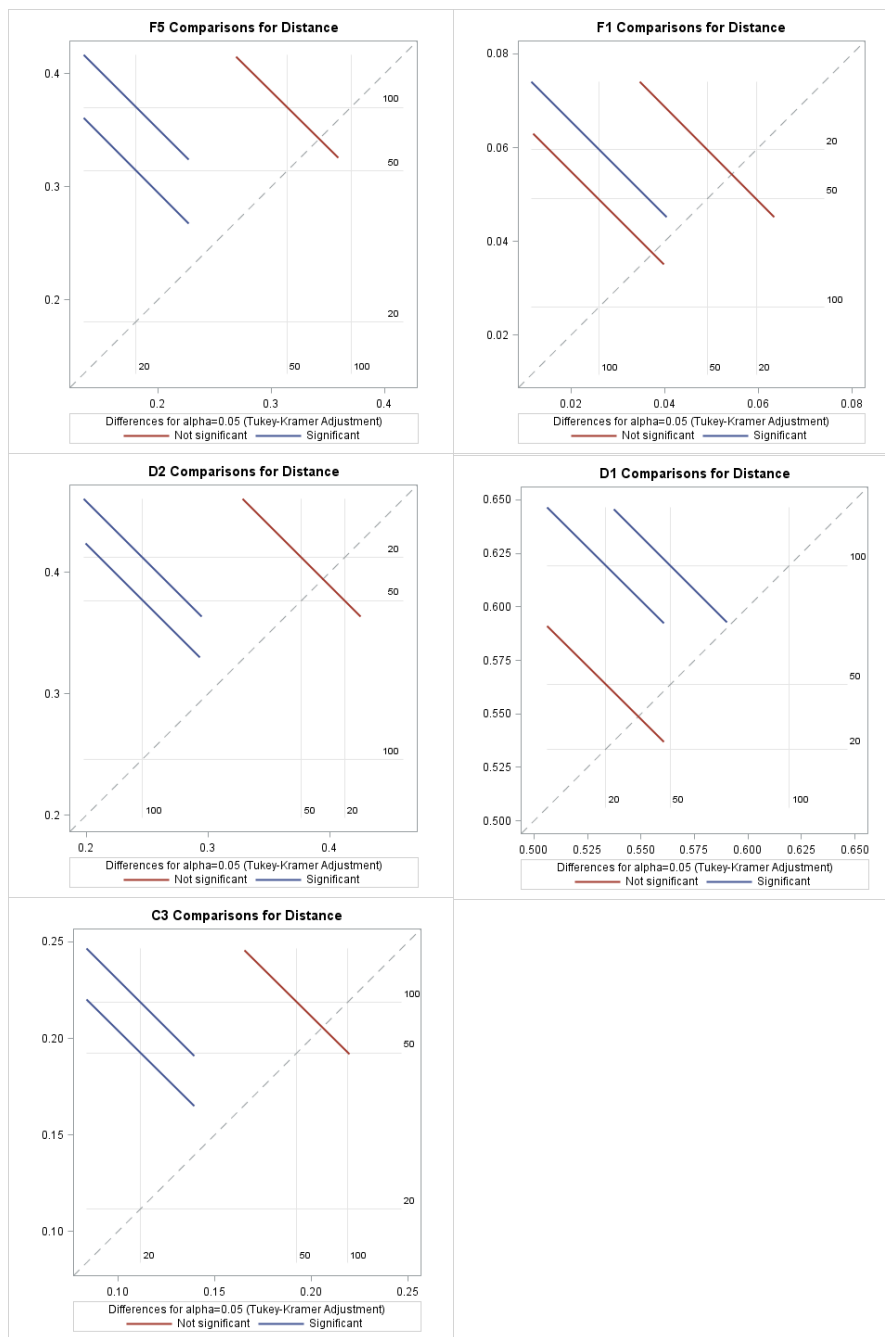
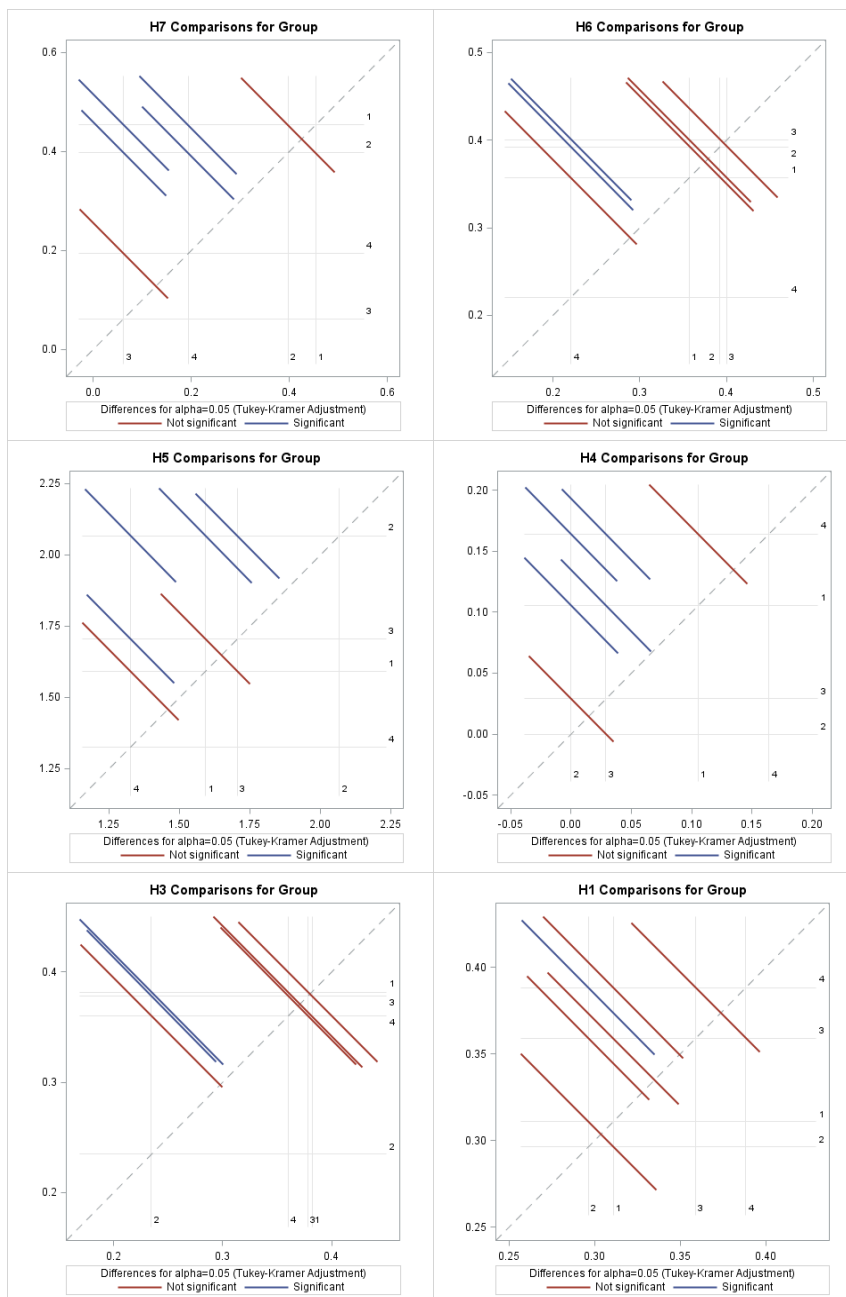


Figure 28. Continued.



**Figure 29.** Significant effects of production group on duration, family, growth, habit, and photosynthetic pathway. Each plot shows LS-means adjusted pairwise differences between groups, their significance levels and their individual confidence limits. The LS-means of each pair meet at their intercept (center of line). Blue line = significant differences between groups; red line = similar LS-mean. Axis ticks indicate mean diversity values. Group 1 = PR1, 2 = PA, 3 = PR2, 4 = PR3. Family F1 = AST, F2 = BRA, F3 = CAC, F4 = FAB, F5 = MAL, F6 = PLA, F7 = POA, F8 = EUP, F9 = NYC, F10 = ONA, F11 = AMA, F12 = VRB, F13 = CYP, F14 = BOR, F15 = LIN, F16 = PLG, F17 = POR, F18 = CAP, F19 = SOL, F20 = ZYG, F21 = SCR, F22 = CNV, F23 = CON, F24 = CHE, F25 = CMM. Duration: D1 = perennial, D2 = annual D3 = annual biennial, D4 = annual biennial perennial, D5 = annual perennial, D6 = biennial, D7 = biennial perennial. Growth Habit = H1 = forb herb, H2 = forb herb shrub subshrub, H3 = forb herb subshrub, H4 = forb herb vine, H5 = graminoid, H6 = shrub, H7 = subshrub. Photosynthetic Pathway: C1 = C3, C2 = C4, C3 = CAM.

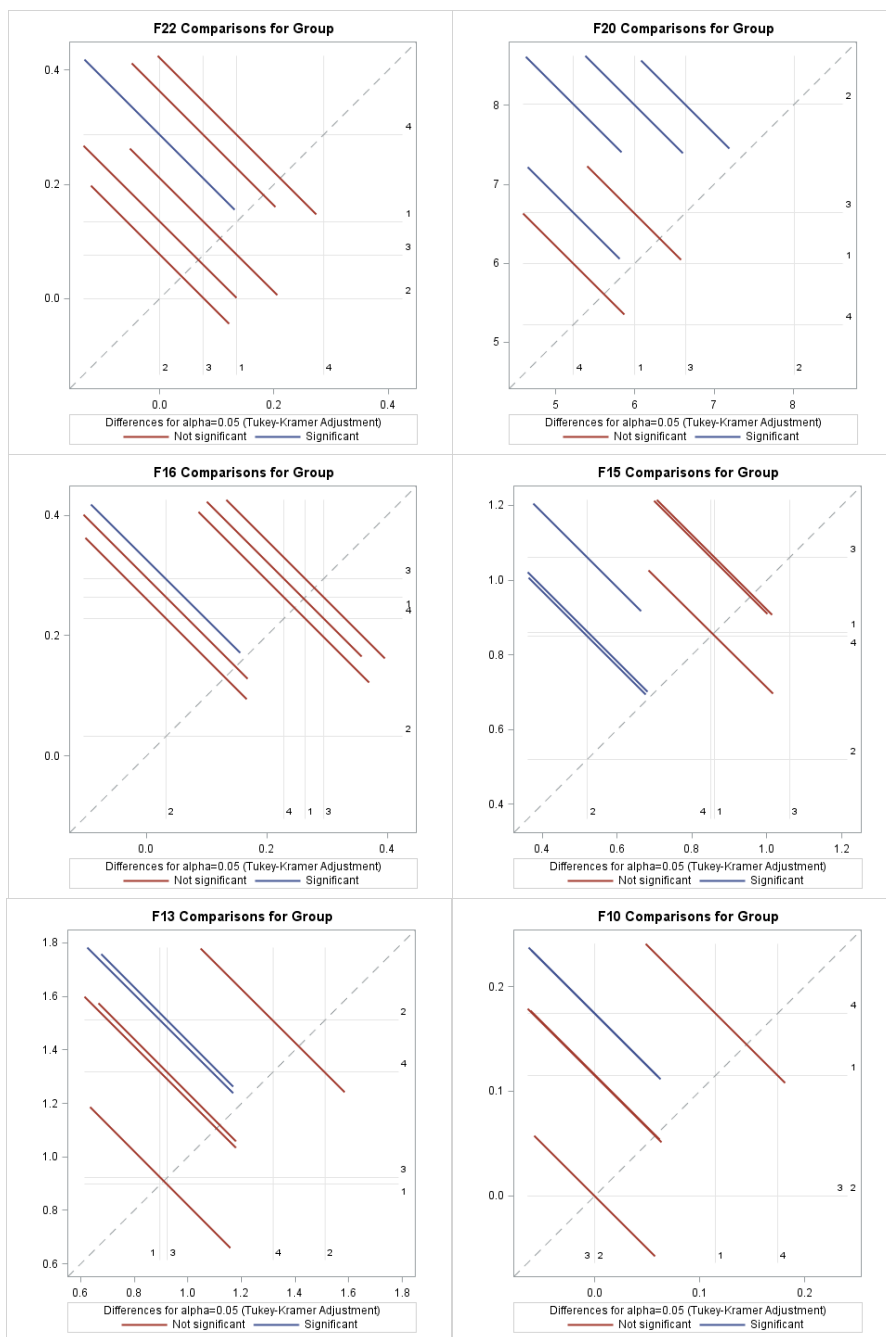


Figure 29. Continued.

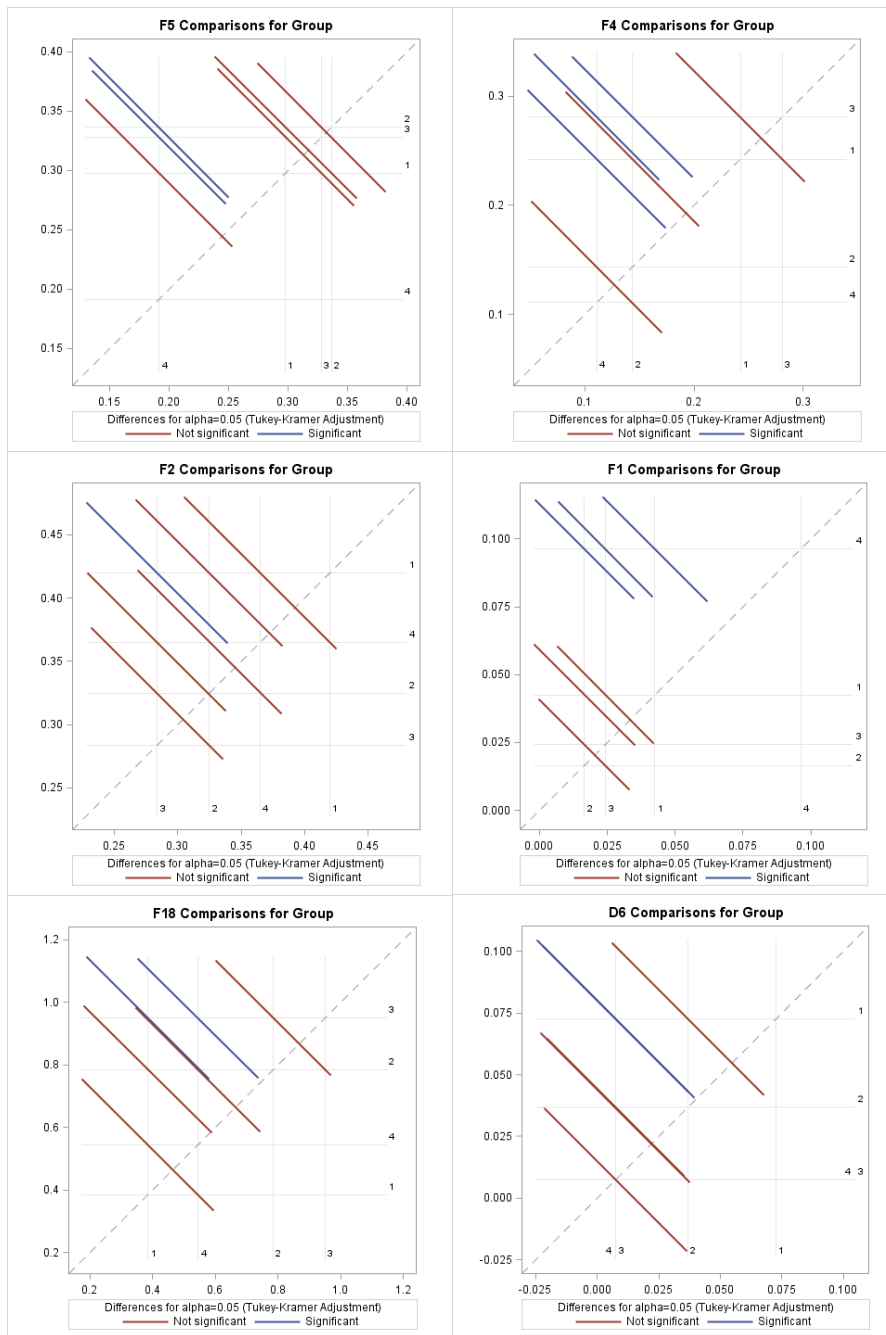


Figure 29. Continued.



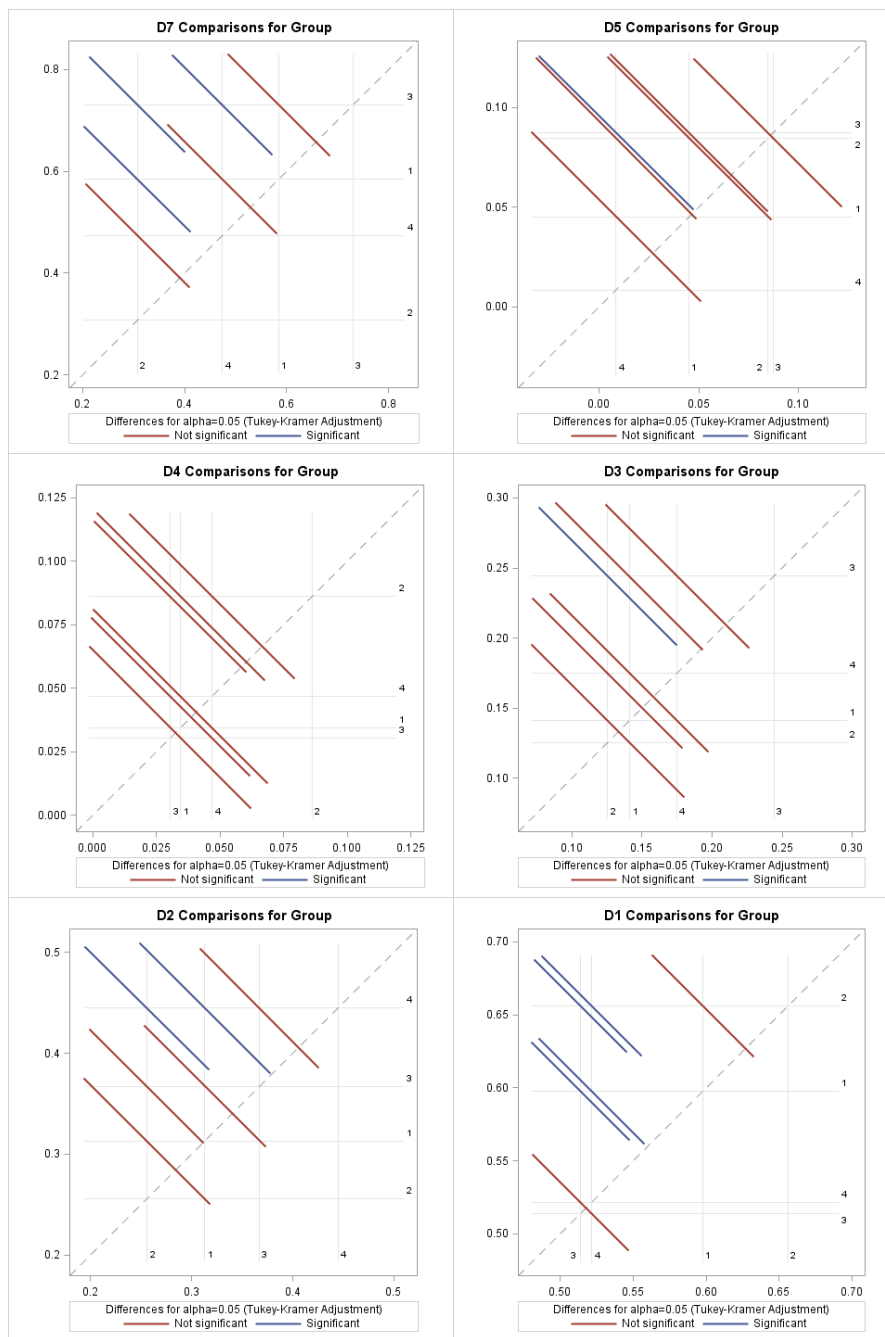


Figure 29. Continued.

The older, more recovered PA and PR sites had a higher abundance of perennials, biennials, subshrubs and species from the family FAB (Figure 29). The PA sites were also higher in CYP and ZYG Families and had a lower abundance of biennial perennials than other groups. The PR2 sites were similar to PA sites in that they both had a high abundance of shrubs. PR1 and PR2 sites both had a high abundance of forb/ herb/ subshrubs and species belonging to the LIN family. The PR2 sites had many annual biennials (defined on the USDA Plants database as a species found as either an annual or biennial), biennial perennials, annuals and species from EUP, CAP and PLG Families (Figure 29). The newest sites were the PR3 sites, which contained significantly fewer C<sub>3</sub> species than any other site and had higher abundances of annuals, forb/herb/vines and species belonging to families LIN, ONA, AST and CNV (Figure 29).

Frequencies can also be revealing, as PR2 sites had the highest frequency of biennial perennial forbs a trend also shown by Nasen et al. (2011). The frequency of POA was ~11% for PA and PR2 sites and only ~6% for PR1 and PR3. The PA 100 sites were predominately composed of graminoids and did not contain vines. The PR3 group did not contain any shrubs or shrub/subshrubs and contained the highest frequency of vines. The highest frequency of photosynthetic pathway on all sites was C<sub>3</sub>, followed by C<sub>4</sub> and then CAM photosynthetic pathway (see Appendix D for frequency Figures).

Before deleting the outliers, a Multivariate Analysis of Variance (MANOVA) test determined effects on species richness (S), evenness (E), Shannon diversity (H) and Simpson's diversity (D) were significant for Group (Wilks' Lambda = 0.58,  $F(12, 437) = 8.2$ ,  $p < 0.0001$ ), Distance (Wilks' Lambda = 0.88,  $F(8, 330) = 2.7$ ,  $p = 0.0065$ ) and Group\*Distance interaction (Wilks' Lambda = 0.68,  $F(24, 576) = 2.73$ ,  $p < 0.0001$ ).

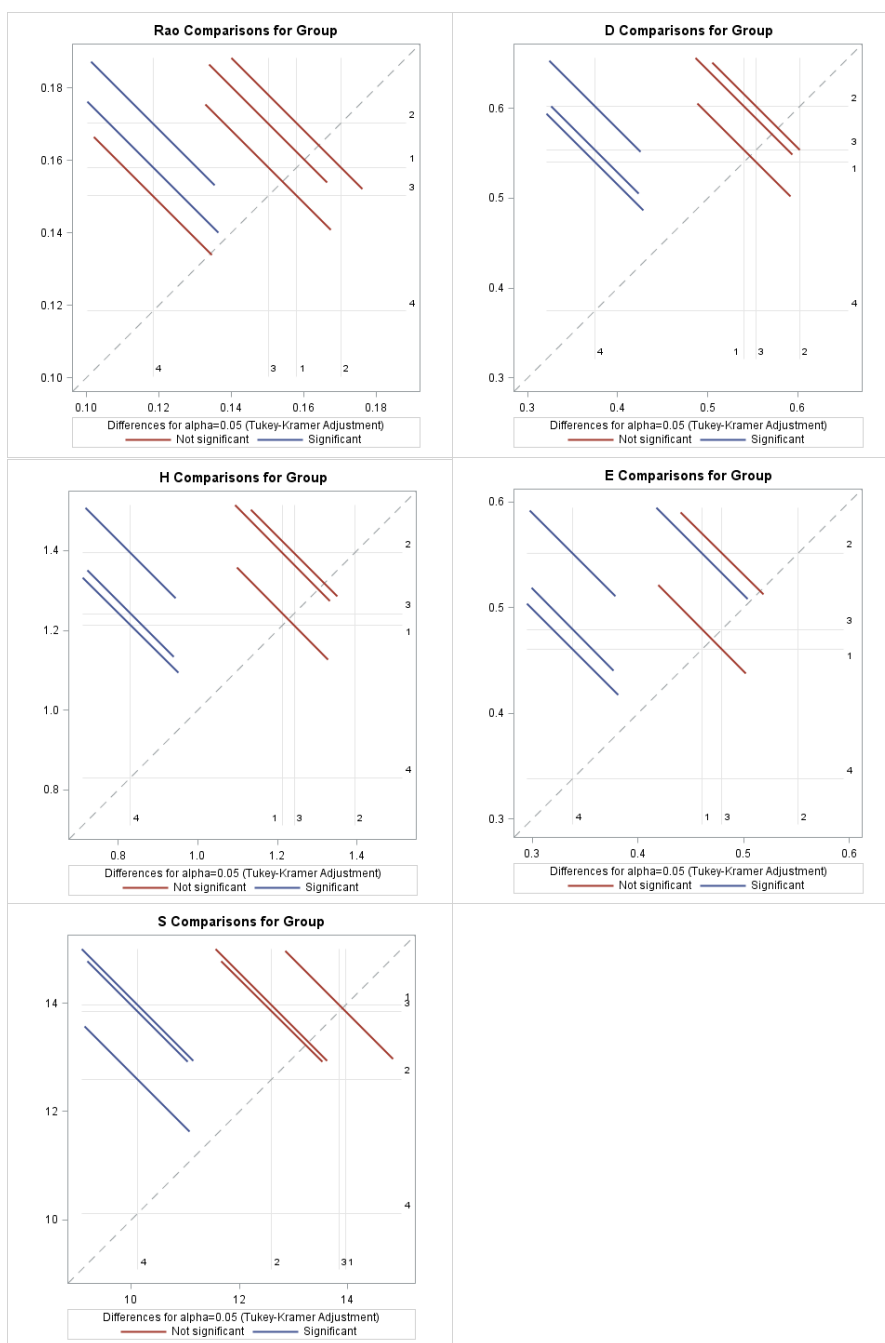
Univariate ANOVAs, with Tukey-Kramer Least Squares Means Adjustments for Multiple Comparisons, determined significant Group, Distance and Distance\*Group interaction effects on  $FD_Q$  FR and diversity indices (i.e., S, E, H and D; Table 19; Figures 30-32).

Table 19

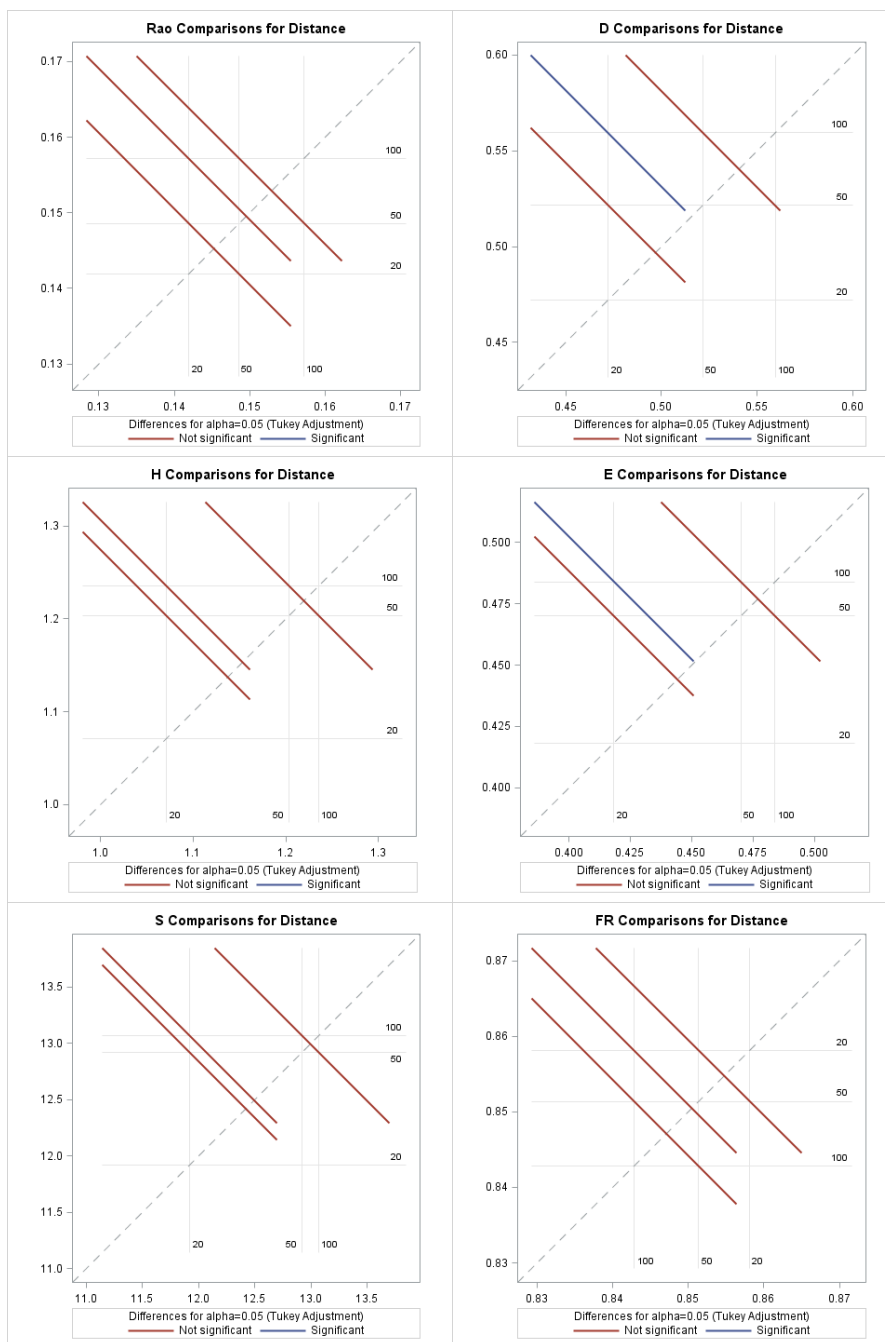
*Analysis of Variance (ANOVA) Results for Functional Diversity ( $FD_Q$ ), Functional Redundancy (FR), and Diversity Indices*

	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
FD <sub>Q</sub>	Model	11	0.16	0.01	3.68	0.0001 **
	Group	3	0.07	0.02	5.63	0.0011 **
	Distance	2	0.01	0.00	0.89	0.414
	Group*Dist	6	0.08	0.01	3.52	0.0026 **
S	Model	11	723.24	65.75	5.19	<.0001 ***
	Group	3	431.03	143.68	11.35	<.0001 ***
	Distance	2	45.85	22.93	1.81	0.1667
	Group*Dist	6	246.17	41.03	3.24	0.0049 **
E	Model	11	1.51	0.14	6.18	<.0001 ***
	Group	3	1.07	0.36	16.04	<.0001 ***
	Distance	2	0.14	0.07	3.17	0.0446
	Group*Dist	6	0.28	0.05	2.08	0.0576
H	Model	11	10.98	1.00	5.84	<.0001 ***
	Group	3	7.81	2.60	15.23	<.0001 ***
	Distance	2	0.90	0.45	2.63	0.0753
	Group*Dist	6	2.13	0.36	2.08	0.0583
D	Model	11	1.97	0.18	5.2	<.0001 ***
	Group	3	1.34	0.45	12.95	<.0001 ***
	Distance	2	0.23	0.11	3.27	0.0403 *
	Group*Dist	6	0.37	0.06	1.81	0.0995
FR	Model	11	7342	667	3.66	<.0001 ***
	Group	3	2484	828	5.42	<.0014 **
	Distance	2	1293	646	4.78	0.0096**
	Group*Dist	6	3462	577	3.9	0.0012**

Notes: \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$



*Figure 30.* Significant effects of production group on diversity indices. Diversity indices include richness (S), evenness (E), Shannon's diversity (H), Simpson's diversity (D), Functional diversity (Rao), and Functional redundancy (FR). Each plot shows LS-means adjusted pairwise differences between groups, their significance levels and their individual confidence limits. The LS-means of each pair meet at their intercept (center of line). Blue line indicates means are significantly different between groups, red line indicates groups have similar means. Axes indicate mean diversity value. Group 1 = PR1, 2 = PA, 3 = PR2, 4 = PR3.



*Figure 31.* Significant effects of distance (from the wellhead) on diversity indices. Diversity indices include richness (S), evenness (E), Shannon's diversity (H), Simpson's diversity (D), Functional diversity (Rao), and Functional redundancy (FR). Each plot shows LS-means adjusted pairwise differences between groups, their significance levels and their individual confidence limits. The LS-means of each pair meet at their intercept (center of line). Blue line indicates means are significantly different between distances, red line indicates groups have similar means. Axes indicated mean diversity value. Distance 1 = 20 m, 2 = 50 m, 3 = 100 m.

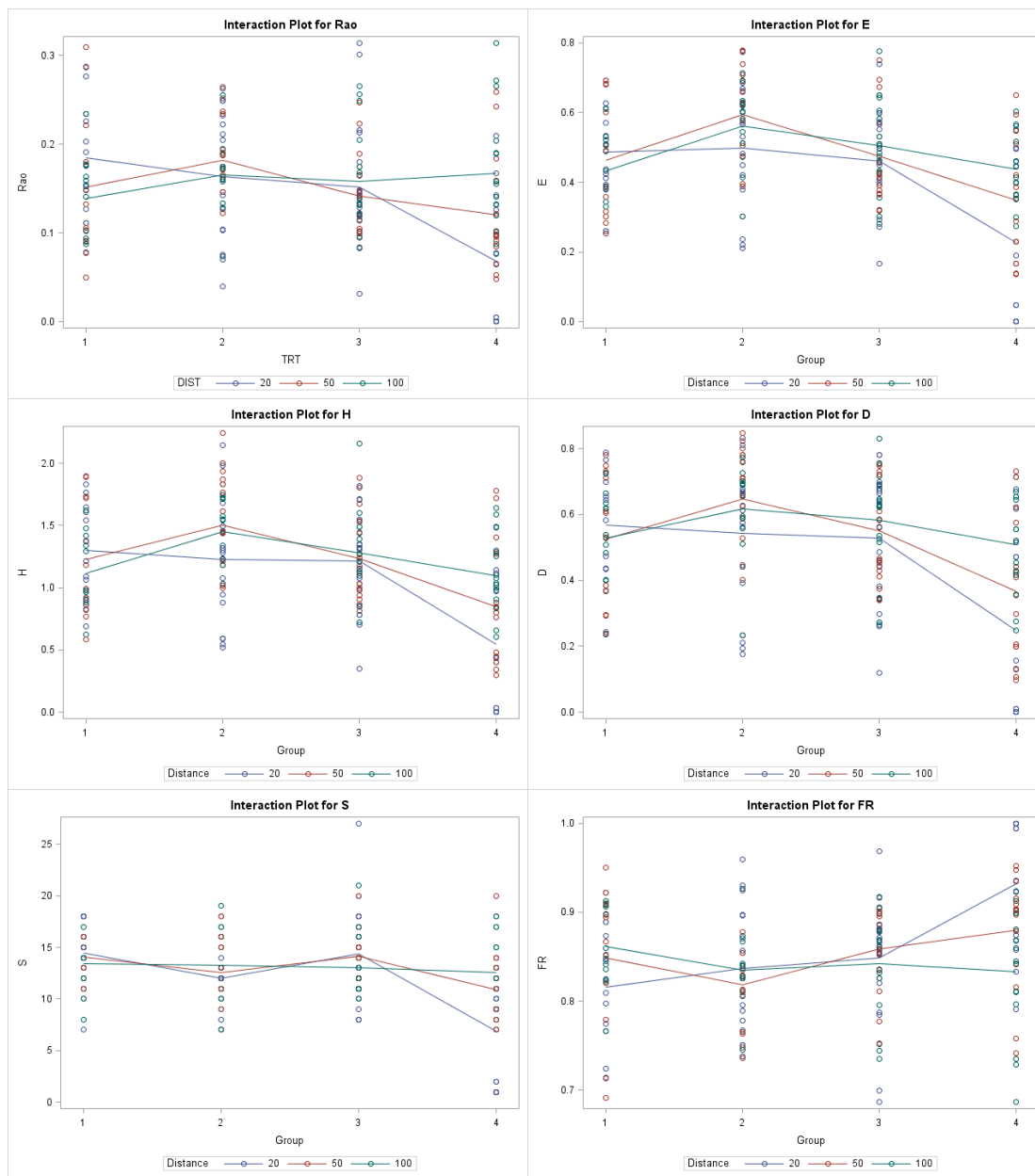
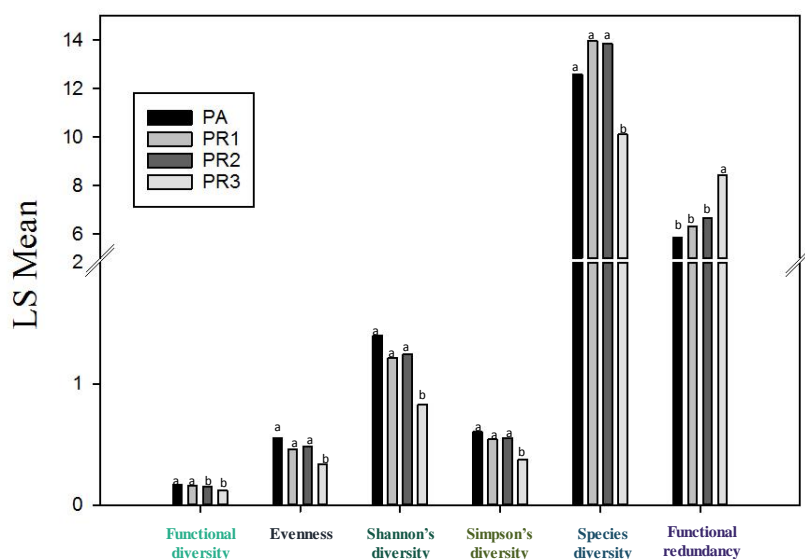


Figure 32. Interaction plots of production group\*distance for diversity indices. Diversity indices include richness (S), evenness (E), Shannon's diversity (H), Simpson's diversity (D), Functional diversity (Rao), and Functional redundancy (FR). Blue = 20 m, red = 50 m, and green = 100 m. Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3). In the figure, group 1 = PR1, 2 = PA, 3 = PR2, and 4 = PR3.

In general, PR3 sites had the lowest scores for all diversity indices, while PA sites had the highest scores for all indices (Figure 30). Across distances, only D and E had significant differences, with higher scores at 100 m compared to 20 m for both indices (Figure 30). When we examined the interaction plots between distance and production group, there was a clear trend. Group 4 (PR3) sites were always lower than other sites and always in a very specific order, with 100 m the highest and 20 m the lowest for all diversity and functional indices except functional redundancy (Figure 31). Richness was highest on PR1 and PR2 sites, but was not significantly higher than PA sites (Figure 33). Mean species richness (S) was 12.6 species per plot, mean evenness (E) was 0.46, mean Shannon diversity (H) was 1.17 and Simpson diversity (D) was 0.52 for O&NG sites including PA, PR1, PR2 and PR3 (Figure 33). Functional Redundancy (FR) was measured as the inverse of functional diversity and was highest on PR3 sites (Figure 33).

To determine if these scores were comparable to typical, PNG diversity indices, scores were compared to data (Franklin, unpublished) collected on 43 control sites with similar soil and vegetation structure, but without O&NG development. On these sites mean diversity values were  $S = 24$ ,  $E = 0.53$ ,  $H = 1.64$  and  $D = 0.69$ . The scores on control sites were slightly higher than the mean scores for O&NG sites (PA, PR1, PR2, and PR3), although the plot sizes were slightly larger (0.1 ha). When we compared these scores to the fully reclaimed PA sites with mean scores of  $S = 14$ ,  $E = 0.46$ ,  $H = 1.21$ ,  $D = 0.54$ , we discovered PA sites were only slightly lower in diversity indices. The percent mean bare ground for the 43 control plots was 19.2% or 80.8% cover, which is quite high for the PNG. Following the 80% cover guideline for restoration, the O&NG sites would need 64.4% cover or less than 35.6% bare ground to satisfy requirements for successful

restoration. Our mean percent bare ground on PA sites was only 5.52 %, meeting the requirement for restoration. These levels of bare ground are exceptionally low and could be due to higher than normal levels of precipitation in 2015 (637.3 mm) and in 2016 (431 mm) when data was collected (Colorado Climate Center 2017). High levels of precipitation during the spring and summer months could have temporarily increased percent cover on sites.



*Figure 33.* Least square means of diversity and functionality indices across production groups. Indices include Richness (S), evenness (E), Shannon's diversity (H), Simpson's diversity (D), Functional Diversity (FD), Functional Redundancy (FR). Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3)

## Discussion

It was hypothesized that vegetation composition would differ significantly with distance from the wellhead and time since well completion indicated by production group. Our data support both of these predictions. In general, PA 100 m sites were distinctly different from PR sites, including PR1 sites, which were partially (interim)



reclaimed more than 30 years ago. This evidence supports species and functional shifts in vegetation over long periods on O&NG production sites. We hypothesized that reclamation would be successful by the BLM standards and found support for this hypothesis. We also found indices of species diversity and functionality to be higher on PA sites than PR sites. Nasen et al. (2011) also found that desirable species diversity and richness declined with high producing sites, and the opposite was true for PA and reference sites.

Site quality was determined using three of nine restoration parameters recommended by the Society for Ecological Restoration, including species diversity, presence of indigenous species and presence of functional groups for long-term stability. As expected, at 20 m and 50 m, sites had substantially more bare ground and introduced species than at 100 m. PR3 sites had the highest percentage of bare ground, which was still on average as high as 10%, 100 m from the wellhead, while other sites had only 2-5% bare ground. Two decades ago, less than 50% of the ground on the shortgrass steppe was covered by vegetation (Hazlet 1998); however, on our study PA sites vegetation cover was substantially higher than this estimate. High vegetation cover on sites in 2015 and 2016 could be due to drought release. Only time will tell if this is a temporary or long-term trend caused by variation in temperature and precipitation.

Total, there were 16.5% non-native individuals on all plots combined and thus we expected to find a significant presence of invasive species in these highly disturbed areas as well. Only 2% of species sampled were invasive and factors Distance and O&NG Group did not effect on the presence of invasive species. It was surprising to find invasive species on PA sites, 100 m from wells that had been “recovered” for over 30

years. This might speak to reclamation methods used in the 1980s, before there were recommendations for reseeding. It is likely that the non-native grass AGRCRI was seeded to prevent erosion, although we cannot be certain.

Reclamation cannot be examined on PR2 and PR3 sites 25 m from the wellhead, as vegetation is often removed and sprayed with herbicide to prevent fires and to allow for access to sites. Even so, resistant and resilient species survive and reproduce between disturbances, allowing for vegetation monitoring at these distances. Many of these sites have also been released from grazing due to fences and are potentially receiving excess nutrients from O&NG production (Asia et al. 2007). Satisfactory reclamation was achieved at 50 m on PR1 and PR2 sites as vegetation was at 80% total cover when compared to 100 m. The PR3 sites did not meet this requirement, but 50 m, depending on the specific site, could be considered an interim reclamation (still in progress) or part of the wellpad. As to whether the vegetation is “desirable” or not is arguable. Desirable is a vague description and depending on the forest service ranger giving approval, could be interpreted differently. If we examine the range of diversity indices across groups, we can distinguish that the PA sites were the highest in diversity across all indices and that PR3 were the lowest. Thus, it seems recovery over time is possible.

In the last decade, there has been a push to move away from managing landscapes and ecosystems from a species diversity perspective and to focus research, conservation and management projects on functional diversity. Instead of focusing on morphological traits within taxa, we can more accurately predict community stability by focusing on shared functional traits across taxa and biogeochemical cycles (nutrient flow, carbon, water and energy). Plant functional types (PFTs) have been shown as the necessary link

between biodiversity and biogeochemical cycles, the two drivers of ecosystem productivity and services (Chapin et al. 2000; Wardle et al. 2004; Díaz et al. 2007; Cadotte et al. 2009; 2011; Lavorel 2013). To date, multiple studies have used functional traits to show the response of plants and animals to environmental factors such as climate change, and the impacts on ecosystem functions, community composition and ecosystem services (e.g., McKenzie et al. 2007; Douma et al. 2012; Pompe et al. 2014). Due to restrictions on time and funding, we were not able to collect vegetation structure (e.g., height, leaf area index) data on individuals, although we were able to use other characteristics to determine species functionality.

In the current study, when we compared O&NG sites across time and space, they were not composed of functionally similar species. For example, indicator species for PA sites exhibited a variety of growth forms, including a non-native, C<sub>3</sub> grass (AGRCRI), two native C<sub>3</sub> grasses (HESCOM; PASSMI), two leguminous forbs (PSOTEN and MELOFF) and two native C<sub>4</sub> grasses (BOUDAC and SCHPAN). These sites have been reclaimed for over 35 years and have very different vegetation from the PR3 sites. For the PR3 sites, the indicator species include one leguminous forb (ASTMOL), introduced C<sub>4</sub> grass (DIGSAN), two native annual forbs (AMAPOW and XANSTR) and three introduced C<sub>4</sub> annual forbs (AMASPI, BASSCO and SALTRA). One potential concern is that some of these unpalatable forbs with deep roots, such as XANSTR (cockle bur), might become established and spread if O&NG companies are not diligent in their mechanical and chemical control (Stohlgren et al. 2002).

Some have shown that the shortgrass steppe is a low diversity system, with a few dominate graminoids guiding and maintaining community structure (Adler and Lauenroth

2000). It has been shown by others that grasslands have high diversity of species and high functional redundancy, creating community resilience after frequent perturbations such as cattle grazing (Pillar et al. 2013). It has been suggested that functional groups with little or no redundancy warrant priority for reclamation and conservation efforts (Walker 1992). Functional redundancy in assemblages ensures ecosystem processes with the replacement of similar species, preventing shifts in dominant system functionality. We did not find high functional redundancy on our O&NG sites; instead, we found high species diversity and high functional diversity on PA sites. This begs the question, is the system as resistant to change as we think it is? Can a C<sub>4</sub> dominated system under resource and grazing release caused by O&NG development withstand C<sub>3</sub> competitors?

Others have shown that population stability is positively correlated with diversity, if stability is a precursor and not a response to diversity (Dovčiak and Halpern 2010). If high functional redundancy in grasslands enhances community stability that would imply our system is not as stable, yet this system has withstood decades of drought, grazing, fires and is still resilient. Vertical O&NG production has been occurring on the grasslands since the 1950s. With new technological advances in slick horizontal fracturing, we are seeing an unprecedented increase in frequency and magnitude of O&NG production, causing a novel impact on native flora and fauna (Bjorge 1987; Ingelfinger and Anderson 2004; Holloran 2005; Walker et al. 2007). Development of O&NG has a resulting footprint (well pads, infrastructure, tanks, roads) equating to a measurable loss of habitat (Watkins et al. 2007).

After O&NG site reclamation, vegetation will assumedly return to a similar state as before the disturbance and will function in a similar manner. It is interesting to note

that PA sites did not show high functional redundancy (Figure 33). This indicates that when a dominant C<sub>4</sub> grass, such as BOUGRA, is removed from an area due to O&NG development and production, there might not be a comparable C<sub>4</sub> grass nearby to fill its functional role in the environment. We often see this replacement on O&NG sites with BOUDAC in the short term, but it appears C<sub>4</sub> grasses could be replaced with C<sub>3</sub> grasses, such as AGRCRI in the long term as seen on PA sites, especially if fences and roads deter grazers and O&NG companies are reseeded with C<sub>3</sub> species. Porensky et al. (2017) found that over the past 72 years, C<sub>3</sub> perennial graminoids on the shortgrass steppe have increased more than five-fold in the absence and presence of cattle grazing (enclosures). They attribute this shift from C<sub>4</sub> to C<sub>3</sub> graminoids to drought recovery since the dust bowl and elevated atmospheric CO<sub>2</sub> concentrations. The long term changes in resources and climate, such as annual and seasonal precipitation levels (Lauenroth and Sala 1992; Köchy and Wilson 2004, Concilio et al. 2015), will likely play a role in future vegetation communities (Fay et al. 2011; Yang et al. 2011; McCluney et al. 2012). Invasive species, in particular, may have traits that will allow them to benefit from climate change (Dukes and Mooney 1999, Hellmann et al. 2008, Bradley 2009). Gardner (1950) showed that a moderately grazed desert grassland in New Mexico was not recovered after thirty years of protection from grazing. Depending on the intensity and frequency of disturbances, vegetation biomass may drastically shift from season to season, which is why careful, long-term and sustainable management strategies must be followed (Gardner 1991). Long-term experiments in natural field settings are required to understand these complex systems and to develop management and restoration strategies (Suttle et al. 2007).

The synergistic effects of nutrient deficiency, VOC loading, changes in magnitude and frequency of precipitation events, rising CO<sub>2</sub> levels, habitat loss and fragmentation due to O&NG production, could affect community balance on the shortgrass steppe leading to a slow shift in the relative abundance of species and overall functionality of the system (Sala 2001). Roads and structures associated with O&NG development have created a fractured landscape across the shortgrass steppe at an unprecedented frequency and magnitude. A plugged and abandoned O&NG site (PA) is arguably more disturbed than an old farming field with barren ground. It typically takes an old farm 20-40 years to recover to a community of shortgrass prairie, but this depends on factors such as length of cultivation, cropping history, intensity of cropping, soil type, degree and kind of erosion, method and degree of grazing, distance from seed source, amount of soil deposition and climatic cycle (Judd 1974). How then, can we assess the long-term impacts of O&NG with only minimal, short-term monitoring? Successful reclamation can only come when time and money are invested in careful, long-term sampling to uncover and map plant replacement patterns (Cooke and Johnson 2002).

On patches of land disturbed by O&NG, C<sub>3</sub> grasses and introduced forb abundances remain intact for longer periods due to fences. These communities with greater spatial heterogeneity than BOUGRA-dominated sites could persist with the abandonment of sites (Adler and Lauenroth 2000). We have seen this trend in the current study with AGRCRI on PA sites. Although the Forest Service has a No Surface Occupancy lease stipulation for new O&NG sites, preventing land surface disturbances, the PNG is composed of private and public lands (USDA 2014a). The Forest Service has little to no control over adjacent private property owner's land use (e.g., habitat loss,

fragmentation, percent bare ground, percent invasive) within the PNG. These novel surface disturbances are allowing introduced species to expand into new areas (Bradford and Lauenroth 2006), change fire regimes, outcompete native vegetation and cattle forage, and influence reclamation cost and success (Pilkington and Redente 2006). The inability to assess the cumulative impacts of O&NG occurring on private property is a major obstacle to understanding the impact of O&NG on the entire ecosystem (Naugle 2011).

To understand cumulative impact we must communicate with private landowners the importance of disturbance research and expand data collection to include all parcels within the PNG. We suggest using the current study as a baseline for data collection. Continued, detailed field experimentation is required to identify small-scale plant-plant replacement as well as larger scale community shifts to discover the mechanisms and tolerances producing slow shifts.

## CHAPTER VI

### SUMMARY OF RESULTS AND CONCLUSION

In 2013, there was little information on the impacts of O&NG production to waters, soils, air, animals or vegetative communities. Shortgrass steppe habitat quality, functional biodiversity and ecosystem services were potentially reduced or changed by O&NG construction, production and related infrastructure on the PNG. We were interested in examining O&NG production specifically, by examining proximate air and vegetation. We were able to quantify O&NG fugitive emissions in the air (VOCs) and deposition onto proximate vegetation (VOCs and minerals). We were also able to estimate reclamation success post-production and determine changes in vegetation structure. Each project bringing us a step closer to understanding the potential impacts to the region.

Volatile organic compounds have very complicated mixing chemistries and are difficult to estimate and track throughout the day. The current research was not able to show the significant effects of distance and direction on VOC concentrations, although Pumping sites consistently increased in VOC concentration throughout the day. The only VOC that seemed to have a decreasing trend was nitrous oxide. Most VOCs we identified had concentrations significantly lower in the Mountain group (sites located in Roosevelt National Forest) such as the alkenes, alkanes, sulfides and oxygenated species. The mountain group was consistently lower than other groups across the board, including control sites on the PNG. This indicates that air quality is reduced on the PNG due to



O&NG production and high VOC background concentrations are playing a regional role in air quality.

Greenhouse gases were also a major concern in the current study. Methane, an alkane and a GHG, reached 5.7 ppm and carbon monoxide reached 7.1 ppm on Pumping sites, indicating leaks. Overall, our Nojack group (dry production) had higher average concentrations of VOCs than other groups, including significantly higher levels for benzene, ethylbenzene and acrolein. The VOC with the highest relative abundance was carbon disulfide, which was found mostly on Pumping and Nojack sites, and can form highly toxic hydrogen disulfide and GHG Carbon Dioxide (Rich and Patel 2015). We created a model to predict concentrations of benzene and exceedances of TWA PELs. All VOCs with available inhalation reference concentrations (RfCs) had exceedances in their mean concentrations, but not for every treatment group (e.g., carbon disulfide was only > 0 in the Pumping group). There were two VOCs, acrolein and benzene, which exceeded their TWA PELs. In total, there were 23 individual observations out of 900 with exceedances of benzene. This is a health hazard considering benzene is a well know carcinogenic compound causing leukemia (IRIS 2002). The RfC for benzene is 0.03 ppb. In our study, we found that the mean concentration of benzene was ~0.09 ppb for all sites combined, which is three times the reference concentration. Concentrations of benzene were as high as 1.31 ppm (Nojack) and 1.26 ppm (Pumping). Although the majority of VOCs do not exceed PELs, concentrations are still of potential concern if they deposit onto surrounding media (e.g., water, soil, vegetation). All compounds found at these sites have the potential to deposit onto soil, water and in some cases, accumulate on the waxy

cuticles or in the tissues of plants. This environment presents a complex mixture of VOCs with multiple pathways of exposure.

Two extraneous variables we first considered as sources of VOCs were agriculture and vehicle exhaust. The suite of VOCs produced from agricultural activities were deemed too different from those released during O&NG production, with the exception of methane (Parker et al. 2007; Trabue et al. 2011; Hales et al. 2012). As for vehicle exhaust, there were 20 sites with a ratio of *i*-pentane to *n*-pentane, at or below one, suggesting O&NG source as opposed to traffic, and these sites had concentrations of VOCs at biologically relevant levels. The data not only confirm that O&NG emissions are impacting the region, but also that this influence is present at all sites, including controls. These data clearly indicate that governmental agencies should require increased monitoring on O&NG sites. Even the newest sites with the most expensive and innovative technology (PR3 sites) have shown fugitive emissions. Our recommendations are to monitor sites in the Spring and Winter when concentrations of VOCs are potentially at their greatest and to use instantaneous monitoring such as an FTIR gas analyzer or IR survey camera. Long term monitoring would also be beneficial although it is more costly. O&NG companies could work with existing monitoring stations, such as those run by the National Oceanic and Atmospheric Administration (NOAA), to keep track of monthly and yearly VOC concentrations. Lastly, monitored species should extend beyond NAAQS six principal pollutants (carbon monoxide, lead, nitrogen dioxide, ozone, PM<sub>2.5</sub> and sulfur dioxide) and include biologically relevant species such as BTEX.

In this body of work, we not only studied BTEX in the air, but also their deposition onto flora. We found that concentrations of BTEX were higher on vegetation near newer wells and the monthly mean volume of oil a site produced strongly correlated with the concentration of benzene found on proximate flora. This indicates that high oil producing sites have higher fugitive emissions of benzene. Correspondingly, sites that were more active (i.e., actively pumping) had higher concentrations of benzene and xylenes. We also found a strong correlation between increasing natural gas concentrations and o-xylene concentrations on vegetation, indicating that high natural gas production sites are the source of emissions. The average concentration for benzene on all sites combined, including the PA sites, was 13.18 ppbv, which exceeds the RFC, RFD and MCL for benzene. Concentrations on proximate vegetation were as high as 176 ppbv. This is arguably a biologically relevant concentration based on previous human impact research (McKenzie et al. 2012; Colburn et al., 2014; Thompson et al. 2014; Bolden et al. 2015), cattle research (Bechtel et al., 2009; Waldner and Clark, 2009) and a combination of the two (Bamberger and Oswald 2012).

Unfortunately, there likely unknown substances depositing onto proximate flora. It is difficult to understand cumulative impacts in this system when there are so many gaps in our knowledge. One disturbance that we did expect to find on the O&NG sites was increased mineral (heavy metal) deposition. Although there were not clear trends for all minerals across all sites, there were statistical differences among O&NG production groups as well as distances (25 m, 50 m and 100 m). What was most surprising were concentrations of Pb and Hg on all sites, including PA and PR1 sites that have undergone reclamation of soil (addition of top soil) and vegetation (reseeding) over 35 years ago.

We expected to find values above general dietary guideline concentrations for cattle and similar to those found in vegetation supplemented with sewage sludge, and we did for most micro minerals. Dietary guidelines indicate that vegetation was potentially deficient in Se and Zn essential nutrients. Potassium levels were also especially low on O&NG sites when compared to native sites and other studies.

There are two major issues with nutrient deficiency in the target species (blue grama and buffalo grass). First, mineral deficiency in general indicates that plants are stressed. Extra physiological stress on top of environmental stressors can inhibit an organism's ability to grow and reproduce. This alone can affect species dynamics and overall success on the shortgrass steppe. Add an additional heavy metal toxicity (e.g., Pb or Hg) to the nutrient deficiency and the effects are exacerbated. The other issue with nutrient deficiency is that these species supply grazers with a large portion of their dry mass daily intake. Ranchers often assume nutrients such as phosphorous are available in prairie vegetation and focus is placed exclusively on quantifying protein (CP) and Energy Fiber (NDF) content. Analysis of other common and palatable shortgrass steppe species is required to calculate an accurate daily intake of nutrients. For good performance and health of range cattle consuming these grass species, ration formulations should provide mineral supplementations, especially during summer months and during periods of drought (Ramírez et al 2004).

Many studies have shown that warm season grasses benefit from reduced nutrients, increased stress conditions and grazing disturbances (Coffin and Lauenroth 1988; Paschke et al. 2000; Cherwin et al. 2009). Long-lived C<sub>4</sub> grasses such as blue grama (*Bouteloua gracilis*) dominate under the characteristically dry conditions of the

shortgrass steppe by efficiently accessing available water. With new technological advances in slick horizontal fracturing, we are seeing an unprecedented increase in frequency and magnitude of O&NG production, causing a novel disturbance to native flora and fauna (Bjorge 1987; Ingelfinger and Anderson 2004; Holloran et al. 2005; Walker et al. 2007). Our research indicates that PA 100 m sites were distinctly different from PR sites, including PR1 sites, which had been reclaimed more than 30 years ago. This evidence supports species and functional shifts in vegetation over long periods of time on O&NG production sites. Although reclamation was successful by the BLM standards, we found indices of species diversity and functionality to be higher on PA sites than PR sites. Nasen et al. (2011) also found desirable species diversity and richness declined with high producing O&NG sites and increased on PA and reference sites. Functionally, species on the new, high producing sites were very dissimilar from our older sites, even within producing groups. For example, indicator species for PA sites included a non-native, C<sub>3</sub> grass (AGRCRI), two native C<sub>3</sub> grasses (HESCOM ;PASSMI), two leguminous forbs (PSOTEN and MELOFF) and two native C<sub>4</sub> grasses (BOUDAC and SCHPAN). These sites have been reclaimed for over 35 years and are composed of mainly graminoids and legumes. For our PR3 sites, the indicator species include one leguminous forb (ASTMOL), introduced C<sub>4</sub> grass (DIGSAN), two native annual forbs (AMAPOW and XANSTR) and three introduced C<sub>4</sub> annual forbs (AMASPI, BASSCO and SALTRA). There was also up to 95% bare ground on some of the PR3 plots. At one point in time, the PA sites likely had similar vegetation to the PR3 sites, but with time, it appears these sites can recover to a similarly functioning community of plants.

There is one noticeable difference between the reclaimed PA sites and the older, but not yet fully reclaimed PR1 sites. Both were high in all of the diversity indices including richness (S), evenness (E), Shannon's diversity (H), Simpson's diversity (D) and Functional Diversity (FDQ (FD)); however, the PA sites had a relative abundance score of 66 % for AGRCRI whilst the PR1 group had a score of 0 % for AGRCRI (see Appendix D). This non-native grass outcompeted the native species such as BOUGRA and BOUDAC, which were much more frequent, yet less abundant on sites (see Appendix D). It is likely that the PA sites were reseeded during reclamation with a C<sub>3</sub>/C<sub>4</sub> mix, as is often used today. Once vegetation had become established the fences were removed and roads were allowed to naturally revegetate. As in all complex systems, this species success is likely a combination of environmental variables (precipitation, temperature, soil, grazing), species traits (fast growing, drought resistant), as well as anthropogenic variables (O&NG roads and fences). The success of AGRCRI just goes to show the importance of reseeded techniques on O&NG sites. It is imperative companies use local, native seeds with C<sub>4</sub> species to re-establish vegetative communities.

As a scientist and advocate for transparency, it has been my duty the last four years to unearth the truths of O&NG production. It is my hope that the Forest Service can pursue energy development while protecting the Pawnee National Grassland shortgrass steppe, its flora and fauna from undue harm. It is my professional opinion that further development and production on the PNG risks permanently fracturing the ecosystem. A reduction of air pollution, including BTEX and greenhouse gas emissions are required to ensure the safety of wildlife, cattle, O&NG workers and outdoor enthusiasts. Further

investigation of soils, waters and habitat fragmentation are required to understand the full impact of O&NG production on the PNG.

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**APPENDIX A**  
**SUPPLEMENTAL MATERIALS FOR CHAPTER II**

Table 1.

*Technical Details of DX4040 FTIR Gas Analyzer*

Cell Temperature (controlled)	50 ° C
Flow rate	2.0 – 6.0 l/min
Water vapor (V/V%)	0 – 5%
Sample cell volume	400 mL
Operating time (Bluetooth ON)	2.5 h
Interface	External computer via RS-232C cable
Wavenumber range	900 – 4200 cm-1
Wavelength: Mid-IR region	12 μm – 2.5 μm

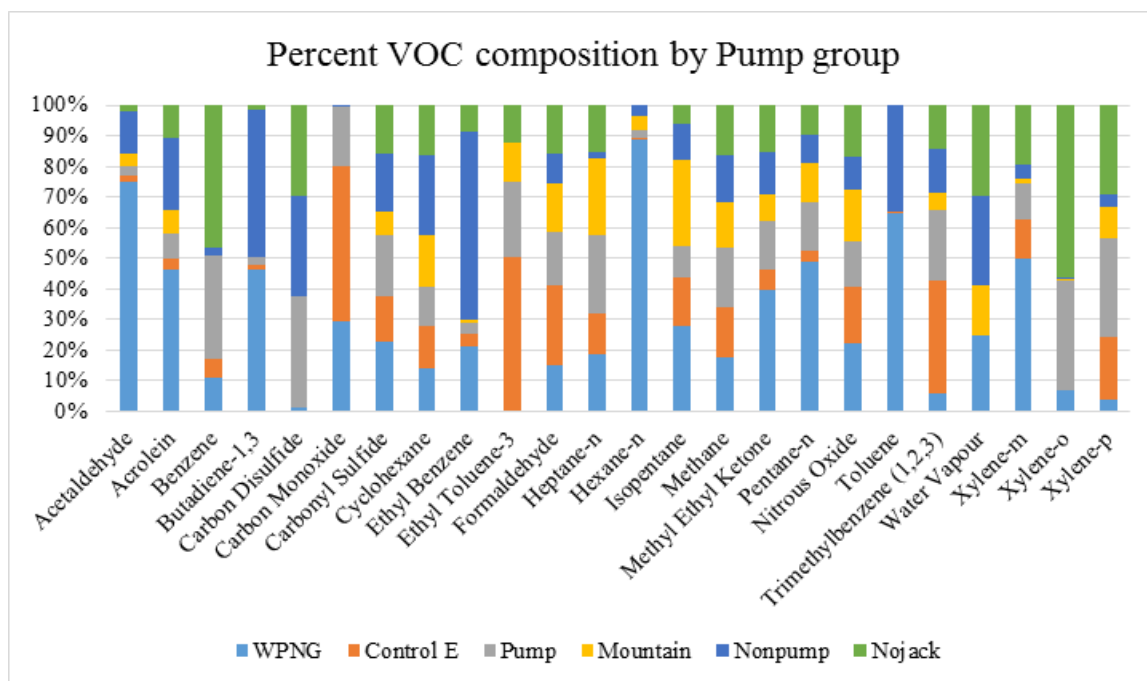
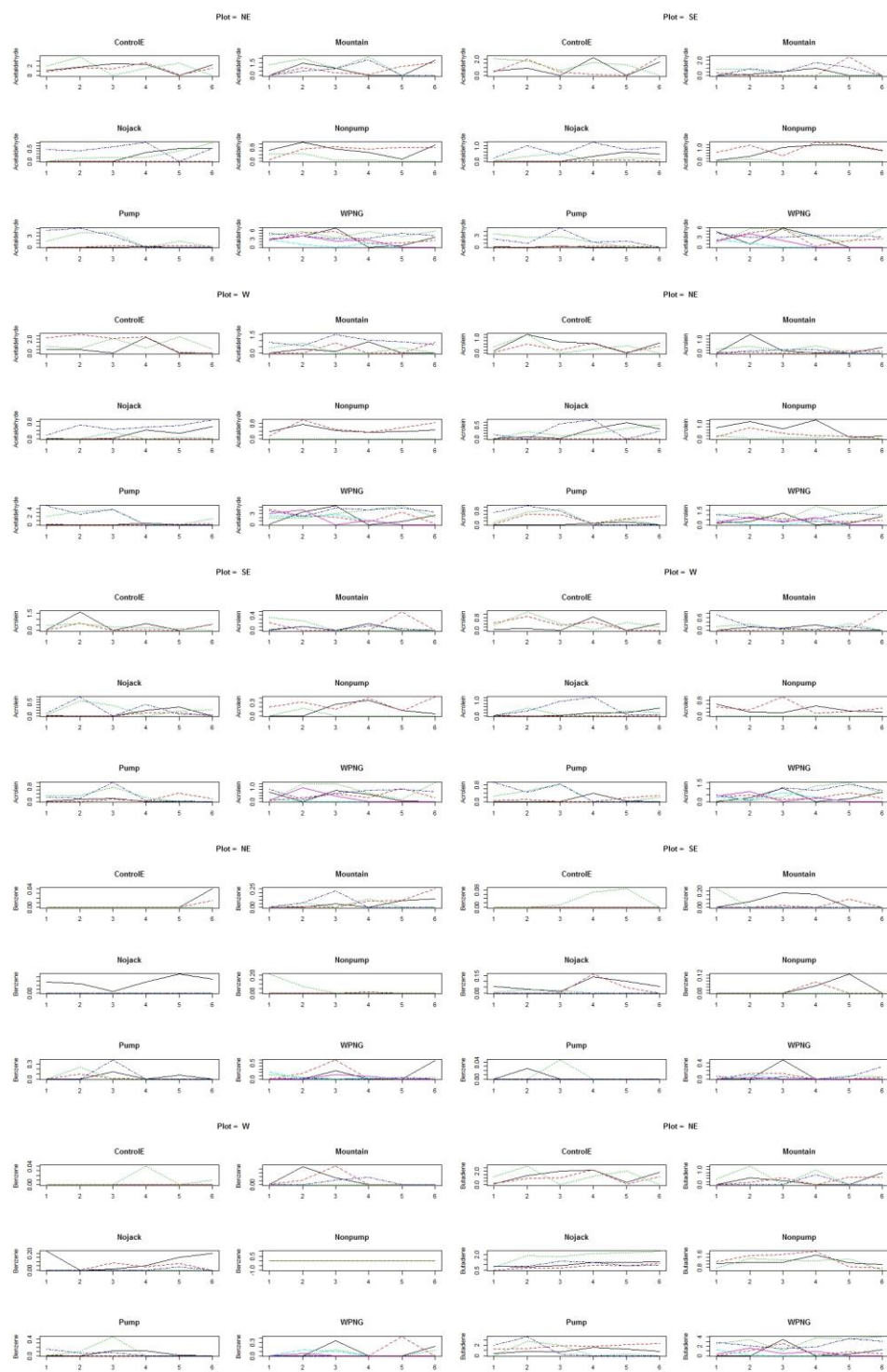


Figure 1. Percent relative abundance of individual Volatile Organic Compounds VOCs per pumping group.



*Figure 2.* Hourly (8:00 a.m. to 2:00 p.m.) Volatile Organic Compound (VOC) concentrations (ppbv) per Direction (NE, SE, and W) and production or control group (ControlE, Mountain, Nojack, Nonpump, Pump, WPNG).



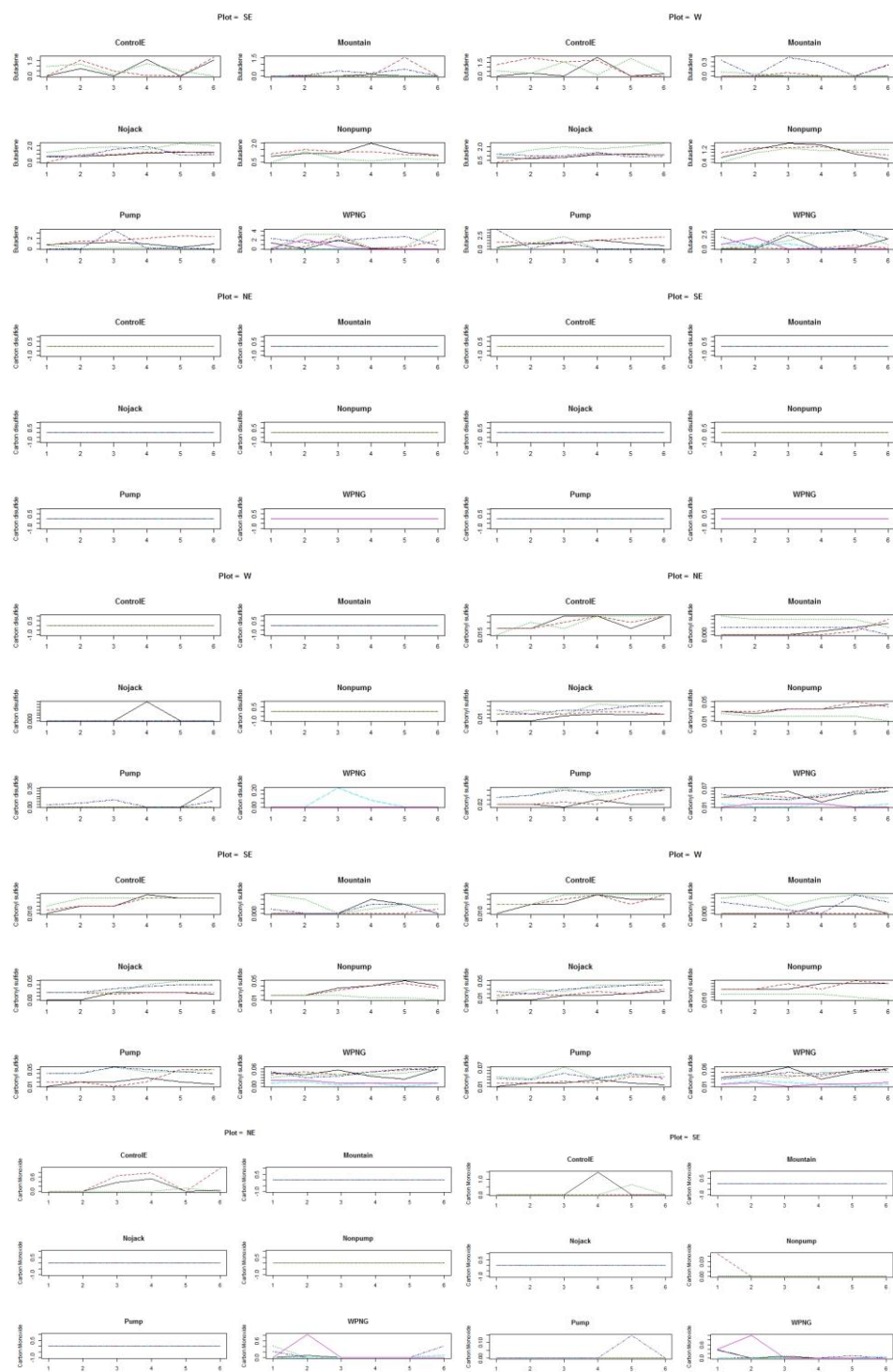


Figure 2. Continued.

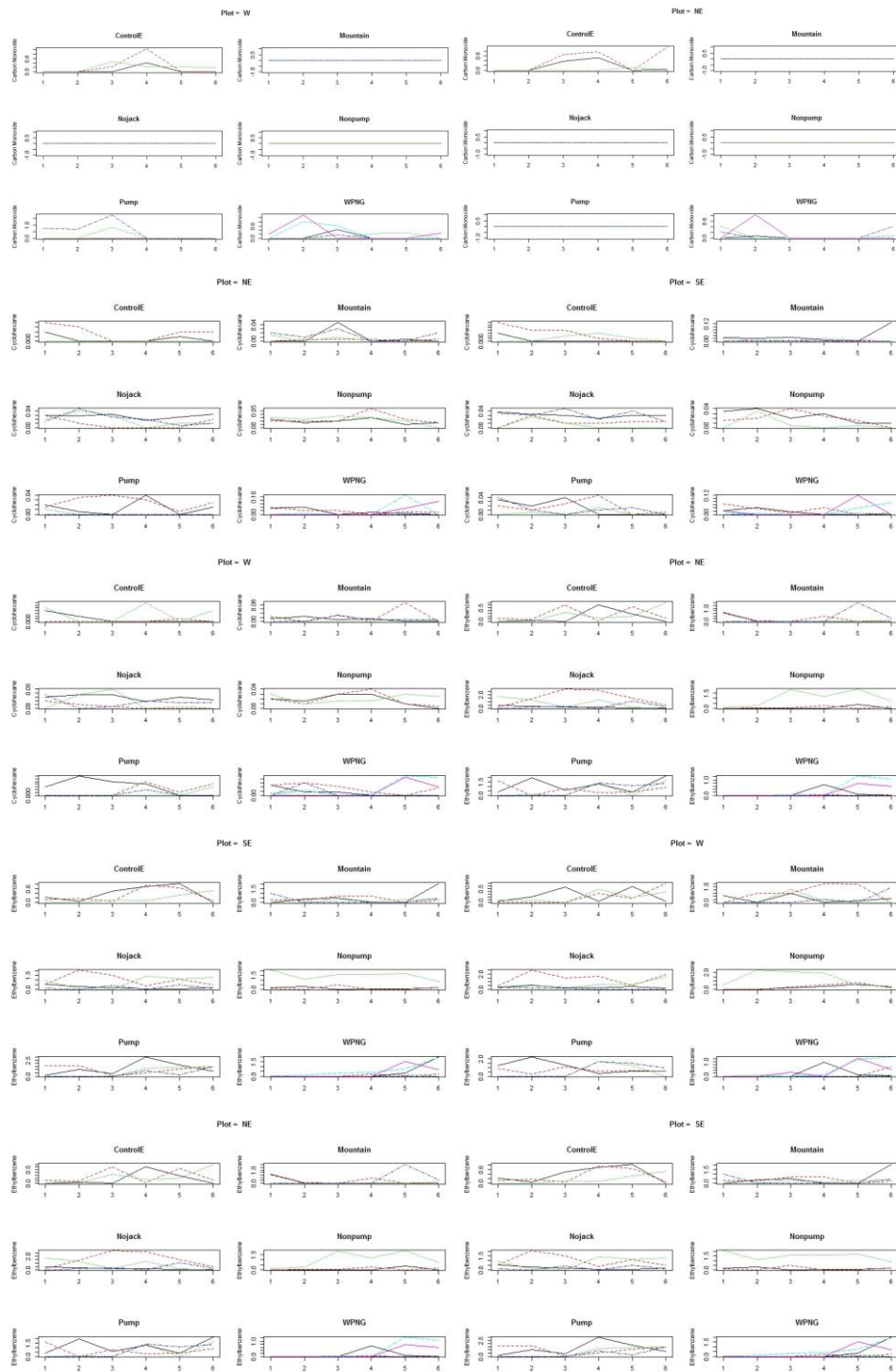


Figure 2. Continued.

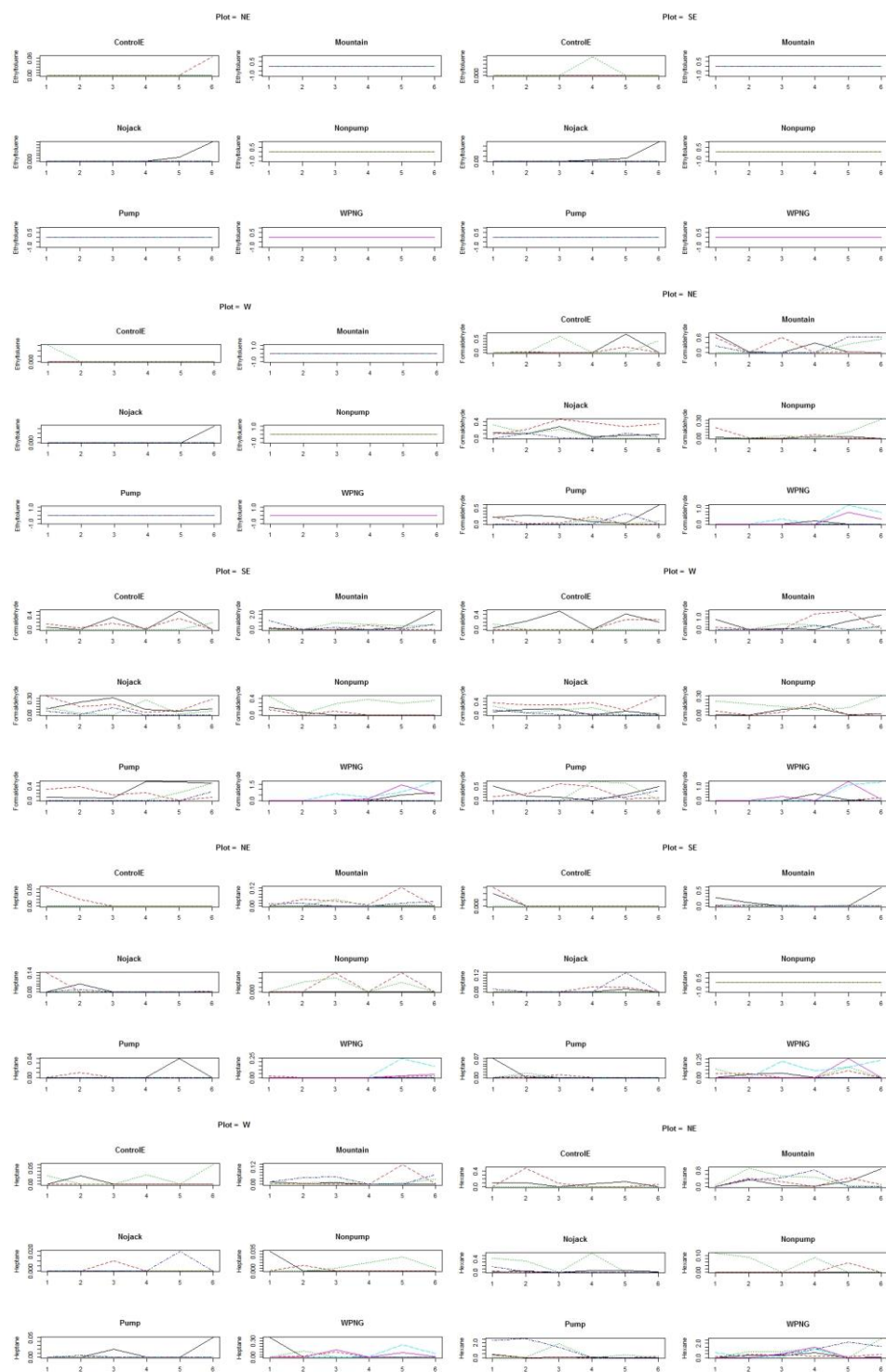


Figure 2. Continued.

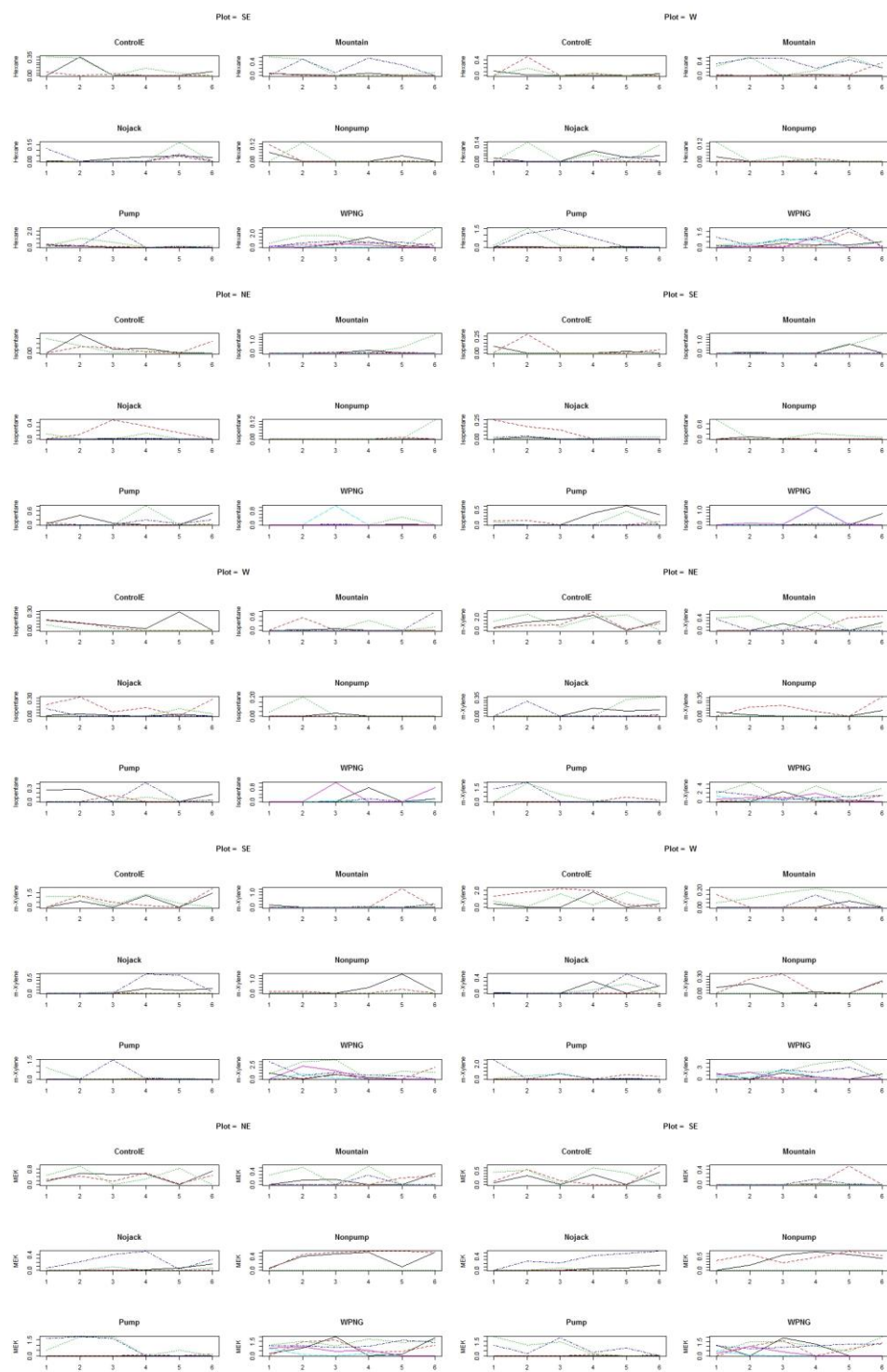


Figure 2. Continued.

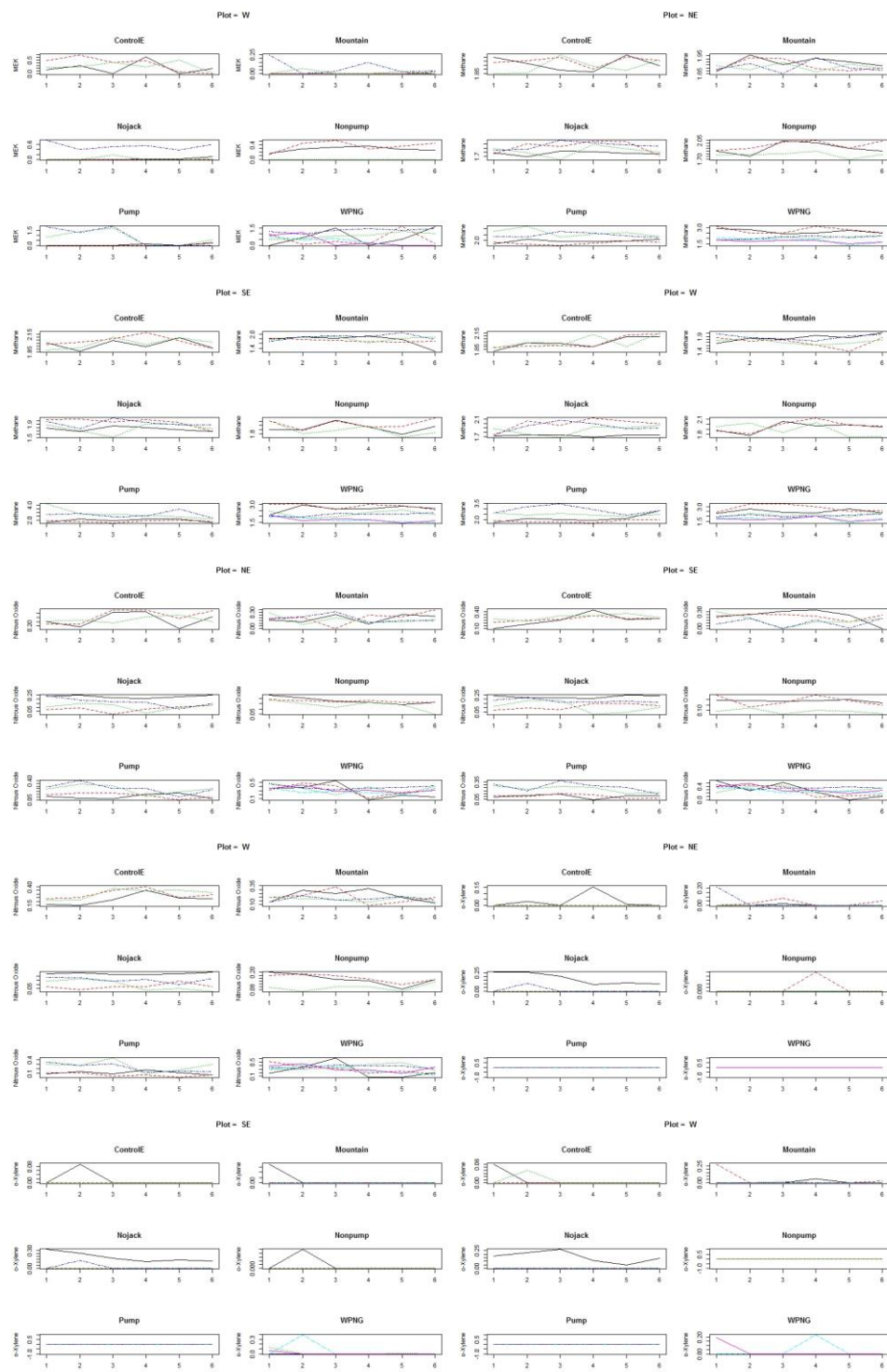


Figure 2. Continued.

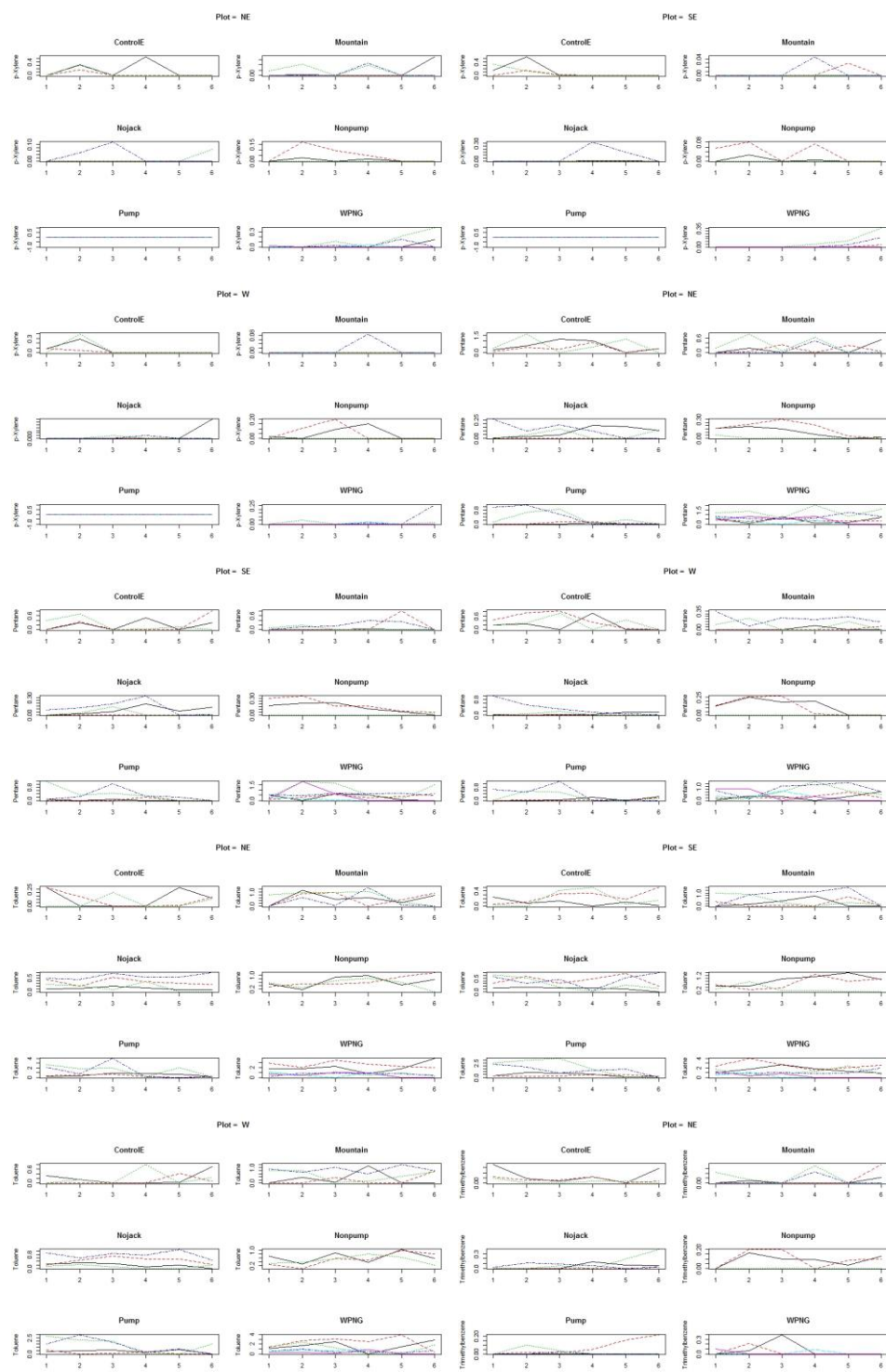


Figure 2. Continued.

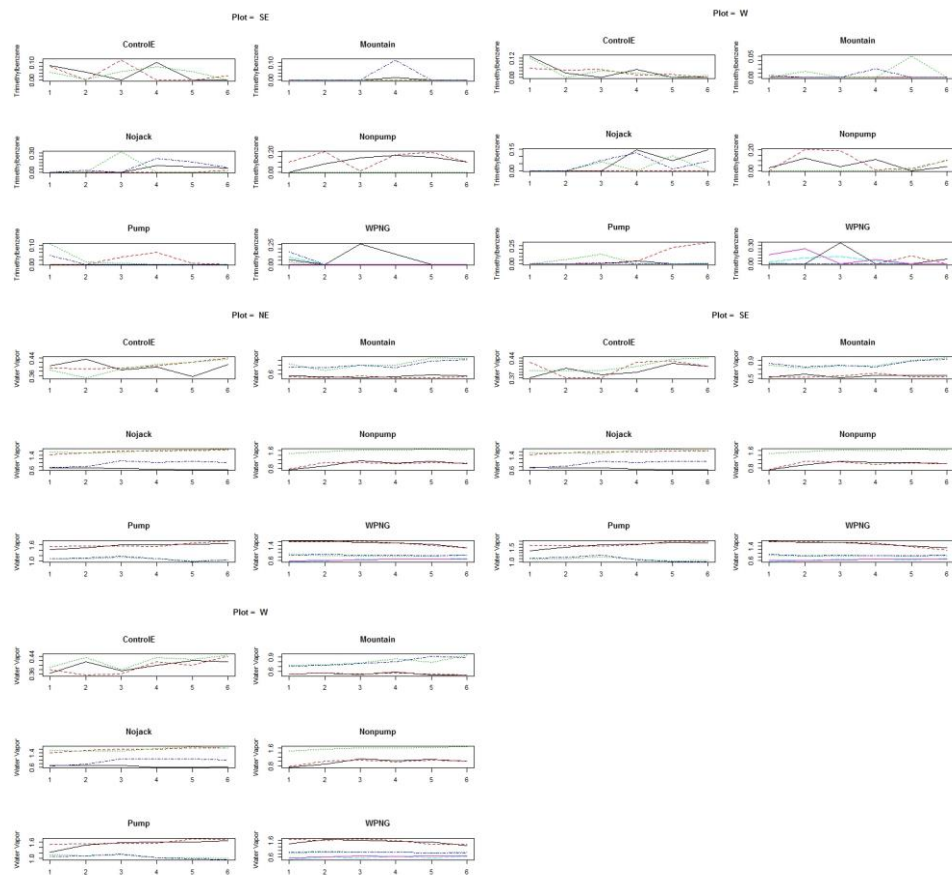


Figure 2. Continued.

Table 2

*Descriptive Statistics for All Sites Combined*

<i>Carbon Dioxide (ppm)</i>		<i>Methane(ppm)</i>		<i>Nitrous Oxide(ppm)</i>		<i>Carbon Monoxide(ppm)</i>	
Mean	360.78	Mean	2.10	Mean	0.23	Mean	0.06
Standard Error	0.63	Standard Error	0.01	Standard Error	0.00	Standard Error	0.01
Median	363.04	Median	1.99	Median	0.24	Median	0.00
Mode	374.73	Mode	1.89	Mode	0.24	Mode	0.00
Standard Deviation	18.89	Standard Deviation	0.37	Standard Deviation	0.05	Standard Deviation	0.25
Sample Variance	356.90	Sample Variance	0.14	Sample Variance	0.00	Sample Variance	0.06
Kurtosis	1.64	Kurtosis	11.90	Kurtosis	1.24	Kurtosis	55.92
Skewness	-0.41	Skewness	2.21	Skewness	-1.00	Skewness	6.13
Range	165.41	Range	4.29	Range	0.34	Range	3.57
Minimum	269.43	Minimum	1.41	Minimum	0.00	Minimum	0.00
Maximum	434.84	Maximum	5.70	Maximum	0.34	Maximum	3.57
Sum	324705.39	Sum	1890.83	Sum	204.57	Sum	57.40
Count	900.00	Count	900.00	Count	900.00	Count	900.00
<i>Benzene (ppm)</i>		<i>Toluene(ppm)</i>		<i>Ethyl Benzene(ppm)</i>		<i>m-Xylene (ppm)</i>	
Mean	0.09	Mean	0.01	Mean	0.03	Mean	0.17
Standard Error	0.01	Standard Error	0.00	Standard Error	0.00	Standard Error	0.02
Median	0.00	Median	0.00	Median	0.00	Median	0.00
Mode	0.00	Mode	0.00	Mode	0.00	Mode	0.00
Standard Deviation	0.23	Standard Deviation	0.06	Standard Deviation	0.10	Standard Deviation	0.54
Sample Variance	0.05	Sample Variance	0.00	Sample Variance	0.01	Sample Variance	0.29
Kurtosis	11.63	Kurtosis	420.48	Kurtosis	50.36	Kurtosis	19.81
Skewness	3.31	Skewness	18.75	Skewness	5.60	Skewness	4.28
Range	1.31	Range	1.48	Range	1.42	Range	4.08
Minimum	0.00	Minimum	0.00	Minimum	0.00	Minimum	0.00
Maximum	1.31	Maximum	1.48	Maximum	1.42	Maximum	4.08
Sum	80.44	Sum	4.75	Sum	28.84	Sum	154.16
Count	900.00	Count	900.00	Count	900.00	Count	900.00
<i>o-Xylene (ppm)</i>		<i>p-Xylene(ppm)</i>		<i>Acrolein (ppm)</i>		<i>Acetaldehyde (ppm)</i>	
Mean	0.04	Mean	0.14	Mean	0.07	Mean	0.10
Standard Error	0.00	Standard Error	0.01	Standard Error	0.00	Standard Error	0.00
Median	0.00	Median	0.04	Median	0.00	Median	0.05
Mode	0.00	Mode	0.00	Mode	0.00	Mode	0.00
Standard Deviation	0.11	Standard Deviation	0.19	Standard Deviation	0.12	Standard Deviation	0.13
Sample Variance	0.01	Sample Variance	0.04	Sample Variance	0.01	Sample Variance	0.02
Kurtosis	8.80	Kurtosis	1.68	Kurtosis	3.75	Kurtosis	6.66
Skewness	2.96	Skewness	1.43	Skewness	2.00	Skewness	2.09
Range	0.68	Range	0.95	Range	0.69	Range	0.96
Minimum	0.00	Minimum	0.00	Minimum	0.00	Minimum	0.00
Maximum	0.68	Maximum	0.95	Maximum	0.69	Maximum	0.96
Sum	37.62	Sum	130.23	Sum	62.23	Sum	86.80
Count	900.00	Count	900.00	Count	900.00	Count	900.00



Table 2 Continued.

<i>Formaldehyde (ppm)</i>		<i>1,3-Butadiene (ppm)</i>		<i>Isopentane (ppm)</i>		<i>Pentane (ppm)</i>	
Mean	0.17	Mean	0.03	Mean	0.03	Mean	0.11
Standard Error	0.00	Standard Error	0.00	Standard Error	0.00	Standard Error	0.01
Median	0.16	Median	0.00	Median	0.00	Median	0.00
Mode	0.00	Mode	0.00	Mode	0.00	Mode	0.00
Standard Deviation	0.11	Standard Deviation	0.06	Standard Deviation	0.13	Standard Deviation	0.29
Sample Variance	0.01	Sample Variance	0.00	Sample Variance	0.02	Sample Variance	0.08
Kurtosis	0.43	Kurtosis	7.30	Kurtosis	103.82	Kurtosis	19.27
Skewness	0.65	Skewness	2.64	Skewness	9.17	Skewness	4.12
Range	0.68	Range	0.38	Range	1.76	Range	2.13
Minimum	0.00	Minimum	0.00	Minimum	0.00	Minimum	0.00
Maximum	0.68	Maximum	0.38	Maximum	1.76	Maximum	2.13
Sum	152.36	Sum	27.20	Sum	28.41	Sum	97.69
Count	900.00	Count	900.00	Count	900.00	Count	900.00
<i>Hexane (ppm)</i>		<i>Heptane(ppm)</i>		<i>3-Ethyl Toluene (ppm)</i>		<i>1-3-5 Trimethylbenzene (ppm)</i>	
Mean	0.01	Mean	0.04	Mean	0.10	Mean	0.12
Standard Error	0.00	Standard Error	0.00	Standard Error	0.01	Standard Error	0.01
Median	0.00	Median	0.00	Median	0.00	Median	0.00
Mode	0.00	Mode	0.00	Mode	0.00	Mode	0.00
Standard Deviation	0.05	Standard Deviation	0.06	Standard Deviation	0.20	Standard Deviation	0.18
Sample Variance	0.00	Sample Variance	0.00	Sample Variance	0.04	Sample Variance	0.03
Kurtosis	38.65	Kurtosis	12.97	Kurtosis	8.23	Kurtosis	8.97
Skewness	5.70	Skewness	2.71	Skewness	2.61	Skewness	2.39
Range	0.49	Range	0.57	Range	1.34	Range	1.57
Minimum	0.00	Minimum	0.00	Minimum	0.00	Minimum	0.00
Maximum	0.49	Maximum	0.57	Maximum	1.34	Maximum	1.57
Sum	10.92	Sum	33.08	Sum	89.86	Sum	105.44
Count	900.00	Count	900.00	Count	900.00	Count	900.00
<i>Cyclohexane</i>		<i>Methyl Ethyl Ketone (ppm)</i>		<i>Carbon Disulfide (ppm)</i>		<i>Carbon disulfide (ppm)</i>	
Mean	0.01	Mean	0.21	Mean	0.00	Mean	0.02
Standard Error	0.00	Standard Error	0.01	Standard Error	0.00	Standard Error	0.00
Median	0.00	Median	0.16	Median	0.00	Median	0.02
Mode	0.00	Mode	0.00	Mode	0.00	Mode	0.02
Standard Deviation	0.02	Standard Deviation	0.22	Standard Deviation	0.03	Standard Deviation	0.01
Sample Variance	0.00	Sample Variance	0.05	Sample Variance	0.00	Sample Variance	0.00
Kurtosis	27.26	Kurtosis	0.76	Kurtosis	344.86	Kurtosis	-0.55
Skewness	3.93	Skewness	1.13	Skewness	17.65	Skewness	0.12
Range	0.28	Range	1.09	Range	0.71	Range	0.07
Minimum	0.00	Minimum	0.00	Minimum	0.00	Minimum	0.00
Maximum	0.28	Maximum	1.09	Maximum	0.71	Maximum	0.07
Sum	11.07	Sum	192.78	Sum	2.12	Sum	21.63
Count	900.00	Count	900.00	Count	900.00	Count	900.00

**APPENDIX B**  
**SUPPLEMENTAL MATERIALS FOR CHAPTER III**

Table 1

*Site Description Parameters for All Sites*

	Site	Wellbore Completed	Oil (bbl)	NG (McF)	Status Code	PA date
PR1	1	4/15/1988	27	0	PR	
	2	2/15/1986	105	41	PR	
	3	10/22/1987	53	0	PR	
	6	10/5/1988	28	0	PR	
	8	7/27/1987	0	39,643	PR	
PA	7	9/29/1982	0	0	PA	3/23/2000
	9	3/8/1984	0	0	PA	9/22/1994
	10	3/7/1986	0	0	PA	7/1/2002
	11	4/18/1985	0	0	PA	9/11/1986
	12	1/16/1985	0	0	PA	3/12/1990
PR2	13	5/3/2005	24	211	PR	
	14	3/25/2002	252	355	PR	
	15	6/7/2001	106	210	PR	
	16	6/26/2001	120	9	PR	
	17	4/28/2000	113	44	PR	
PR3	19	12/4/2008	92	160	PR	
	18	6/2/1990	117	0	PR	
	20	8/6/2013	577	2565	PR	
	21	2/14/2013	312	3028	PR	
	22	9/19/2013	176	11,555	PR	

*Note:* Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3). Wellbore completed is the official date the well finished completion (beginning of production phase). Oil is the amount of oil produced the month of data collection. NG is the amount of natural gas produced the month of data collection. PA date is the date the sites were plugged and abandoned.

Table 2

*Oil and Natural Gas Production by Site and  
Corresponding Mean BTEX (ppbv)*

Site	Oil	NG	benzene (ppbv)	toluene (ppbv)	Ethylbenz ene (ppbv)	m,p- xylene (bbpv)	o- xylene (ppbv)
1	27	0	12.66	0.82	0.00	0.00	0.00
2	105	41	0.00	2.06	0.00	1.49	34.05
3	53	0	0.00	0.00	0.00	0.00	0.78
6	28	0	1.61	0.00	0.00	0.00	0.00
8	0	39,643	40.02	6.36	0.00	0.00	56.81
7	0	0	28.11	1.54	0.00	0.00	6.16
9	0	0					
10	0	0	0.00	0.00	0.00	0.00	0.00
11	0	0	0.43	0.00	0.00	1.42	1.98
12	0	0	0.00	0.25	0.00	0.00	0.00
13	24	211	0.00	0.75	0.00	0.00	0.00
14	252	355	6.63	0.29	0.00	0.07	1.00
15	106	210	0.52	0.37	0.00	9.38	4.19
16	120	9	0.80	3.02	0.00	0.00	6.84
17	113	44	14.19	0.78	0.00	0.00	22.85
19	92	160	1.24	0.15	0.00	0.00	0.00
18	117	0	22.77	6.34	0.00	1.54	12.09
20	577	2565	60.22	6.96	0.00	0.00	8.58
21	312	3028	55.92	8.68	0.00	0.00	12.37
22	176	11,555	38.06	10.46	0.00	0.00	11.86

*Note:* Oil is the amount of oil produced the month of data collection. NG is the amount of natural gas produced the month of data collection.

Table 3

Gas Chromatography with Flame Ionization Detector Specs and method.

Column: HP 1, GC Capillary Column

Phase: 100% Dimethylpolysiloxane

Dimensions: 30 meters x 0.53 mm x 1.5  $\mu$ mOven Profile: 35°C for 3 min to 60°C at 5°C/min  
for 5 min, heat at 220°C for 1 min. 9 min total run.

Carrier Gas: Constant Flow Helium, 2.5 mL/min

Injection: Split 100:1 1  $\mu$ L @ 150°C

Detection: Flame Ionization (FID; 300°C)

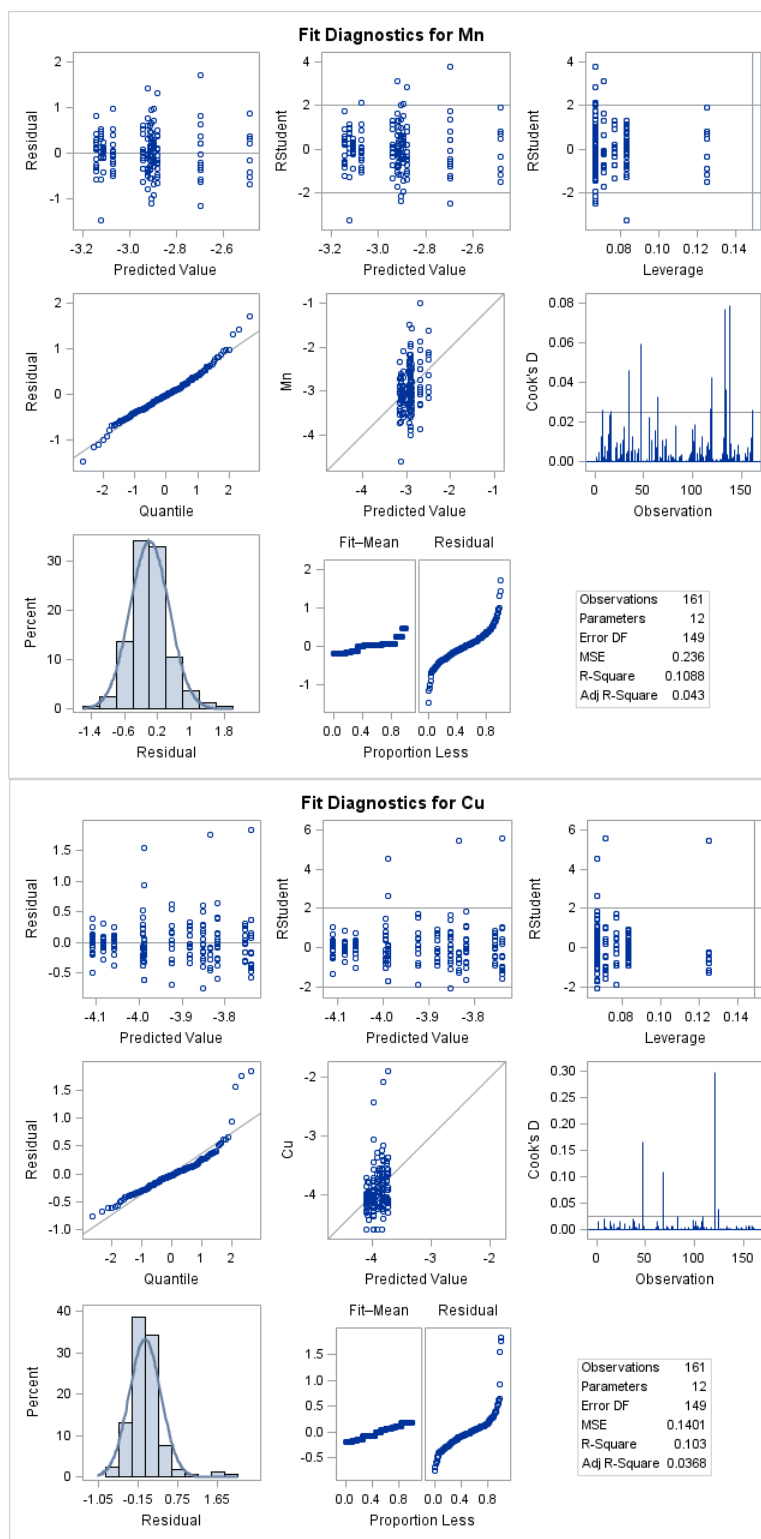
Equation 1. *Calculations for BTEX concentrations.*

BTEX concentrations, determined by GCFID analysis, were multiplied by methanol volume and divided by tissue weight.

$$\left(\frac{0.01 \text{ mg Benzene}}{g \text{ tissue}}\right) \left(\frac{0.02 \text{ L Methanol}}{1.37 \text{ g tissue}}\right) = 1.4 \text{ E}^{-4} \frac{\text{mg Benzene}}{g \text{ tissue}}$$

To convert to ppm \*1000 or ppb \*1000000

**APPENDIX C**  
**SUPPLEMENTAL MATERIALS FOR CHAPTER IV**



*Figure 1.* Fit Diagnostics for nutrients. Residual plots indicate all nutrients were normally distributed after log transformation.

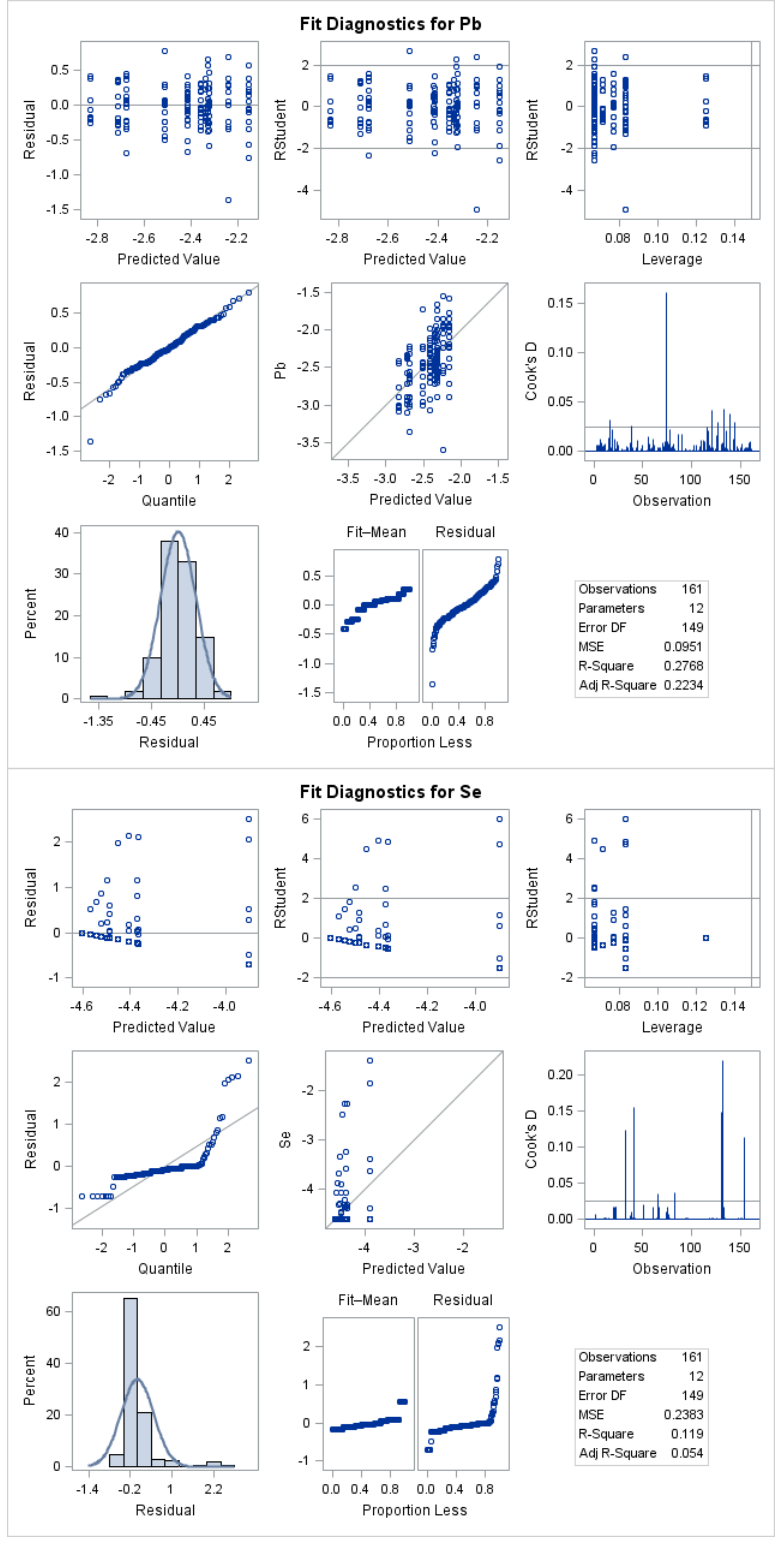


Figure 1. Continued.



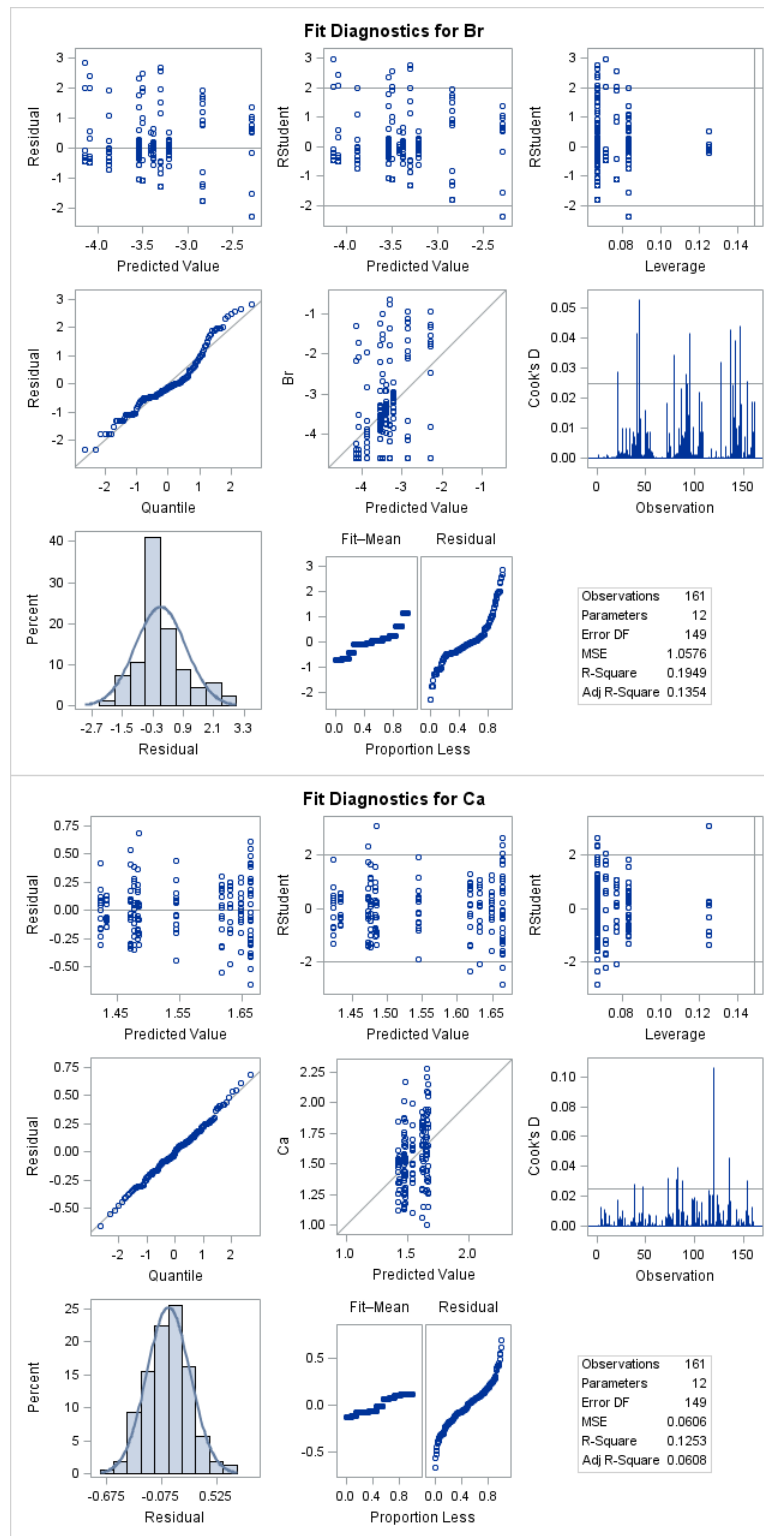


Figure 1. Continued.

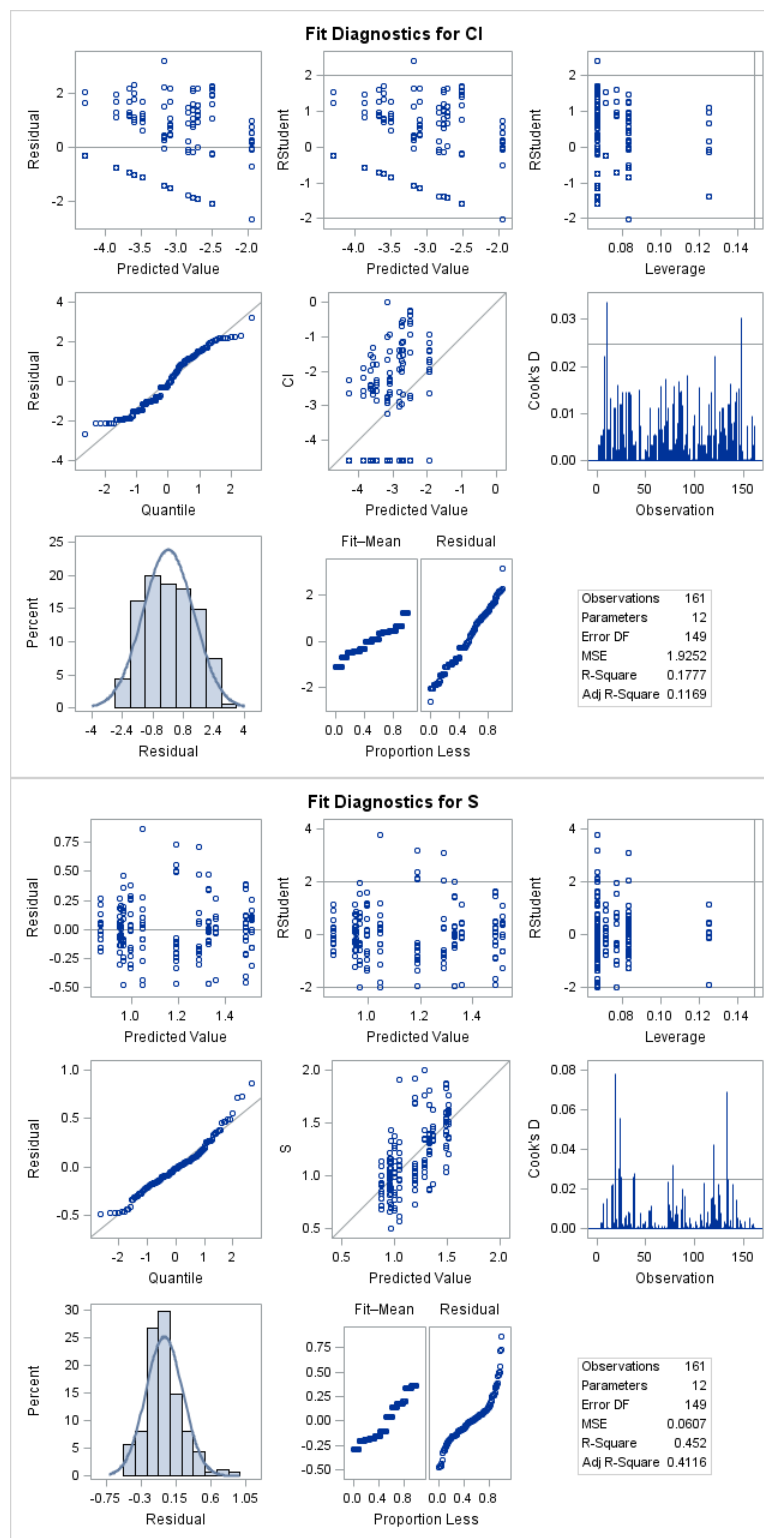


Figure 1. Continued.

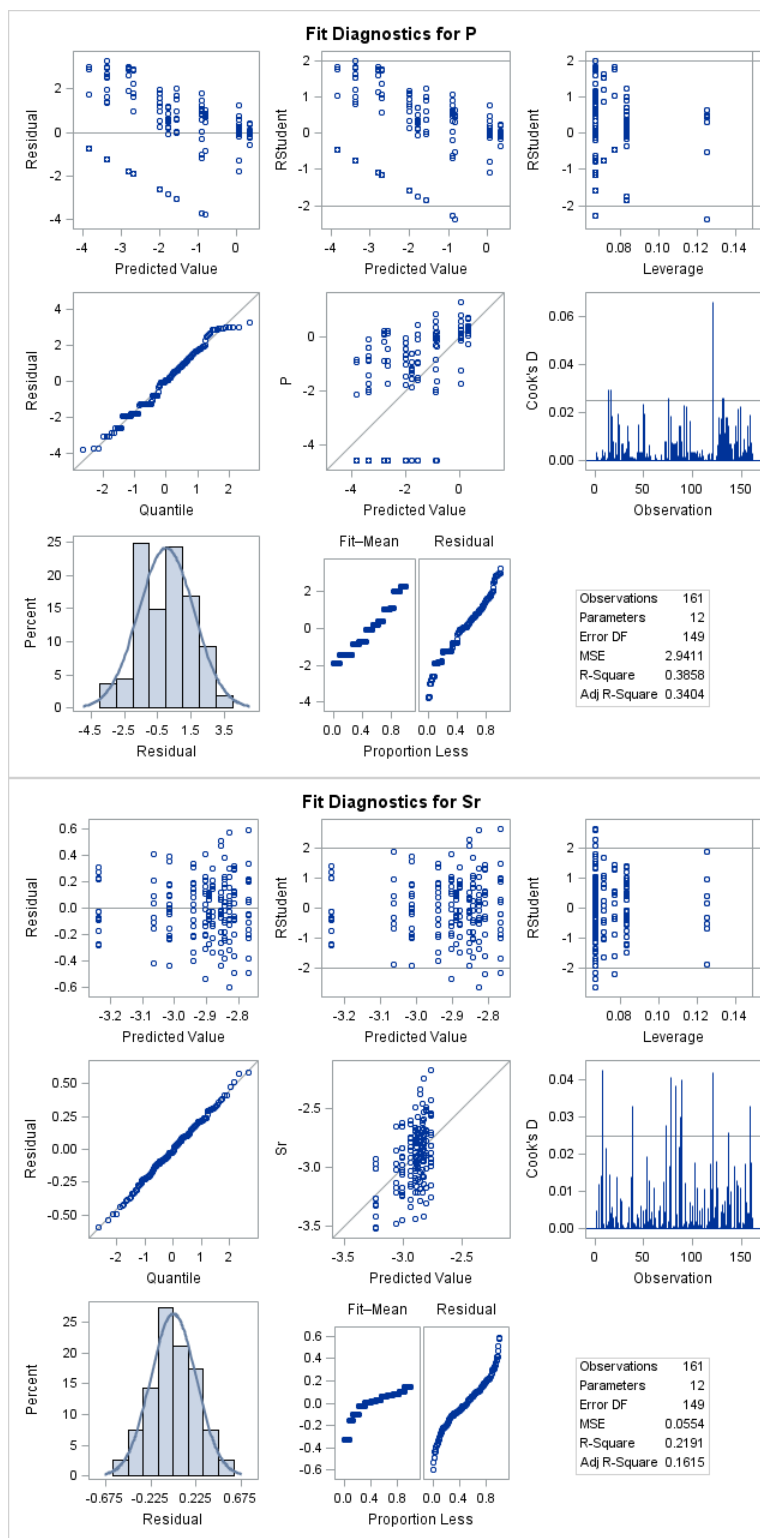


Figure 1. Continued.

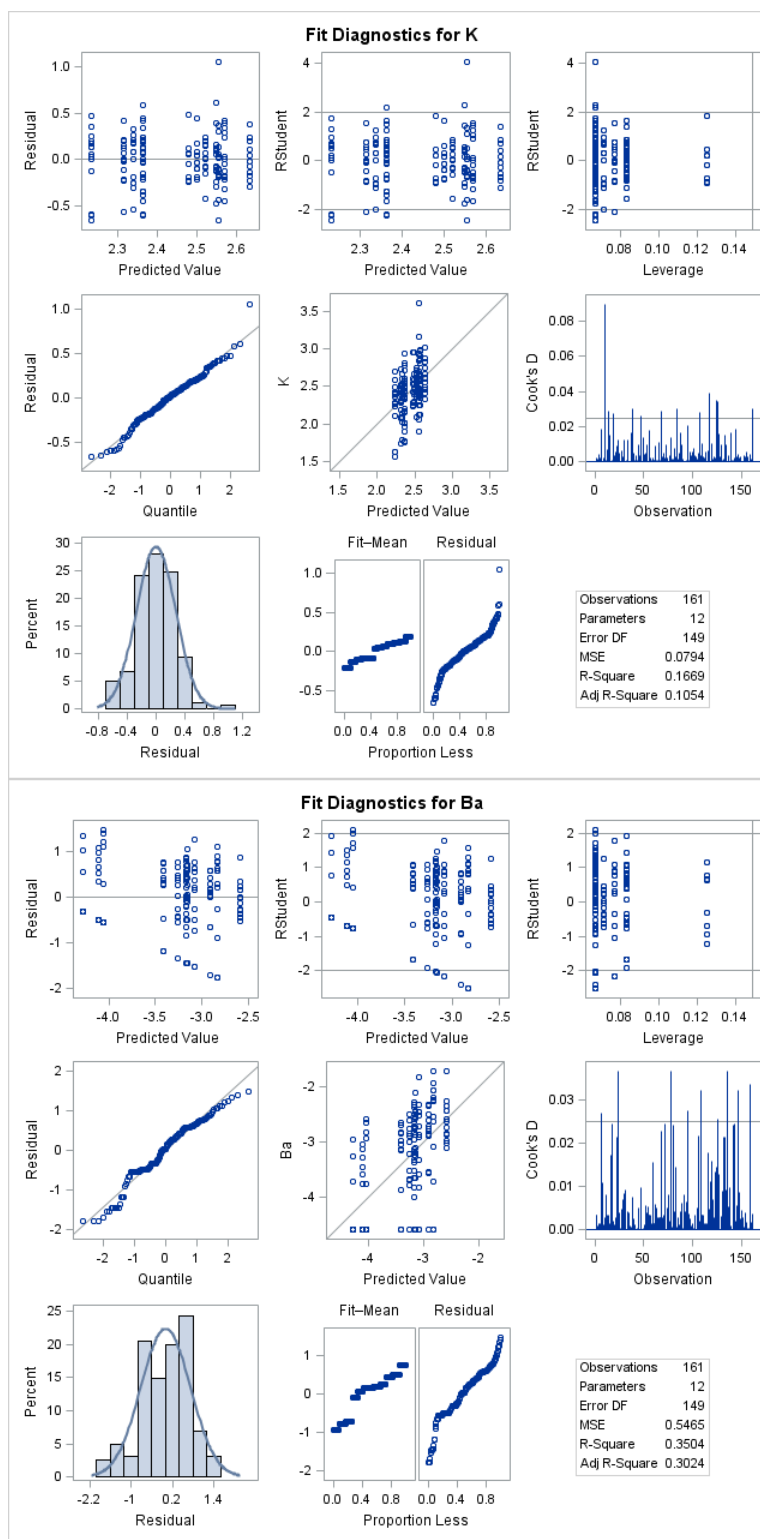


Figure 1. Continued.

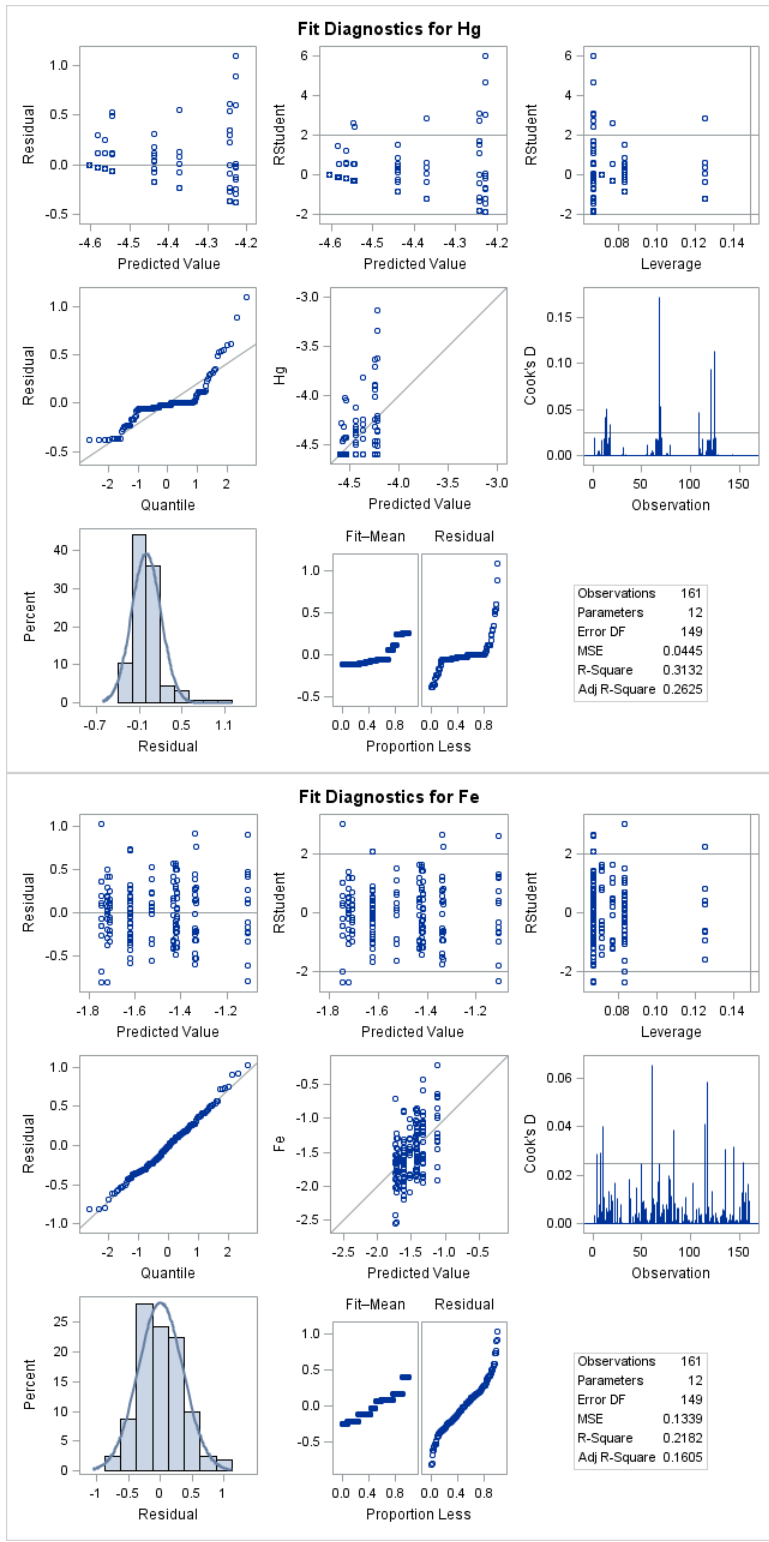


Figure 1. Continued.

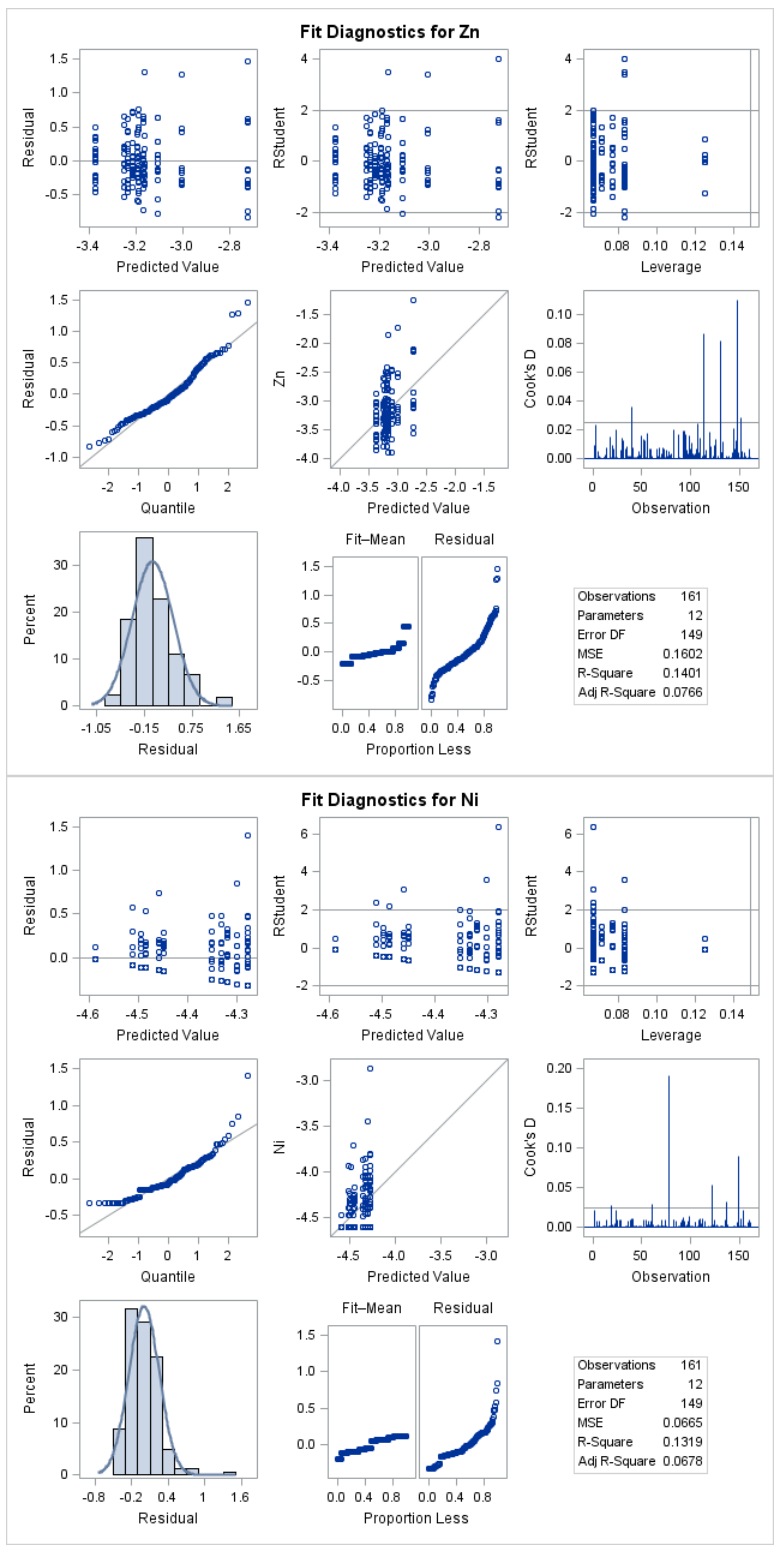


Figure 1. Continued.

**APPENDIX D**  
**SUPPLEMENTAL MATERIALS FOR CHAPTER V**

Table 1

*Relative Abundance of Species by Oil and Natural Gas Production Group*

Species	% Relative Abundance <sup>a</sup>								Species	% Relative Abundance <sup>a</sup>							
	Avg	Max	MaxGrp <sup>b</sup>	PA	PR1	PR2	PR3	Avg		Max	MaxGrp <sup>b</sup>	PA	PR1	PR2	PR3		
ABRFRA	25	100	3	0	100	0	0	HETVIL	25	58	1	8	2	32	58		
AGRCRI	25	66	2	66	0	23	11	HORJUB	25	63	3	4	63	23	10		
AGRDES	25	59	4	0	0	59	41	HORVUL	25	95	4	0	5	95	0		
AGRSTO	25	99	3	1	99	0	0	IVAAXI	25	62	2	62	0	0	38		
AMAALB	25	33	1	26	26	15	33	LACSER	25	68	2	68	32	0	0		
AMAPOW	25	100	4	0	0	100	0	LAPOCC	25	61	2	61	17	23	0		
AMASPI	25	94	4	0	6	94	0	LEPDEN	25	73	4	0	0	73	27		
AMBPSI	25	97	4	0	0	97	3	LICHEN	25	44	1	0	41	16	44		
ANTPAR	25	100	2	100	0	0	0	LINRIG	25	77	3	23	77	0	0		
ARIPUR	25	74	3	21	74	0	5	LITINC	25	62	2	62	0	0	38		
ARTDRA	25	57	4	0	43	57	0	LUPARG	25	100	1	0	0	0	100		
ARTFIL	25	100	1	0	0	0	100	LUPPUS	25	100	3	0	100	0	0		
ARTFRI	25	40	2	40	6	15	38	LYGJUN	25	37	4	16	14	37	33		
ASTER	25	71	1	29	0	0	71	MACPIN	25	44	4	0	32	44	24		
ASTLOT	25	64	3	0	64	17	19	MACTAN	25	56	1	44	0	0	56		
ASTMOL	25	100	4	0	0	100	0	MEDLUP	25	69	1	31	0	0	69		
ASTPEC	25	100	4	0	0	100	0	MELOFF	25	100	2	100	0	0	0		
BASSCO	25	89	4	0	3	89	8	MIRLIN	25	36	3	3	36	27	34		
BOUDAC	25	32	2	32	26	17	26	OENSUF	25	35	4	18	26	35	22		
BOUGRA	25	34	1	14	32	21	34	OPUPOL	25	35	2	35	28	20	17		
BRIEUP	25	55	2	55	45	0	0	OXYSER	25	49	1	46	5	0	49		
BROINE	25	99	1	0	1	0	99	PACPLA	25	53	3	0	53	47	0		
BROTEC	25	69	3	2	69	4	25	PACTRI	25	56	1	0	19	25	56		
CALBER	25	93	3	0	93	7	0	PANCAP	25	60	1	0	40	0	60		
CARROS	25	100	2	100	0	0	0	PANVIR	25	100	3	0	100	0	0		
CARSIM	25	43	1	7	10	40	43	PASSMI	25	40	3	25	40	23	11		
CHAGLY	25	90	1	2	7	1	90	PENALB	25	52	4	0	19	52	29		
CHEBER	25	66	1	0	15	20	66	PHYFEN	25	33	3	28	33	8	31		
CHELEP	25	80	2	80	0	0	20	PICOPP	25	53	4	47	0	53	0		
CHOTEN	25	100	1	0	0	0	100	PLAPAT	25	34	3	31	34	17	18		
CIRCAN	25	51	1	10	34	6	51	POROLE	25	82	1	0	18	0	82		
CIRFLO	25	55	1	39	0	6	55	PSOTEN	25	84	2	84	6	2	9		
CIROCH	25	69	1	0	18	12	69	QUILOB	25	100	1	0	0	0	100		
CIRSCO	25	100	2	100	0	0	0	RATCOL	25	55	3	41	55	4	0		
CIRUND	25	77	1	0	0	23	77	SALTRA	25	69	4	0	16	69	14		
CLESER	25	75	3	0	75	25	0	SCHPAN	25	78	2	78	17	0	5		
CONARV	25	67	1	0	0	33	67	SECCER	25	38	4	34	28	38	0		
CONCAN	25	56	2	56	14	20	10	SENSPA	25	58	1	23	19	0	58		
CRYCIN	25	56	3	44	56	0	0	SISALT	25	45	3	0	45	10	45		
DESSOP	25	44	1	6	24	26	44	SOLCAN	25	43	2	43	24	33	0		
DIGSAN	25	95	4	0	1	95	4	SOLROS	25	78	1	0	13	9	78		
ECHVIR	25	86	1	0	14	0	86	SOLTRI	25	80	4	0	20	80	0		
ELYCAN	25	61	3	0	61	2	37	SONOLE	25	45	4	0	34	45	21		
ELYELY	25	46	1	15	3	36	46	SOPNUT	25	43	3	29	43	16	12		
ERICAN	25	55	2	55	45	0	0	SPHCOC	25	32	1	17	31	21	32		
ERILON	25	87	3	0	87	9	5	SPOAIR	25	93	1	7	0	0	93		
ERINAU	25	62	1	21	1	16	62	SPOCRY	25	54	3	41	54	0	5		
ERYASP	25	65	3	11	65	5	19	TAROFF	25	57	1	0	25	17	57		
ESCVIV	25	59	1	12	29	0	59	THLARV	25	45	3	24	45	19	13		
FESOC	25	58	3	29	58	8	5	TRADUB	25	37	1	15	27	20	37		
GRISQU	25	53	4	47	0	53	0	TRIREP	25	71	2	71	29	0	0		
GUTSAR	25	74	1	7	6	12	74	TRITER	25	91	1	0	9	0	91		
HELANN	25	53	1	0	0	47	53	VERBRA	25	63	1	8	18	12	63		
HESCOM	25	51	3	42	51	0	7	VICAME	25	48	1	0	16	36	48		
HESSPA	25	72	4	0	15	72	13	XANSTR	25	100	4	0	0	100	0		

Note: Production groups: PA= Plugged and abandoned in the 1980's, PR1= Producing since 1980-1990, PR2= Producing since 2000-2005, PR3= Producing since 2006-2013

<sup>a</sup> Relative abundance in group, % of perfect indication (average abundance of a given species in a given group over the average abundance of that species in all samples expressed as a %)

<sup>b</sup> MaxGrp is the group containing the maximum relative abundance for each species



Table 2.

*Relative frequency of species by oil and natural gas production group.*

Species	% Relative Frequency <sup>a</sup>				% Relative Frequency <sup>a</sup>										
	Avg	Max	MaxGrp <sup>b</sup>	PA	PR1	PR2	PR3	Species	Avg	Max	MaxGrp <sup>b</sup>	PA	PR1	PR2	PR3
ABRFRA	1	4	3	0	4	0	0	HETVIL	21	42	1	9	2	30	42
AGRCRI	30	58	2	58	4	35	22	HORJUB	11	24	3	2	24	8	8
AGRDES	6	14	1	2	0	8	14	HORVUL	1	3	4	0	2	3	0
AGRSTO	3	11	3	2	11	0	0	IVAAXI	2	4	2	4	0	0	3
AMAALB	9	11	1	9	9	8	11	LACSER	5	11	2	11	7	0	0
AMAPOW	3	13	4	0	0	13	0	LAPOCC	3	7	2	7	2	3	0
AMASPI	7	25	4	0	2	25	0	LEPDEN	3	8	4	0	0	8	3
AMBPSI	2	6	1	0	0	3	6	LICHEN	8	14	1	0	13	5	14
ANTPAR	1	4	2	4	0	0	0	LINRIG	2	7	3	2	7	0	0
ARIPUR	8	17	3	11	17	0	3	LITINC	2	4	2	4	0	0	3
ARTDRA	1	3	4	0	2	3	0	LUPARG	1	6	1	0	0	0	6
ARTFIL	2	8	1	0	0	0	8	LUPPUS	2	9	3	0	9	0	0
ARTFRI	34	56	1	51	13	15	56	LYGJUN	14	20	4	11	9	20	17
ASTER	2	6	1	2	0	0	6	MACPIN	3	5	4	0	4	5	3
ASTLOT	4	9	3	0	9	3	3	MACTAN	1	3	1	2	0	0	3
ASTMOL	2	8	4	0	0	8	0	MEDLUP	3	9	2	9	0	0	3
ASTPEC	1	5	4	0	0	5	0	MELOFF	5	20	2	20	0	0	0
BASSCO	18	31	1	0	13	28	31	MIRLIN	17	24	3	2	24	18	22
BOUDAC	82	96	3	93	96	63	75	OENSUF	10	13	3	4	13	13	11
BOUGRA	84	93	2	93	93	68	83	OPUPOL	67	80	2	80	78	48	61
BRIEUP	1	2	2	2	2	0	0	OXYSER	4	11	1	2	2	0	11
BROINE	2	6	1	0	2	0	6	PACPLA	2	6	3	0	6	3	0
BROTEC	16	25	1	11	13	15	25	PACTRI	2	6	1	0	2	3	6
CALBER	3	9	3	0	9	3	0	PANCAP	1	3	1	0	2	0	3
CARROS	1	4	2	4	0	0	0	PANVIR	2	7	3	0	7	0	0
CARSIM	13	22	1	11	11	8	22	PASSMI	50	59	3	58	59	40	42
CHAGLY	11	19	3	4	19	3	17	PENALB	7	15	4	0	6	15	8
CHEBER	3	8	1	0	2	3	8	PHYFEN	16	20	3	18	20	5	19
CHELEP	3	11	2	11	0	0	3	PICOPP	4	8	4	7	0	8	0
CHOTEN	1	6	1	0	0	0	6	PLAPAT	49	70	3	56	70	40	31
CIRCAN	8	17	1	4	7	3	17	POROLE	1	3	1	0	2	0	3
CIRFLO	3	6	1	2	0	3	6	PSOTEN	33	69	2	69	13	15	36
CIROCH	4	8	1	0	4	3	8	QUILOB	1	6	1	0	0	0	6
CIRSCO	1	4	2	4	0	0	0	RATCOL	10	20	3	16	20	3	0
CIRUND	1	3	1	0	0	3	3	SALTRA	11	20	4	0	15	20	11
CLESER	2	4	3	0	4	3	0	SCHPAN	5	11	2	11	6	0	3
CONARV	8	18	4	0	0	18	14	SECCER	2	3	4	2	2	3	0
CONCAN	28	40	4	24	28	40	19	SENSPA	2	6	1	2	2	0	6
CRYCIN	3	6	3	4	6	0	0	SISALT	5	11	1	0	7	3	11
DESSOP	7	11	1	2	9	5	11	SOLCAN	4	7	2	7	4	5	0
DIGSAN	6	15	4	0	2	15	6	SOLROS	4	11	1	0	4	3	11
ECHVIR	3	11	1	0	2	0	11	SOLTRI	2	8	4	0	2	8	0
ELYSAN	13	28	1	0	20	3	28	SONOLE	13	22	3	0	22	15	14
ELYELY	22	29	2	29	6	28	28	SOPNUT	35	54	3	40	54	28	19
ERICAN	1	2	2	2	2	0	0	SPHCOC	72	94	3	44	94	70	81
ERILON	12	39	3	0	39	5	3	SPOAIR	4	14	1	2	0	0	14
ERINAU	17	28	1	18	2	20	28	SPOCRY	7	18	2	18	7	0	3
ERYASP	17	31	3	11	31	5	19	TAROFF	4	8	1	0	4	3	8
ESCVIV	5	11	1	2	6	0	11	THLARV	17	30	3	16	30	13	8
FESOCT	30	53	2	53	44	5	17	TRADUB	17	22	1	11	20	15	22
GRISQU	1	3	4	2	0	3	0	TRIREF	2	4	2	4	2	0	0
GUTSAR	7	11	1	4	4	8	11	TRITER	1	3	1	0	2	0	3
HELANN	1	3	1	0	0	3	3	VERBRA	11	15	3	7	15	10	14
HESCOM	24	51	2	51	19	0	25	VICAME	15	25	4	0	11	25	22
HESSPA	3	6	3	0	6	5	3	XANSTR	4	15	4	0	0	15	0

Note: Production groups: PA= Plugged and abandoned in the 1980's, PR1= Producing since 1980-1990, PR2= Producing since 2000-2005, PR3= Producing since 2006-2013

<sup>a</sup>Relative frequency in group, % of perfect indication (% of samples in given group where given species is present)

<sup>b</sup>MaxGrp is the group containing the maximum relative frequency for each species

Table 3

*Summary Table of Percent Bare Ground Per Plot Plots are Sorted By Production Group and Distance*

Group	Distance	N Obs	Lower 95% CL for Mean	Upper 95% CL for Mean	Mean	Std Error
PA	20	15	3.80	10.27	7.03	1.51
	50	15	2.96	7.24	5.10	1.00
	100	15	3.12	5.75	4.43	0.61
	total	45	4.22	6.82	5.52	0.64
PR1	20	12	6.45	39.55	23.00	7.52
	50	12	-1.15	11.82	5.33	2.95
	100	12	-0.79	12.29	5.75	2.97
	total	36	5.04	17.68	11.36	3.11
PR2	20	19	2.47	17.48	9.97	3.57
	50	17	1.76	4.18	2.97	0.57
	total	54	3.08	8.59	5.83	1.37
	100	18	1.62	6.71	4.17	1.21
PR3	20	16	31.49	79.38	55.44	11.23
	50	14	15.48	65.73	40.61	11.63
	100	15	4.24	19.56	11.90	3.57
	total	45	24.10	48.52	36.31	6.06
Total		180	5.67	6.98	6.32	0.34

*Note:* Sites were grouped according to production (date and amount) including sites that were plugged and abandoned in the 1980s (PA), producing since 1980-1990 (PR1), producing since 2000-2005 (PR2) and producing since 2006-2013 (PR3). Bare ground was coded 0-9 for each plot and then converted to mean percentages. Bare ground scores were defined as the following mean percentages: 9 = 95 %; 8 = 82.5%; 7 = 62.5%; 6 = 37.5%; 5 = 17.5%; 4 = 7.5%; 3 = 3.5%; 2 = 1.5%; 1 = 0.5%.

Table 4

*Secondary Matrix Pearson and Kendall Correlations with Ordination*

Index	Axis								
	1			2			3		
	r	r-sq	tau	r	r-sq	tau	r	r-sq	tau
S	0.391	0.153	0.217	-0.102	0.01	0.021	-0.01	0	-0.049
E	0.084	0.007	-0.005	-0.061	0.004	0.23	0.133	0.018	0.079
H	0.162	0.026	0.053	-0.045	0.002	0.248	0.084	0.007	0.042
D	0.128	0.016	0.04	-0.111	0.012	0.203	0.124	0.015	0.048
Rao	0.246	0.061	0.095	0.089	0.008	0.224	-0.071	0.005	-0.039
C3	0.343	0.117	0.181	0.022	0	0.111	-0.034	0.001	-0.053
C4	0.152	0.023	0.045	-0.202	0.041	-0.111	-0.064	0.004	-0.093
CAM	0.111	0.012	0.096	-0.399	0.159	-0.315	-0.042	0.002	-0.017
AST	0.189	0.036	0.097	-0.034	0.001	-0.01	-0.238	0.057	-0.163
BRA	0.194	0.038	0.142	-0.097	0.009	-0.085	0.188	0.035	0.146
CAC	0.045	0.002	0.031	-0.477	0.228	-0.332	-0.024	0.001	0.056
FAB	-0.481	0.231	-0.243	0.063	0.004	-0.049	-0.182	0.033	-0.15
MAL	0.26	0.067	0.139	-0.386	0.149	-0.262	0.135	0.018	0.154
PLA	0.036	0.001	0.003	-0.136	0.018	-0.05	-0.093	0.009	-0.039
POA	-0.204	0.042	-0.218	0.006	0	0.135	0.311	0.097	0.231
EUP	0.05	0.003	0.062	0.088	0.008	0.079	0.034	0.001	-0.025
NYC	0.127	0.016	0.126	-0.224	0.05	-0.211	0.046	0.002	0.024
ONA	0.191	0.037	0.175	-0.054	0.003	-0.009	0.081	0.007	0.052
AMA	0.103	0.011	0.126	0.412	0.17	0.087	-0.088	0.008	-0.088
VRB	0.192	0.037	0.174	0.174	0.03	0.045	-0.073	0.005	-0.068
CYP	0.047	0.002	0.06	0.046	0.002	0.054	0.202	0.041	0.184
BOR	0.131	0.017	0.111	-0.014	0	0.035	0.063	0.004	0.039
LIN	-0.009	0	0.013	-0.097	0.009	-0.057	0.014	0	0.022
PLG	-0.037	0.001	-0.031	0.077	0.006	0.068	0.047	0.002	0.06
POR	0.062	0.004	0.048	0.144	0.021	0.112	0.028	0.001	0.027
CAP	-0.01	0	0.032	0.153	0.024	0.086	-0.094	0.009	-0.017
SOL	0.162	0.026	0.125	0.266	0.071	0.172	-0.174	0.03	-0.124
ZYG	0.215	0.046	0.125	0.132	0.017	0.101	-0.125	0.016	-0.052
SCR	-0.007	0	0.021	0.03	0.001	-0.084	-0.117	0.014	-0.054
CNV	-0.043	0.002	-0.039	-0.042	0.002	-0.016	0.041	0.002	0.057
CON	-0.056	0.003	-0.041	0.51	0.26	0.272	-0.126	0.016	-0.089
CHE	0.189	0.036	0.111	0.1	0.01	0.092	0.165	0.027	0.133
CMM	-0.01	0	-0.002	0.092	0.009	0.075	0.098	0.01	0.088
introduced	0.056	0.003	0.025	0.673	0.453	0.355	-0.029	0.001	-0.048
native	-0.085	0.007	-0.064	-0.642	0.412	-0.304	0.03	0.001	0.04
non-invasive	-0.074	0.005	-0.086	-0.316	0.1	-0.14	-0.126	0.016	-0.073
invasive	-0.009	0	0.019	0.49	0.24	0.337	0.138	0.019	0.086
bare ground	-0.178	0.032	-0.039	0.689	0.475	0.343	-0.364	0.133	-0.165

Table 5

## Main Matrix Pearson and Kendall Correlations with Ordination

Species	Axis								
	1			2			3		
	r	r-sq	tau	r	r-sq	tau	r	r-sq	tau
ABRFRA	0.121	0.015	0.106	0	0	0.032	0.017	0	0.045
AGRCRI	-0.181	0.033	-0.226	0.208	0.043	0.395	-0.49	0.24	-0.34
AGRDES	0.124	0.015	0.115	-0.011	0	0.017	-0.267	0.071	-0.161
AGRSTO	0.142	0.02	0.007	0.098	0.01	0	0.057	0.003	0.034
AMAALB	-0.058	0.003	-0.044	0.144	0.021	0.14	-0.05	0.002	-0.059
AMAPOW	-0.02	0	-0.009	0.465	0.216	0.232	-0.226	0.051	-0.192
AMASPI	-0.039	0.002	-0.041	0.398	0.158	0.208	-0.28	0.078	-0.225
AMBPSI	0.219	0.048	0.076	0.119	0.014	0.067	0.125	0.016	-0.044
ANTPAR	0.1	0.01	0.091	-0.086	0.007	-0.084	-0.011	0	-0.029
ARIPUR	-0.011	0	0.021	0.032	0.001	0.06	0.104	0.011	0.053
ARTDRA	0.085	0.007	0.077	-0.026	0.001	-0.029	-0.148	0.022	-0.112
ARTFIL	0.026	0.001	0.031	-0.04	0.002	-0.006	-0.004	0	-0.014
ARTFRI	0.007	0	-0.136	0.059	0.003	-0.007	-0.304	0.092	-0.305
ASTER	0.015	0	0.001	0.028	0.001	0.042	-0.04	0.002	-0.033
ASTLOT	0.017	0	0.007	-0.096	0.009	-0.087	0.041	0.002	0.029
ASTMOL	-0.121	0.015	-0.126	-0.061	0.004	-0.022	-0.026	0.001	0.012
ASTPEC	0.233	0.054	0.141	0.088	0.008	0.083	0.14	0.019	0.121
BASSCO	0.005	0	0.185	0.038	0.001	-0.08	0.222	0.049	0.019
BOUDAC	-0.533	0.284	-0.436	-0.491	0.241	-0.406	0.111	0.012	0.146
BOUGRA	0.549	0.301	0.439	-0.615	0.378	-0.658	-0.017	0	-0.027
BRIEUP	0.01	0	0.001	0.023	0.001	0.055	0.032	0.001	0.057
BROINE	0.012	0	-0.012	0.157	0.025	0.143	0.052	0.003	0.071
BROTEC	0.223	0.05	0.027	0.202	0.041	0.231	0.177	0.031	0.246
CALBER	0.099	0.01	0.083	-0.029	0.001	-0.014	0.017	0	0.009
CARROS	-0.012	0	-0.046	0.094	0.009	0.096	0.096	0.009	0.016
CARSIM	0.09	0.008	0.082	0.092	0.008	0.039	0.176	0.031	0.193
CHAGLY	-0.034	0.001	0.064	0.159	0.025	0.036	0.107	0.011	-0.015
CHEBER	0.083	0.007	0.066	0.073	0.005	0.042	-0.141	0.02	-0.108
CHELEP	-0.006	0	0.01	0.01	0	-0.001	0.156	0.024	0.122
CHOTEN	-0.049	0.002	-0.042	0.19	0.036	0.128	-0.024	0.001	-0.007
CIRCAN	0.112	0.013	0.085	0.151	0.023	0.126	0.056	0.003	0.084
CIRFLO	0.098	0.01	0.067	0.084	0.007	0.086	-0.084	0.007	-0.093
CIROCH	0.232	0.054	0.194	-0.015	0	0.023	-0.048	0.002	-0.061
CIRSCO	-0.029	0.001	-0.011	-0.07	0.005	-0.04	0.021	0	0.044
CIRUND	0.169	0.029	0.024	0.133	0.018	0.093	-0.12	0.014	-0.019
CLESER	0.073	0.005	0.033	0.072	0.005	0.085	-0.023	0.001	-0.017
CONARV	-0.062	0.004	-0.039	0.344	0.118	0.268	0.049	0.002	-0.083
CONCAN	0.18	0.033	0.224	0.125	0.016	0.1	0.004	0	0.149
CRYCIN	-0.012	0	-0.026	-0.009	0	0.034	-0.038	0.001	-0.033
DESSOP	0.153	0.023	0.14	0.148	0.022	0.134	0.179	0.032	0.136
DIGSAN	-0.023	0.001	-0.025	0.5	0.25	0.304	-0.165	0.027	-0.086
ECHVIR	0.067	0.005	0.066	0.104	0.011	0.065	0.114	0.013	0.101
ELYCAN	0.118	0.014	0.114	-0.099	0.01	-0.122	-0.036	0.001	-0.056
ELYELY	0.241	0.058	0.194	0.142	0.02	0.029	0.126	0.016	0.059
ERICAN	0.101	0.01	0.079	0.031	0.001	0.018	-0.023	0.001	-0.036
ERILON	0.142	0.02	0.124	-0.127	0.016	-0.139	0.03	0.001	0.042
ERINAU	0.018	0	-0.035	-0.083	0.007	-0.032	-0.229	0.053	-0.255
ERYASP	0.155	0.024	0.13	-0.073	0.005	-0.23	0.079	0.006	0.116
ESCVIV	0.069	0.005	0.056	-0.067	0.004	-0.077	-0.05	0.003	-0.071
FESOCT	-0.011	0	-0.058	-0.088	0.008	0.044	0.147	0.022	0.085
GRISQU	0.011	0	0.003	0.012	0	0.044	-0.067	0.004	-0.071
GUTSAR	0.008	0	0.003	0.011	0	0.079	-0.121	0.015	-0.162
HELANN	0.297	0.088	0.151	0.179	0.032	0.126	-0.014	0	-0.005
HESCOM	0.02	0	-0.169	-0.013	0	0.095	-0.064	0.004	-0.152
HESSPA	-0.021	0	0.004	-0.03	0.001	-0.003	0.07	0.005	0.118
HETVIL	0.137	0.019	0.074	-0.064	0.004	-0.092	-0.221	0.049	-0.158
HORJUB	0.073	0.005	0.145	-0.048	0.002	0.01	-0.069	0.005	0.14

Table 5. Continued.

Species	Axis								
	1			2			3		
	r	r-sq	tau	r	r-sq	tau	r	r-sq	tau
HORVUL	0.223	0.05	0.126	0.119	0.014	0.096	0.129	0.017	0.087
IVAAXI	-0.122	0.015	-0.119	0.034	0.001	0.021	0.054	0.003	-0.006
LACSER	-0.01	0	-0.029	-0.03	0.001	-0.008	-0.083	0.007	-0.079
LAPOCC	0.188	0.035	0.155	0.028	0.001	0.008	0.071	0.005	0.069
LEPDEN	-0.047	0.002	-0.054	0.014	0	0.06	-0.035	0.001	-0.017
LICHEN	0.189	0.036	0.181	-0.235	0.055	-0.25	0.018	0	0.018
LINRIG	-0.012	0	-0.001	-0.076	0.006	-0.048	0.024	0.001	0.042
LITINC	0.044	0.002	0.06	-0.005	0	0.028	0.06	0.004	0.027
LUPARG	0.121	0.015	0.104	-0.104	0.011	-0.133	0.013	0	0.033
LUPPUS	0.04	0.002	0.038	-0.129	0.017	-0.106	0.009	0	0.012
LYGIUN	0.066	0.004	0.08	-0.031	0.001	-0.047	0.065	0.004	-0.009
MACPIN	-0.023	0.001	-0.031	-0.123	0.015	-0.107	-0.006	0	-0.003
MACTAN	0.053	0.003	0.02	-0.05	0.003	-0.016	-0.04	0.002	-0.066
MEDLUP	-0.075	0.006	-0.13	0.145	0.021	0.049	0.082	0.007	-0.034
MELOFF	-0.146	0.021	-0.138	-0.006	0	0.059	-0.114	0.013	-0.101
MIRLIN	0.147	0.022	0.132	-0.195	0.038	-0.205	0.047	0.002	0.018
OENSUF	0.189	0.036	0.164	-0.036	0.001	-0.005	0.132	0.017	0.082
OPUPOL	0.063	0.004	0.036	-0.205	0.042	-0.275	0.036	0.001	-0.009
OXYSER	0.04	0.002	0.143	0.024	0.001	0.025	-0.068	0.005	-0.042
PACPLA	-0.037	0.001	-0.051	-0.067	0.005	-0.032	0.027	0.001	0.047
PACTRI	0.034	0.001	0.015	-0.074	0.005	-0.06	-0.053	0.003	-0.056
PANCAP	0.135	0.018	0.072	0.133	0.018	0.112	-0.012	0	-0.004
PANVIR	0.031	0.001	0.036	-0.006	0	-0.004	0.085	0.007	0.077
PASSMI	-0.146	0.021	-0.172	0.239	0.057	0.224	0.663	0.44	0.604
PENALB	0.013	0	0.022	-0.061	0.004	-0.086	-0.082	0.007	-0.052
PHYFEN	-0.055	0.003	-0.061	-0.17	0.029	-0.139	-0.104	0.011	-0.12
PICOPP	-0.025	0.001	-0.028	-0.076	0.006	-0.05	0.032	0.001	0.03
PLAPAT	0.091	0.008	0.08	-0.037	0.001	-0.041	-0.088	0.008	-0.082
POROLE	0.112	0.012	0.049	0.146	0.021	0.113	0.006	0	0.026
PSOTEN	-0.126	0.016	-0.16	-0.051	0.003	0.085	-0.07	0.005	-0.281
QUILOB	0.191	0.037	0.144	-0.058	0.003	-0.028	-0.048	0.002	-0.079
RATCOL	0.07	0.005	0.057	-0.052	0.003	-0.033	0	0	-0.008
SALTRA	0.258	0.066	0.218	0.123	0.015	0.072	0.15	0.022	0.099
SCHPAN	-0.165	0.027	-0.176	-0.006	0	0.091	-0.013	0	0.027
SECCER	-0.101	0.01	-0.086	0.147	0.022	0.07	0.033	0.001	0.019
SENSPA	0.184	0.034	0.159	-0.092	0.009	-0.092	0.042	0.002	0.044
SISALT	0.071	0.005	0.117	0.132	0.017	0.09	0.241	0.058	0.141
SOLCAN	-0.056	0.003	-0.065	-0.025	0.001	0.035	-0.011	0	0.015
SOLROS	0.148	0.022	0.145	0.049	0.002	0.112	-0.013	0	-0.008
SOLTRI	-0.032	0.001	-0.039	0.281	0.079	0.176	-0.186	0.034	-0.127
SONOLE	0.045	0.002	0.05	-0.04	0.002	-0.148	0.158	0.025	0.09
SOPNUT	-0.202	0.041	-0.177	-0.134	0.018	-0.021	0.065	0.004	0.07
SPHCOC	0.26	0.067	0.313	-0.18	0.033	-0.229	0.073	0.005	0.122
SPOAIR	0.021	0	0.088	-0.095	0.009	-0.122	-0.002	0	-0.048
SPOCRY	0.021	0	-0.126	0.049	0.002	0.111	-0.096	0.009	-0.095
TAROFF	0.141	0.02	0.118	0.172	0.029	0.144	0.055	0.003	0.04
THLARV	0.144	0.021	0.125	0.033	0.001	0.043	0.135	0.018	0.109
TRADUB	0.12	0.014	0.133	0.15	0.023	0.117	0.068	0.005	0.016
TRIREP	-0.041	0.002	-0.04	-0.001	0	0.023	-0.087	0.008	-0.073
TRITER	0.21	0.044	0.125	0.138	0.019	0.101	-0.144	0.021	-0.052
VERBRA	0.244	0.06	0.187	0.144	0.021	0.022	-0.137	0.019	-0.045
VICAME	0.202	0.041	0.14	-0.226	0.051	-0.233	-0.04	0.002	-0.056
XANSTR	-0.077	0.006	-0.07	0.444	0.197	0.241	-0.325	0.106	-0.228

Table 6

*Species Percent Cover and Percent Bare Ground Per Plot*

Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare
ONE100	AGRSTO	2	5	OSE50	THLARV	1	4	10SE100	OPUPOL	1	2	11NE50	OPUPOL	1	4	11W50	CONCAN	2	3
ONE100	ARIPUR	1	5	OSE50	TRADUB	1	4	10SE100	PASSMI	2	2	11NE50	PHYFEN	1	4	11W50	FESOC	5	3
ONE100	BOUDAC	6	5	OW100	BOUDAC	6	3	10SE100	PSOTEN	4	2	11NE50	PSOTEN	1	4	11W50	HESCOM	1	3
ONE100	BOUGRA	5	5	OW100	BOUGRA	6	3	10SE100	SCHPAN	3	2	11NE50	SCHPAN	2	4	11W50	HETVIL	1	3
ONE100	ERILON	1	5	OW100	ERILON	1	3	10SE100	SOPNUT	1	2	11SE100	AGRCRI	3	3	11W50	LINRIG	1	3
ONE100	LICHEN	1	5	OW100	MIRLIN	1	3	10SE100	SPOCRY	1	2	11SE100	AMAALB	2	3	11W50	OPUPOL	2	3
ONE100	LYGIUN	1	5	OW100	OPUPOL	1	3	10SE20	BOUDAC	6	5	11SE100	ARTFRI	1	3	11W50	PASSMI	3	3
ONE100	OPUPOL	4	5	OW100	PHYFEN	1	3	10SE20	BOUGRA	2	5	11SE100	BOUDAC	6	3	11W50	PLAPAT	3	3
ONE100	PHYFEN	1	5	OW100	PLAPAT	1	3	10SE20	BROTEC	1	5	11SE100	BOUGRA	3	3	11W50	RATCOL	3	3
ONE100	PLAPAT	1	5	OW100	SOPNUT	1	3	10SE20	PASSMI	5	5	11SE100	BROTEC	1	3	11W50	SOLCAN	1	3
ONE100	SOPNUT	1	5	OW100	SPHCOC	1	3	10SE20	PLAPAT	1	5	11SE100	ERINAU	1	3	11W50	SPHCOC	1	3
ONE100	SPHCOC	1	5	OW100	THLARV	1	3	10SE20	PSOTEN	3	5	11SE100	HETVIL	1	3	12NE100	AGRCRI	4	2
ONE20	ARIPUR	3	4	OW20	AGRSTO	6	1	10SE50	ARTFRI	1	3	11SE100	IVAAXI	1	3	12NE100	ARTFRI	2	2
ONE20	ASTLOT	1	4	OW20	BOUDAC	2	1	10SE50	BOUDAC	8	3	11SE100	OPUPOL	1	3	12NE100	BOUDAC	6	2
ONE20	BOUDAC	4	4	OW20	BOUGRA	2	1	10SE50	BOUGRA	3	3	11SE100	PHYFEN	1	3	12NE100	BOUGRA	2	2
ONE20	CALBER	1	4	OW20	BROTEC	6	1	10SE50	OPUPOL	2	3	11SE100	PSOTEN	1	3	12NE100	ERINAU	2	2
ONE20	CHAGLY	1	4	OW20	ELYELY	4	1	10SE50	PASSMI	2	3	11SE100	SCHPAN	3	3	12NE100	FESOC	4	2
ONE20	ERILON	1	4	OW20	ERILON	1	1	10SE50	PSOTEN	2	3	11SE100	SOPNUT	1	3	12NE100	HESCOM	3	2
ONE20	PASSMI	7	4	OW20	LACSER	1	1	10W100	ARTFRI	1	3	11SE100	SPHCOC	2	3	12NE100	LACSER	1	2
ONE20	PHYFEN	1	4	OW20	LYGIUN	1	1	10W100	BOUDAC	8	3	11SE100	TRADUB	1	3	12NE100	PLAPAT	1	2
ONE20	RATCOL	1	4	OW20	MIRLIN	1	1	10W100	BOUGRA	3	3	11SE20	BOUDAC	6	3	12NE100	PSOTEN	3	2
ONE20	SOPNUT	1	4	OW20	PLAPAT	1	1	10W100	OPUPOL	2	3	11SE20	BOUGRA	3	3	12NE100	SPOCRY	1	2
ONE20	SPHCOC	1	4	OW20	SPHCOC	1	1	10W100	PASSMI	2	3	11SE20	ERINAU	1	3	12NE20	AGRCRI	3	4
ONE50	AGRSTO	1	4	OW20	THLARV	1	1	10W100	PSOTEN	2	3	11SE20	FESOC	1	3	12NE20	ARTFRI	1	4
ONE50	BOUDAC	4	4	OW50	AGRSTO	2	2	10W20	ARTFRI	1	5	11SE20	GUTSAR	1	3	12NE20	BOUCUR	2	4
ONE50	BOUGRA	8	4	OW50	ASTLOT	1	2	10W20	BOUDAC	6	5	11SE20	IVAAXI	1	3	12NE20	BOUDAC	6	4
ONE50	ERILON	1	4	OW50	BOUDAC	7	2	10W20	BOUGRA	3	5	11SE20	MIRLIN	1	3	12NE20	BOUGRA	4	4
ONE50	ESCIVIV	1	4	OW50	BOUGRA	6	2	10W20	CARSIM	1	5	11SE20	PSOTEN	7	3	12NE20	FESOC	1	4
ONE50	LICHEN	1	4	OW50	ERILON	1	2	10W20	HESCOM	1	5	11SE20	SOPNUT	1	3	12NE20	GRISOU	1	4
ONE50	OPUPOL	1	4	OW50	LACSER	1	2	10W20	OPUPOL	3	5	11SE20	TRIREP	1	3	12NE20	MELOFF	1	4
ONE50	PHYFEN	1	4	OW50	LICHEN	1	2	10W20	PASSMI	6	5	11SE50	AGRCRI	3	3	12NE20	OPUPOL	2	4
ONE50	PLAPAT	1	4	OW50	OPUPOL	2	2	10W20	PSOTEN	3	5	11SE50	ARTFRI	1	3	12NE20	PASSMI	3	4
ONE50	RATCOL	1	4	OW50	PHYFEN	1	2	10W50	AGRCRI	2	3	11SE50	BOUDAC	5	3	12NE20	PICOPP	1	4
ONE50	SPHCOC	1	4	OW50	PLAPAT	1	2	10W50	ARTFRI	2	3	11SE50	BOUGRA	4	3	12NE20	PLAPAT	1	4
ONE50	TRADUB	1	4	OW50	RATCOL	1	2	10W50	BOUDAC	6	3	11SE50	FESOC	4	3	12NE20	PSOTEN	3	4
OSE100	ARIPUR	2	3	OW50	SOPNUT	2	2	10W50	BOUGRA	3	3	11SE50	HETVIL	1	3	12NE20	SOPNUT	1	4
OSE100	BOUDAC	6	3	OW50	SPHCOC	1	2	10W50	CARSIM	3	3	11SE50	MELOFF	1	3	12NE50	AGRCRI	5	3
OSE100	BOUGRA	6	3	10NE100	AGRCRI	2	4	10W50	HESCOM	1	3	11SE50	OPUPOL	1	3	12NE50	ARTFRI	1	3
OSE100	ELYELY	1	3	10NE100	ARTFRI	2	4	10W50	OPUPOL	2	3	11SE50	PHYFEN	1	3	12NE50	BOUDAC	5	3
OSE100	ERILON	1	3	10NE100	BOUDAC	6	4	10W50	PASSMI	3	3	11SE50	PLAPAT	1	3	12NE50	BOUGRA	4	3
OSE100	LICHEN	1	3	10NE100	BOUGRA	3	4	10W50	PSOTEN	3	3	11SE50	PSOTEN	2	3	12NE50	ERINAU	1	3
OSE100	LYGIUN	1	3	10NE100	BRIEUP	1	4	11NE100	AGRCRI	6	3	11SE50	SOPNUT	2	3	12NE50	FESOC	2	3
OSE100	OPUPOL	1	3	10NE100	FESOC	1	4	11NE100	ARTFRI	1	3	11SE50	SPHCOC	2	3	12NE50	HESCOM	3	3
OSE100	PASSMI	2	3	10NE100	HESCOM	1	4	11NE100	BOUDAC	4	3	11SE50	SPOCRY	1	3	12NE50	HYMFIL	1	3
OSE100	PHYFEN	1	3	10NE100	PASSMI	5	4	11NE100	BOUGRA	2	3	11W100	AGRCRI	6	4	12NE50	OPUPOL	1	3
OSE100	PLAPAT	1	3	10NE100	PICOPP	1	4	11NE100	CHAGLY	1	3	11W100	ARTFRI	1	4	12NE50	PLAPAT	2	3
OSE100	SONOLE	1	3	10NE100	PSOTEN	2	4	11NE100	ERINAU	1	3	11W100	BOUDAC	5	4	12NE50	RATCOL	1	3
OSE100	SPHCOC	1	3	10NE20	ARTFRI	1	4	11NE100	FESOC	4	3	11W100	CRYCIN	1	4	12NE50	SCHPAN	1	3
OSE100	THLARV	1	3	10NE20	BOUDAC	7	4	11NE100	HESCOM	3	3	11W100	FESOC	4	4	12NE50	SOPNUT	1	3
OSE100	TRADUB	1	3	10NE20	BOUGRA	3	4	11NE100	HORIUB	2	3	11W100	LACSER	1	4	12NE50	SPHCOC	1	3
OSE20	AGRCRI	1	5	10NE20	CIRFLO	3	4	11NE100	OPUPOL	3	3	11W100	OPUPOL	3	4	12SE100	AGRCRI	6	4
OSE20	AGRSTO	1	5	10NE20	FESOC	1	4	11NE100	PHYFEN	1	3	11W100	PHYFEN	1	4	12SE100	ARTFRI	1	4
OSE20	ARIPUR	1	5	10NE20	LIAPUN	1	4	11NE100	SOPNUT	1	3	11W100	PLAPAT	1	4	12SE100	BOUDAC	4	4
OSE20	BROTEC	6	5	10NE20	MELOFF	1	4	11NE100	SPHCOC	1	3	11W100	SOPNUT	1	4	12SE100	BOUGRA	4	4
OSE20	CHAGLY	1	5	10NE20	PASSMI	5	4	11NE100	SPOCRY	2	3	11W100	SPOCRY	1	4	12SE100	ERINAU	2	4
OSE20	CONCAN	1	5	10NE20	PSOTEN	3	4	11NE100	TRIREP	1	3	11W100	TRADUB	1	4	12SE100	FESOC	5	4
OSE20	ERILON	1	5	10NE20	SPHCOC	1	4	11NE20	ARTFRI	2	3	11W20	AGRCRI	2	4	12SE100	HESCOM	4	4
OSE20	HESSPA	1	5	10NE50	ARTFRI	1	4	11NE20	BOUDAC	5	3	11W20	AMAALB	1	4	12SE100	MELOFF	1	4
OSE20	MIRLIN	1	5	10NE50	BOUDAC	7	4	11NE20	BOUGRA	4	3	11W20	ARTFRI	2	4	12SE100	OPUPOL	3	4
OSE20	PASSMI	6	5	10NE50	BOUGRA	3	4	11NE20	CHAGLY	1	3	11W20	BOUDAC	5	4	12SE100	PLAPAT	1	4
OSE20	RATCOL	1	5	10NE50	FESOC	2	4	11NE20	FESOC	4	3	11W20	BOUGRA	5	4	12SE100	PSOTEN	1	4
OSE20	SALTRA	1	5	10NE50	MELOFF	1	4	11NE20	HESCOM	3	3	11W20	CARSIM	1	4	12SE100	SPOCRY	1	4
OSE20	SOPNUT	1	5	10NE50	OPUPOL	2	4	11NE20	LACSER	1	3	11W20	CONCAN	1	4	12SE20	AGRCRI	3	5
OSE20	SPHCOC	1	5	10NE50	PASSMI	1	4	11NE20	MELOFF	1	3	11W20	FESOC	5	4	12SE20	BOUDAC	6	5
OSE20	THLARV	1	5	10NE50	PHYFEN	1	4	11NE20	OPUPOL	2	3	11W20	LACSER	2	4	12SE20	BOUGRA	4	5
OSE20	TRADUB	1	5	10NE50	PLAPAT	1	4	11NE20	OXYSER	3	3	11W20	MELOFF	1	4	12SE20	BROTEC	1	5
OSE20	VERBRA	1	5	10NE50	PSOTEN	2	4	11NE20	PHYFEN	1	3	11W20	OPUPOL	1	4	12SE20	FESOC	1	5
OSE50	ARIPUR	1	4	10NE50	SCHPAN	1	4	11NE20	PLAPAT	1	3	11W20	PLAPAT	2	4	12SE20	HESCOM	1	5
OSE50	BOUDAC	6	4	10NE50	SOLCAN	1	4	11NE20	PSOTEN	3	3	11W20	PSOTEN	1	4	12SE20	OPUPOL	2	5
OSE50	BOUGRA	6	4	10SE100	AGRCRI	3	2	11NE20	RATCOL	1	3	11W20	RATCOL	1	4	12SE20	PASSMI	1	5
OSE50	CHAGLY	1	4	10SE100	AMAALB	1	2	11NE20	SOPNUT	1	3	11W20	SOPNUT	1	4	12SE20	PLAPAT	1	5
OSE50	ELYELY	2	4	10SE100	ARTFRI	1	2	11NE50	AGRCRI	6	4	11W20	SPHCOC	2	4	12SE20	PSOTEN	2	5
OSE50	ERILON	1	4	10SE100	BOUDAC	7	2	11NE50	ARTFRI	1	4	11W20	SPOCRY	1	4	12SE20	SOLCAN	1	5
OSE50	HESSPA	1	4	10SE100	BOUGRA	3	2	11NE50	BOUDAC	5	4	11W50	AGRCRI	3	3	12SE20	SOPNUT	1	5
OSE50	OPUPOL	1	4	10SE100	CARSIM	2	2	11NE50	BOUGRA	3	4	11W50	AMAALB	1	3	12SE20	SPHCOC	1	5
OSE50	PASSMI	5	4	10SE100	ERINAU	1	2	11NE50	ESCIVIV	1	4	11W50	ARTFRI	3	3	12SE50	AGRCRI	4	3
OSE50	PLAPAT	1	4	10SE100	FESOC	2	2	11NE50	FESOC	2	4	11W50	BOUDAC	5	3	12SE50	ARTABS	1	3
OSE50	SONOLE	1	4	10SE100	HESCOM	1	2	11NE50	HESCOM	4	4	11W50	BOUGRA	4	3	12SE50	BOUDAC	5	3
OSE50	SPHCOC	1	4	10SE100	LACSER	1	2	11NE50	HETVIL	1	4	11W50	BROTEC	1	3	12SE50	BOUGRA	5	3

Notes: Plots are coded by site-direction-distance. Cover is the species percent cover per plot. Bare is

Table 6. Continued.

Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare
11W50	CONCAN	2	3	12SE50	FESOC	5	3	13SE100	ELYCAN	1	2	13W50	SPHCOC	1	2	14SE20	CLESER	2	6
11W50	FESOC	5	3	12SE50	HESCOM	4	3	13SE100	ERILON	1	2	13W50	SUCULNT	1	2	14SE20	CONCAN	1	6
11W50	HESCOM	1	3	12SE50	OPUPOL	2	3	13SE100	LINRIG	1	2	14NE100	AMASPI	1	3	14SE20	CRYCAN	1	6
11W50	HETVIL	1	3	12SE50	PASSMI	2	3	13SE100	MIRLIN	1	2	14NE100	ARIPUR	2	3	14SE20	ELYCAN	1	6
11W50	LINRIG	1	3	12SE50	PLAPAT	1	3	13SE100	OPUPOL	1	2	14NE100	ARTFRI	1	3	14SE20	GUTSAR	1	6
11W50	OPUPOL	2	3	12SE50	PSOTEN	2	3	13SE100	PASSMI	4	2	14NE100	BOUDAC	5	3	14SE20	HORVUL	1	6
11W50	PASSMI	3	3	12SE50	TRADUB	1	3	13SE100	PLAPAT	1	2	14NE100	BOUGRA	7	3	14SE20	MIRLIN	1	6
11W50	PLAPAT	3	3	12W100	AGRCRI	4	3	13SE100	SONOLE	1	2	14NE100	CHAGLY	1	3	14SE20	OENSUF	1	6
11W50	RATCOL	3	3	12W100	ARTFRI	2	3	13SE100	SOPNUT	1	2	14NE100	CONCAN	1	3	14SE20	PASSMI	3	6
11W50	SOLCAN	1	3	12W100	BOUDAC	6	3	13SE100	SPHCOC	1	2	14NE100	ELYCAN	2	3	14SE20	PHYFEN	1	6
11W50	SPHCOC	1	3	12W100	BOUGRA	3	3	13SE100	THLARV	1	2	14NE100	ERILON	1	3	14SE20	PSOTEN	2	6
12NE100	AGRCRI	4	2	12W100	BROTEC	3	3	13SE20	BOUDAC	9	2	14NE100	MACPIN	1	3	14SE20	SALTRA	2	6
12NE100	ARTFRI	2	2	12W100	FESOC	6	3	13SE20	BOUGRA	4	2	14NE100	MIRLIN	1	3	14SE20	SOLROS	1	6
12NE100	BOUDAC	6	2	12W100	HESCOM	1	3	13SE20	ERILON	1	2	14NE100	OPUPOL	1	3	14SE20	SOLTRI	1	6
12NE100	BOUGRA	2	2	12W100	OPUPOL	1	3	13SE20	HETVIL	1	2	14NE100	PSOTEN	1	3	14SE20	SPHCOC	1	6
12NE100	ERINAU	2	2	12W100	PHYFEN	1	3	13SE20	LINRIG	1	2	14NE100	RATCOL	2	3	14SE20	THLARV	1	6
12NE100	FESOC	4	2	12W100	PSOTEN	2	3	13SE20	NA	1	2	14NE100	SALTRA	1	3	14SE20	TRIRIP	1	6
12NE100	HESCOM	3	2	12W100	SPHCOC	1	3	13SE20	OPUPOL	1	2	14NE100	SONOLE	1	3	14SE20	TRITER	1	6
12NE100	LACSER	1	2	12W100	SPOCRY	1	3	13SE20	PLAPAT	1	2	14NE100	SPHCOC	1	3	14SE20	VERBRA	1	6
12NE100	PLAPAT	1	2	12W20	AGRCRI	5	4	13SE20	RATCOL	1	2	14NE100	TRADUB	1	3	14SE20	ARTFRI	1	3
12NE100	PSOTEN	3	2	12W20	ARTFRI	1	4	13SE20	SALTRA	1	2	14NE100	VERBRA	1	3	14SE20	BASSCO	1	3
12NE100	SPOCRY	1	2	12W20	BOUDAC	7	4	13SE20	SPHCOC	1	2	14NE100	VICAME	1	3	14SE20	BOUDAC	6	3
12NE20	AGRCRI	3	4	12W20	BOUGRA	3	4	13SE50	BASSCO	1	2	14NE20	ABRFRA	1	5	14SE20	BOUGRA	7	3
12NE20	ARTFRI	1	4	12W20	HESCOM	1	4	13SE50	BOUDAC	5	2	14NE20	AMAALB	1	5	14SE20	CHAGLY	1	3
12NE20	BOUCUR	2	4	12W20	LYGIUN	1	4	13SE50	BOUGRA	6	2	14NE20	BASSCO	1	5	14SE20	CIROCH	1	3
12NE20	BOUDAC	6	4	12W20	MELOFF	1	4	13SE50	ELYCAN	1	2	14NE20	BOUDAC	3	5	14SE20	ELYCAN	2	3
12NE20	BOUGRA	4	4	12W20	OPUPOL	1	4	13SE50	ERILON	1	2	14NE20	BOUGRA	6	5	14SE20	ERILON	1	3
12NE20	FESOC	1	4	12W20	PASSMI	5	4	13SE50	HESCOM	1	2	14NE20	CALBER	4	5	14SE20	LUPPUS	1	3
12NE20	GRISQU	1	4	12W50	AGRCRI	6	5	13SE50	LINRIG	1	2	14NE20	CHAGLY	1	5	14SE20	OENSUF	1	3
12NE20	MELOFF	1	4	12W50	ARTFRI	3	5	13SE50	OPUPOL	1	2	14NE20	CHEBER	1	5	14SE20	PANVIR	1	3
12NE20	OPUPOL	2	4	12W50	BOUDAC	4	5	13SE50	PASSMI	5	2	14NE20	CLESER	1	5	14SE20	PENALB	1	3
12NE20	PASSMI	3	4	12W50	GUTSAR	1	5	13SE50	PLAPAT	1	2	14NE20	ELYCAN	2	5	14SE20	PHYFEN	1	3
12NE20	PICOPP	1	4	12W50	HESCOM	2	5	13SE50	RATCOL	1	2	14NE20	GUTSAR	1	5	14SE20	PLAPAT	1	3
12NE20	PLAPAT	1	4	12W50	LYGIUN	1	5	13SE50	SONOLE	1	2	14NE20	OENSUF	1	5	14SE20	PSOTEN	1	3
12NE20	PSOTEN	3	4	12W50	MELOFF	1	5	13SE50	SOPNUT	1	2	14NE20	PASSMI	2	5	14SE20	SALTRA	1	3
12NE20	SOPNUT	1	4	12W50	PASSMI	1	5	13SE50	SPHCOC	1	2	14NE20	RATCOL	2	5	14SE20	SPHCOC	1	3
12NE50	AGRCRI	5	3	12W50	PSOTEN	2	5	13SE50	THLARV	1	2	14NE20	SALTRA	1	5	14SE20	THLARV	1	3
12NE50	ARTFRI	1	3	12W50	SOPNUT	1	5	13SE50	TRADUB	1	2	14NE20	SOLROS	1	5	14SE20	VERBRA	1	3
12NE50	BOUDAC	5	3	12W50	SPHCOC	1	5	13SE50	VERBRA	1	2	14NE20	SPHCOC	1	5	14W100	AMAALB	1	5
12NE50	BOUGRA	4	3	13NE100	AMAALB	1	4	13W100	BOUDAC	7	2	14NE20	THLARV	1	5	14W100	ERILON	1	5
12NE50	ERINAU	1	3	13NE100	BOUDAC	6	4	13W100	BOUGRA	4	2	14NE20	VERBRA	1	5	14W100	BOUDAC	2	5
12NE50	FESOC	2	3	13NE100	BOUGRA	6	4	13W100	ERILON	1	2	14NE50	ASTLOT	1	2	14W100	BOUGRA	6	5
12NE50	HESCOM	3	3	13NE100	ERIAN	1	4	13W100	MIRLIN	1	2	14NE50	BASSCO	1	2	14W100	CHAGLY	1	5
12NE50	HYMFIL	1	3	13NE100	ERILON	1	4	13W100	OPUPOL	2	2	14NE50	BOUDAC	3	2	14W100	ERILON	1	5
12NE50	OPUPOL	1	3	13NE100	ERYASP	1	4	13W100	PASSMI	5	2	14NE50	BOUGRA	7	2	14W100	HESCOM	6	5
12NE50	PLAPAT	2	3	13NE100	MIRLIN	1	4	13W100	PLAPAT	1	2	14NE50	CHAGLY	1	2	14W100	OENSUF	1	5
12NE50	RATCOL	1	3	13NE100	OPUPOL	1	4	13W100	SONOLE	1	2	14NE50	ELYCAN	4	2	14W100	OPUPOL	1	5
12NE50	SCHPAN	1	3	13NE100	SOPNUT	1	4	13W100	SOPNUT	2	2	14NE50	ERILON	3	2	14W100	PHYFEN	1	5
12NE50	SOPNUT	1	3	13NE100	SPHCOC	1	4	13W100	SPHCOC	1	2	14NE50	HESCOM	1	2	14W100	PLAPAT	1	5
12NE50	SPHCOC	1	3	13NE100	THLARV	1	4	13W100	TRADUB	1	2	14NE50	MACPIN	1	2	14W100	PSOTEN	1	5
12SE100	AGRCRI	6	4	13NE20	ARIPUR	1	4	13W20	AMAALB	2	7	14NE50	PANVIR	1	2	14W100	SALTRA	1	5
12SE100	ARTFRI	1	4	13NE20	BOUDAC	4	4	13W20	BASSCO	1	7	14NE50	PASSMI	1	2	14W100	SPHCOC	1	5
12SE100	BOUDAC	4	4	13NE20	BOUGRA	3	4	13W20	BOUDAC	4	7	14NE50	PLAPAT	1	2	14W100	THLARV	1	5
12SE100	BOUGRA	4	4	13NE20	CALBER	1	4	13W20	BOUGRA	1	7	14NE50	RATCOL	2	2	14W100	TRADUB	1	5
12SE100	ERINAU	2	4	13NE20	ELYCAN	1	4	13W20	BROINE	1	7	14NE50	SPHCOC	1	2	14W20	BOUDAC	4	4
12SE100	FESOC	5	4	13NE20	ERILON	1	4	13W20	CONCAN	1	7	14NE50	THLARV	1	2	14W20	BOUGRA	8	4
12SE100	HESCOM	4	4	13NE20	ESCIVV	1	4	13W20	ELYCAN	2	7	14NE50	TRADUB	1	2	14W20	CALBER	1	4
12SE100	MELOFF	1	4	13NE20	HESSPA	2	4	13W20	MIRLIN	1	7	14SE100	ARIPUR	2	2	14W20	ELYCAN	1	4
12SE100	OPUPOL	3	4	13NE20	OPUPOL	1	4	13W20	PANVIR	1	7	14SE100	ARTFRI	2	2	14W20	ERILON	1	4
12SE100	PLAPAT	1	4	13NE20	PANVIR	1	4	13W20	PASSMI	4	7	14SE100	BOUDAC	4	2	14W20	HESCOM	1	4
12SE100	PSOTEN	1	4	13NE20	PASSMI	7	4	13W20	POLAVI	1	7	14SE100	BOUGRA	4	2	14W20	OPUPOL	1	4
12SE100	SPOCRY	1	4	13NE20	PLAPAT	1	4	13W20	POROLE	1	7	14SE100	CHAGLY	1	2	14W20	PENALB	1	4
12SE20	AGRCRI	3	5	13NE20	SCHPAN	1	4	13W20	SCHPAN	1	7	14SE100	CIROCH	1	2	14W20	PHYFEN	1	4
12SE20	BOUDAC	6	5	13NE20	SOPNUT	1	4	13W20	SPHCOC	1	7	14SE100	ELYCAN	3	2	14W20	PLAPAT	1	4
12SE20	BOUGRA	4	5	13NE20	SPHCOC	1	4	13W20	THLARV	1	7	14SE100	ERILON	1	2	14W20	PSOTEN	1	4
12SE20	BROTEC	1	5	13NE20	THLARV	1	4	13W20	VERBRA	1	7	14SE100	LYGIUN	1	2	14W20	SALTRA	1	4
12SE20	FESOC	1	5	13NE50	AMAALB	1	2	13W50	BASSCO	1	2	14SE100	OPUPOL	1	2	14W20	SPHCOC	1	4
12SE20	HESCOM	1	5	13NE50	BASSCO	1	2	13W50	BOUDAC	6	2	14SE100	PACTRI	1	2	14W50	ARTDRA	1	2
12SE20	OPUPOL	2	5	13NE50	BOUDAC	7	2	13W50	BOUGRA	6	2	14SE100	PLAPAT	1	2	14W50	ARTFRI	1	2
12SE20	PASSMI	1	5	13NE50	BOUGRA	5	2	13W50	ERYASP	1	2	14SE100	PSOTEN	1	2	14W50	BOUDAC	5	2
12SE20	PLAPAT	1	5	13NE50	ERILON	1	2	13W50	LINRIG	1	2	14SE100	SPHCOC	1	2	14W50	BOUGRA	8	2
12SE20	PSOTEN	2	5	13NE50	MIRLIN	1	2	13W50	MIRLIN	1	2	14SE100	TRADUB	1	2	14W50	CALBER	1	2
12SE20	SOLCAN	1	5	13NE50	OPUPOL	1	2	13W50	OPUPOL	1	2	14SE20	ABRFRA	1	6	14W50	CIRSP	1	2
12SE20	SOPNUT	1	5	13NE50	PASSMI	1	2	13W50	PACPLA	1	2	14SE20	ARTFRI	1	6	14W50	LYGIUN	1	2
12SE20	SPHCOC	1	5	13NE50	SONOLE	1	2	13W50	PASSMI	1	2	14SE20	ASTLOT	1	6	14W50	OPUPOL	1	2
12SE50	AGRCRI	4	3	13NE50	SOPNUT	2	2	13W50	PLAPAT	1	2	14SE20	BOUDAC	2	6	14W50	PLAPAT	1	2
12SE50	ARTABS	1	3	13NE50	SPHCOC	1	2	13W50	RATCOL	2	2	14SE20	BOUGRA	5	6	14W50	PSOTEN	4	2
12SE50	BOUDAC	5	3	13SE100	BOUDAC	5	2	13W50	SONOLE	1	2	14SE20	BRIEUP	1	6	14W50	RATCOL	2	2
12SE50	BOUGRA	5	3	13SE100	BOUGRA	7	2												

Table 6. Continued.

Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare
14W50	THLARV	1	2	15W20	CIRCAN	1	3	16SE50	PLAPAT	1	1	17SE20	PLAPAT	1	2	18NE20	BROTEC	2	2
15NE100	ARIPUR	1	2	15W20	CONCAN	1	3	16SE50	SOPNUT	1	1	17SE20	SOPNUT	1	2	18NE20	CONCAN	1	2
15NE100	BOUDAC	3	2	15W20	ECHVIR	1	3	16SE50	SPHCOC	1	1	17SE20	SPHCOC	1	2	18NE20	ELYELY	1	2
15NE100	BOUGRA	8	2	15W20	ERYASP	1	3	16W100	BOUDAC	6	2	17SE20	SPOCRY	1	2	18NE20	HETVIL	1	2
15NE100	CONCAN	1	2	15W20	FESOC	6	3	16W100	BOUGRA	4	2	17SE20	VICAME	1	2	18NE20	MACPIN	1	2
15NE100	LAPOCC	1	2	15W20	HORJUB	2	3	16W100	CARSIM	2	2	17SE50	ARTFRI	1	2	18NE20	OENSUF	1	2
15NE100	LICHEN	1	2	15W20	OPUPOL	1	3	16W100	ERINAU	1	2	17SE50	ASTLOT	1	2	18NE20	PASSMI	5	2
15NE100	OPUPOL	4	2	15W20	OXYSER	1	3	16W100	ERYASP	1	2	17SE50	BOUDAC	4	2	18NE20	SPHCOC	1	2
15NE100	PASSMI	3	2	15W20	PENALB	1	3	16W100	FESOC	6	2	17SE50	BOUGRA	7	2	18NE50	ASTLOT	1	2
15NE100	SOPNUT	1	2	15W20	PLAPAT	1	3	16W100	OPUPOL	4	2	17SE50	ERYASP	1	2	18NE50	BOUDAC	7	2
15NE100	SPHCOC	2	2	15W20	SPHCOC	1	3	16W100	PASSMI	6	2	17SE50	FESOC	1	2	18NE50	BOUGRA	5	2
15NE20	BOUDAC	2	3	15W50	BOUDAC	2	3	16W100	PLAPAT	1	2	17SE50	HORJUB	1	2	18NE50	CONCAN	1	2
15NE20	BOUGRA	3	3	15W50	BOUGRA	7	3	16W100	SOPNUT	1	2	17SE50	OPUPOL	1	2	18NE50	HETVIL	1	2
15NE20	BROTEC	7	3	15W50	CONCAN	1	3	16W100	TRADUB	1	2	17SE50	PASSMI	1	2	18NE50	MIRLIN	1	2
15NE20	CIRCAN	2	3	15W50	ERYASP	1	3	16W20	BOUDAC	6	1	17SE50	PLAPAT	1	2	18NE50	OPUPOL	1	2
15NE20	CONCAN	1	3	15W50	FESOC	5	3	16W20	BOUGRA	4	1	17SE50	SONOLE	1	2	18NE50	SPHCOC	1	2
15NE20	DESSOP	1	3	15W50	HORJUB	1	3	16W20	FESOC	6	1	17SE50	SOPNUT	1	2	18SE100	AMASPI	1	2
15NE20	DIGSAN	1	3	15W50	LICHEN	1	3	16W20	OPUPOL	1	1	17SE50	SPHCOC	1	2	18SE100	BOUDAC	6	2
15NE20	ERYASP	1	3	15W50	OPUPOL	1	3	16W20	PASSMI	4	1	17SE50	VICAME	1	2	18SE100	BOUGRA	6	2
15NE20	SPHCOC	2	3	15W50	SPHCOC	2	3	16W20	PLAPAT	1	1	17W100	BOUDAC	2	1	18SE100	OPUPOL	1	2
15NE20	SPOCRY	3	3	16NE100	BOUDAC	6	3	16W20	SOPNUT	2	1	17W100	BOUGRA	7	1	18SE100	PENALB	1	2
15NE20	TAROFF	1	3	16NE100	BOUGRA	3	3	16W20	SPHCOC	1	1	17W100	CONCAN	1	1	18SE100	OPUNAL	1	2
15NE50	BOUDAC	4	4	16NE100	CRYCIN	1	3	16W50	BOUDAC	6	2	17W100	CRYCIN	1	1	18SE100	SPHCOC	1	2
15NE50	BOUGRA	3	4	16NE100	ERYASP	2	3	16W50	BOUGRA	4	2	17W100	ERYASP	1	1	18SE100	VICAME	1	2
15NE50	CIRCAN	1	4	16NE100	FESOC	6	3	16W50	FESOC	6	2	17W100	FESOC	2	1	18SE20	AMASPI	1	4
15NE50	CONCAN	1	4	16NE100	HESCOM	1	3	16W50	OPUPOL	3	2	17W100	HORJUB	1	1	18SE20	ASTMOL	1	4
15NE50	HORJUB	5	4	16NE100	OPUPOL	3	3	16W50	PASSMI	6	2	17W100	LUPPUS	1	1	18SE20	BOUDAC	7	4
15NE50	LACSER	1	4	16NE100	PASSMI	2	3	16W50	PLAPAT	1	2	17W100	OENSUF	1	1	18SE20	BOUGRA	4	4
15NE50	SPHCOC	3	4	16NE100	PLAPAT	3	3	16W50	SOPNUT	1	2	17W100	OPUPOL	1	1	18SE20	BROTEC	2	4
15NE50	SPOCRY	3	4	16NE100	SOLCAN	1	3	17NE100	BOUDAC	6	2	17W100	PLAPAT	2	1	18SE20	CONARV	1	4
15NE50	TAROFF	1	4	16NE100	SOPNUT	1	3	17NE100	BOUGRA	6	2	17W100	SISALT	1	1	18SE20	CONCAN	1	4
15NE50	VICAME	1	4	16NE100	SPHCOC	1	3	17NE100	DESSOP	1	2	17W100	SOPNUT	1	1	18SE20	ELYELY	1	4
15SE100	BOUDAC	2	3	16NE20	BOUDAC	6	4	17NE100	FESOC	1	2	17W100	SPHCOC	1	1	18SE20	HETVIL	1	4
15SE100	BOUGRA	6	3	16NE20	BOUGRA	3	4	17NE100	LUPPUS	1	2	17W100	VICAME	1	1	18SE20	LEPDEN	1	4
15SE100	CARSIM	1	3	16NE20	BROTEC	5	4	17NE100	OPUPOL	1	2	17W20	BOUDAC	8	2	18SE20	PASSMI	3	4
15SE100	DESSOP	1	3	16NE20	FESOC	3	4	17NE100	PASSMI	4	2	17W20	BOUGRA	2	2	18SE20	PENALB	1	4
15SE100	ERYASP	1	3	16NE20	HESCOM	5	4	17NE100	PLAPAT	1	2	17W20	ERYASP	1	2	18SE20	PLAPAT	1	4
15SE100	FESOC	6	3	16NE20	HORJUB	1	4	17NE100	SOPNUT	1	2	17W20	ESCIVIV	1	2	18SE20	SALTRA	3	4
15SE100	HORJUB	3	3	16NE20	PASSMI	6	4	17NE100	SPHCOC	1	2	17W20	FESOC	2	2	18SE20	SOPNUT	1	4
15SE100	OPUPOL	1	3	16NE20	PLAPAT	1	4	17NE20	AGRCRI	1	1	17W20	HORJUB	2	2	18SE20	SPHCOC	1	4
15SE100	SPHCOC	2	3	16NE20	SPHCOC	1	4	17NE20	BROTEC	1	1	17W20	LUPPUS	1	2	18SE50	BOUDAC	7	3
15SE20	BOUDAC	2	3	16NE50	BOUDAC	8	3	17NE20	CONCAN	1	1	17W20	OENSUF	1	2	18SE50	BOUGRA	5	3
15SE20	BOUGRA	5	3	16NE50	BOUGRA	1	3	17NE20	FESOC	2	1	17W20	OPUPOL	1	2	18SE50	CONCAN	1	3
15SE20	BROTEC	6	3	16NE50	FESOC	1	3	17NE20	PASSMI	8	1	17W20	PACPLA	1	2	18SE50	ELYELY	2	3
15SE20	CARSIM	1	3	16NE50	HESCOM	2	3	17NE20	SISALT	2	1	17W20	PASSMI	1	2	18SE50	OPUPOL	1	3
15SE20	DESSOP	1	3	16NE50	OPUPOL	1	3	17NE20	SONOLE	1	1	17W20	PLAPAT	2	2	18SE50	SPHCOC	1	3
15SE20	ERYASP	4	3	16NE50	PASSMI	5	3	17NE50	BOUDAC	3	3	17W20	SONOLE	1	2	18SE50	VICAME	1	3
15SE20	FESOC	3	3	16NE50	PLAPAT	1	3	17NE50	BOUGRA	3	3	17W20	SOPNUT	1	2	18W100	BOUDAC	4	3
15SE20	OPUPOL	1	3	16NE50	SCHPAN	2	3	17NE50	CONCAN	1	3	17W20	SPHCOC	1	2	18W100	BOUGRA	8	3
15SE20	PLAPAT	1	3	16NE50	SOLCAN	1	3	17NE50	ERYASP	1	3	17W20	SPOCRY	1	2	18W100	CONCAN	1	3
15SE20	SISALT	1	3	16NE50	SOPNUT	1	3	17NE50	FESOC	2	3	17W20	VERBRA	1	2	18W100	OPUPOL	2	3
15SE20	SPHCOC	1	3	16NE50	SPHCOC	1	3	17NE50	HORJUB	1	3	17W50	BOUDAC	4	2	18W100	PICOPP	1	3
15SE50	BOUDAC	3	2	16SE100	BOUDAC	7	1	17NE50	OENSUF	1	3	17W50	BOUGRA	7	2	18W100	PLAPAT	1	3
15SE50	BOUGRA	5	2	16SE100	BOUGRA	5	1	17NE50	OPUPOL	1	3	17W50	CONCAN	1	2	18W100	SPHCOC	1	3
15SE50	BROTEC	1	2	16SE100	EVONUT	1	1	17NE50	PANCAP	1	3	17W50	ERYASP	1	2	18W100	VICAME	1	3
15SE50	CARSIM	4	2	16SE100	FESOC	5	1	17NE50	PASSMI	7	3	17W50	FESOC	4	2	18W20	AGRCRI	2	3
15SE50	CIRCAN	2	2	16SE100	HESCOM	3	1	17NE50	PLAPAT	1	3	17W50	HORJUB	1	2	18W20	BOUDAC	6	3
15SE50	CONCAN	1	2	16SE100	OPUPOL	3	1	17NE50	SECCER	1	3	17W50	LUPPUS	1	2	18W20	BOUGRA	2	3
15SE50	DESSOP	1	2	16SE100	PASSMI	4	1	17NE50	SOPNUT	1	3	17W50	MIRLIN	1	2	18W20	BROTEC	2	3
15SE50	ERYASP	2	2	16SE100	SOPNUT	1	1	17NE50	SPHCOC	1	3	17W50	OPUPOL	1	2	18W20	CIRUND	1	3
15SE50	FESOC	6	2	16SE100	SPHCOC	1	1	17SE100	BOUDAC	2	2	17W50	PLAPAT	1	2	18W20	HETVIL	1	3
15SE50	HORJUB	3	2	16SE20	AGRSTO	1	1	17SE100	BOUGRA	7	2	17W50	SONOLE	1	2	18W20	LEPDEN	1	3
15SE50	OPUPOL	1	2	16SE20	BOUDAC	7	1	17SE100	ERYASP	1	2	17W50	SOPNUT	1	2	18W20	PASSMI	6	3
15SE50	PASSMI	3	2	16SE20	BOUGRA	3	1	17SE100	FESOC	2	2	17W50	SPHCOC	1	2	18W20	PICOPP	1	3
15SE50	PLAPAT	1	2	16SE20	ERYASP	1	1	17SE100	HORJUB	1	2	18NE100	BOUDAC	5	3	18W20	PLAPAT	1	3
15SE50	SENSPA	1	2	16SE20	FESOC	6	1	17SE100	OPUPOL	1	2	18NE100	BOUGRA	7	3	18W20	SPHCOC	1	3
15SE50	SISALT	1	2	16SE20	OPUPOL	1	1	17SE100	PASSMI	3	2	18NE100	CONCAN	1	3	18W20	VICAME	1	3
15SE50	SPHCOC	3	2	16SE20	PASSMI	3	1	17SE100	PLAPAT	1	2	18NE100	ELYELY	1	3	18W50	BOUDAC	5	2
15SE50	VICAME	1	2	16SE20	PLAPAT	1	1	17SE100	SOPNUT	1	2	18NE100	HETVIL	1	3	18W50	BOUGRA	7	2
15W100	BOUDAC	5	3	16SE20	SOPNUT	1	1	17SE100	SPHCOC	1	2	18NE100	MIRLIN	1	3	18W50	ELYELY	1	2
15W100	BOUGRA	6	3	16SE20	SPHCOC	1	1	17SE20	BOUDAC	5	2	18NE100	OPUPOL	1	3	18W50	MIRLIN	1	2
15W100	CARSIM	3	3	16SE50	AMBTOM	1	1	17SE20	CONCAN	1	2	18NE100	PASSMI	2	3	18W50	OPUPOL	2	2
15W100	FESOC	3	3	16SE50	BOUDAC	5	1	17SE20	FESOC	4	2	18NE100	PENALB	1	3	18W50	PASSMI	1	2
15W100	LICHEN	1	3	16SE50	BOUGRA	3	1	17SE20	HESCOM	4	2	18NE100	PICOPP	1	3	18W50	SPHCOC	1	2
15W100	OPUPOL	3	3	16SE50	FESOC	5	1	17SE20	HORJUB	1	2	18NE100	PLAPAT	1	3	18W50	VICAME	1	2
15W100	SPHCOC	1	3	16SE50	HESCOM	1	1	17SE20	OENSUF	1	2	18NE100	SPHCOC	1	3	19NE100	BOUDAC	6	4
15W20	BOUDAC	3	3	16SE50	OPUPOL	4	1	17SE20	OPUPOL	1	2	18NE20	ASTMOL	1	2	19NE100	BOUGRA	6	4
15W20	BOUGRA	6	3	16SE50	PASSMI	3	1	17SE20	PACPLA	1	2	18NE20	BOUDAC	7	2	19NE100	OPUPOL	4	4
15W20	CARSIM	1	3	16SE50	PHYFEN	1	1	17SE20	PASSMI</										



Table 6. Continued.

Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare
19NE100	SPHCOC	1	4	19W50	OPUPOL	4	4	21NE20	DIGSAN	3	9	22NE100	PLAPAT	2	2	22W50	SOLROS	1	4
19NE100	SPHCOC	1	4	19W50	PASSMI	1	4	21NE20	PLAPAT	1	9	22NE100	SISALT	1	2	22W50	SPHCOC	1	4
19NE100	TRADUB	1	4	19W50	PHYFEN	1	4	21NE20	PSOTEN	1	9	22NE100	SOPNUT	1	2	22W50	THLARV	1	4
19NE100	VICAME	1	4	19W50	PLAPAT	1	4	21NE20	XANSTR	1	9	22NE100	SPHCOC	2	2	2NE100	AGRCRI	1	4
19NE20	BASSCO	3	3	19W50	SPHCOC	1	4	21NE50	AGRCRI	1	9	22NE100	TAROFF	1	2	2NE100	BOUGRA	6	4
19NE20	BOUDAC	2	3	20NE100	AGRCRI	5	6	21NE50	AMAPOW	1	9	22NE20	SOPNUT	1	9	2NE100	CARSIM	1	4
19NE20	BOUGRA	3	3	20NE100	ARTFRI	2	6	21NE50	AMASPI	1	9	22NE50	ASTPEC	1	4	2NE100	CONCAN	1	4
19NE20	BROTEC	1	3	20NE100	BASSCO	1	6	21NE50	CONARV	1	9	22NE50	BOUDAC	2	4	2NE100	FESOCT	5	4
19NE20	CONCAN	1	3	20NE100	BOUGRA	5	6	21NE50	CONCAN	1	9	22NE50	BOUGRA	5	4	2NE100	HORJUB	2	4
19NE20	ELYLY	6	3	20NE100	CIRFLO	1	6	21NE50	GUTSAR	1	9	22NE50	BROTEC	2	4	2NE100	OPUPOL	1	4
19NE20	HETVIL	1	3	20NE100	CONCAN	1	6	21NE50	PLAPAT	1	9	22NE50	CARSIM	6	4	2NE100	PASSMI	1	4
19NE20	PASSMI	6	3	20NE100	ERINAU	1	6	21NE50	PSOTEN	1	9	22NE50	CIROCH	1	4	2NE100	SALTRA	1	4
19NE20	SALTRA	1	3	20NE100	GRISQU	1	6	21NE50	SOLTRI	1	9	22NE50	DESPIN	1	4	2NE100	SENSPA	1	4
19NE20	SONOLE	3	3	20NE100	HETVIL	1	6	21NE50	SPHCOC	1	9	22NE50	DESSOP	2	4	2NE100	SPHCOC	1	4
19NE20	SPHCOC	1	3	20NE100	HORJUB	2	6	21NE50	XANSTR	1	9	22NE50	FESOCT	2	4	2NE20	AGRCCI	5	3
19NE20	TRADUB	1	3	20NE100	KRALAN	1	6	21SE100	AGRDES	6	6	22NE50	HORJUB	3	4	2NE20	BROTEC	4	3
19NE20	VERBRA	1	3	20NE100	LEPDEN	1	6	21SE100	ARTFRI	1	6	22NE50	HYPRAD	1	4	2NE20	CARSIM	4	3
19NE50	BASSCO	1	4	20NE100	PLAPAT	1	6	21SE100	ARTFRI	1	6	22NE50	OENSUF	2	4	2NE20	CONCAN	1	3
19NE50	BOUGRA	2	4	20NE100	SALTRA	2	6	21SE100	ASTMOL	1	6	22NE50	OPUPOL	1	4	2NE20	DESSOP	1	3
19NE50	CONCAN	1	4	20NE100	SPHCOC	2	6	21SE100	BOUDAC	5	6	22NE50	PASSMI	1	4	2NE20	ECHVIR	1	3
19NE50	ELYLY	3	4	20NE100	TRADUB	1	6	21SE100	GUTSAR	1	6	22NE50	SALTRA	1	4	2NE20	HORJUB	2	3
19NE50	LYGJUN	1	4	20NE100	VERBRA	1	6	21SE100	OPUPOL	1	6	22NE50	SOLCAN	1	4	2NE20	OPUPOL	1	3
19NE50	PASSMI	7	4	20NE50	AGRCCI	1	8	21SE100	PLAPAT	1	6	22NE50	SOPNUT	1	4	2NE20	PASSMI	5	3
19NE50	SONOLE	1	4	20NE50	AMAPOW	1	8	21SE100	SOPNUT	1	6	22NE50	SPHCOC	1	4	2NE20	PLAPAT	1	3
19NE50	SPHCOC	1	4	20NE50	AMASPI	1	8	21SE100	SPHCOC	1	6	22NE50	TRADUB	1	4	2NE20	SISALT	1	3
19NE50	THLARV	1	4	20NE50	BASSCO	1	8	21SE100	THEMEG	1	6	22SE100	BOUDAC	8	4	2NE20	SPHCOC	1	3
19NE50	VICAME	1	4	20NE50	BOUDAC	1	8	21SE100	VICAME	1	6	22SE100	BOUGRA	5	4	2NE20	TRADUB	1	3
19SE100	BASSCO	1	2	20NE50	DIGSAN	4	8	21SE20	AGRCCI	2	8	22SE100	ERILON	1	4	2NE50	AGRCCI	1	3
19SE100	BOUDAC	4	2	20SE100	AGRCCI	6	6	21SE20	AMAPOW	1	8	22SE100	ERINAU	1	4	2NE50	BOUGRA	1	3
19SE100	BOUGRA	9	2	20SE100	ARTFRI	1	6	21SE20	AMASPI	1	8	22SE100	LYGJUN	1	4	2NE50	BROTEC	2	3
19SE100	LYGJUN	1	2	20SE100	ASTGRA	1	6	21SE20	CLESER	1	8	22SE100	MACPIN	1	4	2NE50	CARSIM	5	3
19SE100	MIRLIN	1	2	20SE100	BOUGRA	1	6	21SE20	CONARV	1	8	22SE100	MIRLIN	1	4	2NE50	CONCAN	1	3
19SE100	OPUPOL	3	2	20SE100	ERINAU	1	6	21SE20	DIGSAN	1	8	22SE100	OPUPOL	1	4	2NE50	DESSOP	1	3
19SE100	SONOLE	1	2	20SE100	HETVIL	1	6	21SE20	PSOTEN	1	8	22SE100	PACTRI	1	4	2NE50	ECHVIR	1	3
19SE100	VICAME	1	2	20SE100	LYGJUN	1	6	21SE20	SOLTRI	1	8	22SE100	PASSMI	1	4	2NE50	HORJUB	1	3
19SE20	BASSCO	7	3	20SE100	OENSUF	1	6	21SE20	SOPNUT	1	8	22SE100	PHYFEN	1	4	2NE50	OPUPOL	2	3
19SE20	BOUDAC	2	3	20SE100	OPUPOL	1	6	21SE20	XANSTR	1	8	22SE100	PLAPAT	1	4	2NE50	PASSMI	6	3
19SE20	BOUGRA	2	3	20SE100	SPHCOC	1	6	21SE50	AGRCCI	2	9	22SE100	RATCOL	1	4	2NE50	SALTRA	1	3
19SE20	LYGJUN	2	3	20SE50	AGRCCI	1	8	21SE50	AMASPI	1	9	22SE100	SOPNUT	1	4	2NE50	SISALT	1	3
19SE20	PASSMI	5	3	20SE50	AMAPOW	1	8	21SE50	CONARV	1	9	22SE100	SPHCOC	1	4	2NE50	SPHCOC	1	3
19SE20	SALTRA	2	3	20SE50	BASSCO	1	8	21SE50	ERINAU	1	9	22SE100	TRADUB	1	4	2NE50	TRADUB	1	3
19SE50	BASSCO	1	3	20SE50	CONARV	1	8	21SE50	PSOTEN	1	9	22SE20	SOPNUT	1	9	2SE100	BOUDAC	3	3
19SE50	BOUDAC	6	3	20SE50	DIGSAN	4	8	21SE50	SOLTRI	1	9	22SE50	BOUDAC	4	3	2SE100	BOUGRA	8	3
19SE50	BOUGRA	5	3	20SE50	VERBRA	1	8	21SE50	SOPNUT	1	9	22SE50	BOUGRA	8	3	2SE100	CIRCAN	1	3
19SE50	BROTEC	2	3	20SE50	XANSTR	1	8	21SE50	XANSTR	1	9	22SE50	ELYCAN	1	3	2SE100	CONCAN	1	3
19SE50	CONCAN	1	3	20W100	AGRCCI	7	5	21W100	AGRCCI	2	3	22SE50	ERILON	1	3	2SE100	HETVIL	1	3
19SE50	ELYLY	3	3	20W100	ARTFRI	2	5	21W100	AGRDES	8	3	22SE50	ERINAU	1	3	2SE100	LICHEN	1	3
19SE50	ERYASP	1	3	20W100	ERINAU	1	5	21W100	ARTFRI	3	3	22SE50	ERYASP	1	3	2SE100	OPUPOL	1	3
19SE50	HESSPA	4	3	20W100	HETVIL	1	5	21W100	BOUDAC	2	3	22SE50	HETVIL	1	3	2SE100	PASSMI	1	3
19SE50	PASSMI	4	3	20W100	OPUPOL	1	5	21W100	BOUGRA	4	3	22SE50	LYGJUN	1	3	2SE100	SISALT	1	3
19SE50	PLAPAT	1	3	20W100	PENALB	1	5	21W100	CALBER	1	3	22SE50	OPUPOL	1	3	2SE100	SPHCOC	2	3
19SE50	SONOLE	1	3	20W100	PLAPAT	1	5	21W100	CONCAN	1	3	22SE50	PLAPAT	1	3	2SE20	BOUDAC	2	7
19SE50	SPHCOC	2	3	20W100	SPHCOC	1	5	21W100	ERINAU	1	3	22SE50	SONOLE	1	3	2SE20	BROTEC	1	7
19SE50	THLARV	1	3	20W100	XANSTR	1	5	21W100	OPUPOL	1	3	22SE50	SOPNUT	1	3	2SE20	CARSIM	4	7
19W100	BASSCO	1	4	20W50	AGRCCI	1	8	21W100	PSOTEN	1	3	22SE50	SPHCOC	1	3	2SE20	CONARV	3	7
19W100	BOUDAC	5	4	20W50	AMAALB	1	8	21W100	SPHCOC	1	3	22W100	BOUDAC	5	3	2SE20	DIGSAN	1	7
19W100	BOUGRA	7	4	20W50	AMASPI	1	8	21W100	VICAME	1	3	22W100	BOUGRA	6	3	2SE20	PASSMI	3	7
19W100	MIRLIN	1	4	20W50	DIGSAN	4	8	21W50	AGRCCI	2	9	22W100	CARSIM	2	3	2SE50	BRINE	5	3
19W100	OPUPOL	3	4	20W50	PENALB	1	8	21W50	AMASPI	1	9	22W100	DESSOP	1	3	2SE50	BROTEC	3	3
19W100	PASSMI	1	4	20W50	SECCER	1	8	21W50	CHEBER	1	9	22W100	HETVIL	1	3	2SE50	CARSIM	3	3
19W100	SALTRA	1	4	21NE100	AGRCCI	3	4	21W50	CONARV	1	9	22W100	LICHEN	1	3	2SE50	CIRCAN	1	3
19W100	SPHCOC	1	4	21NE100	AGRDES	7	4	21W50	CONCAN	1	9	22W100	LINPUB	1	3	2SE50	CONARV	2	3
19W100	THLARV	1	4	21NE100	AMASPI	1	4	21W50	DIGSAN	2	9	22W100	LYGJUN	1	3	2SE50	DESSOP	2	3
19W100	VERBRA	1	4	21NE100	ARTDRA	1	4	21W50	GUTSAR	1	9	22W100	OENSUF	1	3	2SE50	DIGSAN	1	3
19W20	BASSCO	1	5	21NE100	ARTFRI	1	4	21W50	PLAPAT	1	9	22W100	OPUPOL	3	3	2SE50	IVAAXI	1	3
19W20	BOUDAC	1	5	21NE100	BOUGRA	3	4	21W50	PSOTEN	1	9	22W100	PACPLA	1	3	2SE50	PASSMI	5	3
19W20	BOUGRA	1	5	21NE100	ERINAU	1	4	21W50	VERBRA	1	9	22W100	PACPLA	2	3	2SE50	SPHCOC	1	3
19W20	CHAGLY	1	5	21NE100	HETVIL	2	4	22NE100	BOUDAC	2	2	22W100	PENALB	1	3	2SE50	TAROFF	1	3
19W20	CONCAN	1	5	21NE100	OPUPOL	1	4	22NE100	BOUGRA	5	2	22W100	SOPNUT	1	3	2W100	ARIPUR	1	4
19W20	ELYLY	6	5	21NE100	PLAPAT	1	4	22NE100	CARSIM	1	2	22W100	SPHCOC	2	3	2W100	BOUDAC	4	4
19W20	PASSMI	7	5	21NE100	SOLCAN	1	4	22NE100	CIRCAN	1	2	22W50	AMAALB	1	4	2W100	BOUGRA	8	4
19W20	SALTRA	1	5	21NE100	SONOLE	1	4	22NE100	CONCAN	1	2	22W50	AMBPSI	6	4	2W100	ECHVIR	1	4
19W20	THLARV	1	5	21NE100	SPHCOC	1	4	22NE100	CRYMIN	1	2	22W50	ASTPEC	1	4	2W100	HETVIL	1	4
19W50	BASSCO	1	4	21NE100	TRADUB	1	4	22NE100	FESOCT	6	2	22W50	CONCAN	1	4	2W100	LICHEN	1	4
19W50	BOUDAC	5	4	21NE20	AGRCCI	1	9	22NE100	HORJUB	3	2	22W50	HELANN	1	4	2W100	LUPARG	1	4
19W50	BOUGRA	7	4	21NE20	AMAALB	1	9	22NE100	LAPOCC	1	2	22W50	HORVUL	4	4	2W100	OPUPOL	2	4
19W50	ELYLY	2	4	21NE20	AMAPOW	1	9	22NE100	LICHEN	1	2	22W50	LYGJUN	1	4	2W100	OXYSER	1	4
19W50	HESSPA	2	4	21NE20	AMASPI	2	9	22NE100	OENSUF	1	2	22W50	SALTRA	4	4	2W100	PASSMI	3	4
19W50	MIRLIN	1	4	21NE20	CONARV	1	9	22NE100	PASSMI	2	2	22W50	SE						

Table 6. Continued.

Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare
2W100	VICAME	1	4	3SE20	ELYCAN	2	2	6NE20	SOLROS	1	7	6W100	PLAPAT	1	2	7SE100	PLAPAT	1	3
2W20	AGRSCRI	3	5	3SE20	ELYCAN	2	2	6NE20	TRITER	3	7	6W100	QUILOB	1	2	7SE100	PSOTEN	2	3
2W20	BOUDAC	3	5	3SE20	ERINAU	3	2	6NE20	VERBRA	4	7	6W100	SPHCOC	1	2	7SE100	THLARV	1	3
2W20	BOUGRA	3	5	3SE20	ERYASP	1	2	6NE50	ARTFRI	1	2	6W100	SPOAIR	1	2	7SE20	AGRSCRI	3	3
2W20	BROINE	6	5	3SE20	HETVIL	1	2	6NE50	BASSCO	1	2	6W100	VICAME	2	2	7SE20	ASTER	1	3
2W20	BROTEC	4	5	3SE20	MACPIN	1	2	6NE50	BOUGRA	8	2	6W20	AGRDES	6	1	7SE20	BOUDAC	7	3
2W20	CIRCAN	1	5	3SE20	OPUPOL	1	2	6NE50	BROTEC	4	2	6W20	BOUGRA	6	1	7SE20	BOUGRA	4	3
2W20	CONCAN	1	5	3SE20	SPOAIR	1	2	6NE50	ERINAU	2	2	6W20	CHAGLY	1	1	7SE20	MACTAN	1	3
2W20	ECHVIR	1	5	3SE50	ARTFRI	1	1	6NE50	ERYASP	1	2	6W20	CHEBER	1	1	7SE20	MEDLUP	1	3
2W20	OXYSER	2	5	3SE50	BASSCO	1	1	6NE50	HETVIL	1	2	6W20	CIROCH	2	1	7SE20	OPUPOL	1	3
2W20	PASSMI	1	5	3SE50	BOUDAC	8	1	6NE50	OENSUF	1	2	6W20	ELYELY	6	1	7SE20	PASSMI	1	3
2W20	SISALT	1	5	3SE50	BOUGRA	4	1	6NE50	OPUPOL	1	2	6W20	ERINAU	2	1	7SE20	PSOTEN	1	3
2W20	TAROFF	1	5	3SE50	CHAGLY	1	1	6NE50	PHYFEN	1	2	6W20	HETVIL	2	1	7SE20	THLARV	1	3
2W50	BOUDAC	3	3	3SE50	ELYCAN	2	1	6NE50	PSOTEN	1	2	6W20	MACTAN	1	1	7SE20	VERBRA	1	3
2W50	BOUGRA	8	3	3SE50	ERYASP	1	1	6NE50	SPHCOC	1	2	6W20	OENSUF	1	1	7SE50	AGRSCRI	5	3
2W50	BROTEC	1	3	3SE50	OPUPOL	1	1	6NE50	SPOAIR	1	2	6W20	OXYSER	1	1	7SE50	AMBART	1	3
2W50	CARSIM	2	3	3SE50	PHYFEN	1	1	6NE50	VICAME	1	2	6W20	PACTRI	1	1	7SE50	BOUDAC	5	3
2W50	CONCAN	1	3	3W100	ARTFRI	1	1	6SE100	ARTFRI	1	6	6W20	PLAPAT	1	1	7SE50	BOUGRA	4	3
2W50	ERYASP	1	3	3W100	BOUDAC	7	1	6SE100	BASSCO	1	6	6W20	QUILOB	1	1	7SE50	HESCOM	1	3
2W50	FESOCT	1	3	3W100	BOUGRA	6	1	6SE100	BOUDAC	1	6	6W20	SALTRA	1	1	7SE50	MEDLUP	1	3
2W50	HETVIL	1	3	3W100	LYGUN	1	1	6SE100	BOUGRA	9	6	6W20	SPHCOC	2	1	7SE50	PSOTEN	1	3
2W50	LICHEN	1	3	3W100	OPUPOL	1	1	6SE100	ESCIV	1	6	6W20	TRADUB	1	1	7SE50	THLARV	1	3
2W50	LUPARG	1	3	3W100	PHYFEN	1	1	6SE100	HESCOM	1	6	6W50	AGRDES	3	3	7W100	AGRSCRI	3	2
2W50	OPUPOL	2	3	3W100	SONOLE	1	1	6SE100	HETVIL	2	6	6W50	BASSCO	1	3	7W100	AGRDES	1	2
2W50	PASSMI	1	3	3W100	SPHCOC	1	1	6SE100	MIRLIN	1	6	6W50	BOUDAC	4	3	7W100	ARIPUR	1	2
2W50	SENSPA	1	3	3W100	THLARV	1	1	6SE100	OENSUF	1	6	6W50	BOUGRA	8	3	7W100	BOUDAC	1	2
2W50	SPHCOC	2	3	3W20	ARTFRI	1	2	6SE100	OPUPOL	1	6	6W50	CONARV	1	3	7W100	BOUGRA	3	2
3NE100	ARTFRI	1	1	3W20	BOUDAC	7	2	6SE100	PLAPAT	1	6	6W50	ELYELY	3	3	7W100	CARROS	5	2
3NE100	BASSCO	1	1	3W20	BOUGRA	6	2	6SE100	PSOTEN	1	6	6W50	HETVIL	1	3	7W100	CIRCAN	1	2
3NE100	BOUDAC	7	1	3W20	CHAGLY	1	2	6SE100	SPHCOC	1	6	6W50	MIRLIN	1	3	7W100	CONCAN	2	2
3NE100	BOUGRA	6	1	3W20	CHEBER	1	2	6SE100	SPOAIR	1	6	6W50	OPUPOL	1	3	7W100	ELYELY	3	2
3NE100	ELYCAN	1	1	3W20	ELYCAN	2	2	6SE100	VERBRA	1	6	6W50	SONOLE	1	3	7W100	HESCOM	1	2
3NE100	HESCOM	1	1	3W20	ERINAU	1	2	6SE100	VICAME	2	6	6W50	SPHCOC	1	3	7W100	LAPOCC	1	2
3NE100	MIRLIN	1	1	3W20	HETVIL	2	2	6SE20	AGRDES	5	6	6W50	VICAME	1	3	7W100	OPUPOL	1	2
3NE100	PHYFEN	1	1	3W20	OPUPOL	1	2	6SE20	ARTFRI	1	6	7NE100	ARIPUR	1	3	7W100	PASSMI	6	2
3NE100	PLAPAT	1	1	3W20	PLAPAT	1	2	6SE20	BASSCO	1	6	7NE100	BOUGRA	2	3	7W100	PSOTEN	2	2
3NE100	PSOTEN	2	1	3W20	PSOTEN	1	2	6SE20	BOUDAC	2	6	7NE100	CONCAN	5	3	7W100	RATCOL	1	2
3NE100	SPHCOC	1	1	3W20	SPHCOC	1	2	6SE20	BOUGRA	1	6	7NE100	ELYELY	5	3	7W100	THLARV	1	2
3NE20	ARTFRI	1	2	3W20	SPOAIR	3	2	6SE20	BROTEC	5	6	7NE100	ERICAN	1	3	7W100	TRADUB	1	2
3NE20	BOUDAC	8	2	3W20	THLARV	1	2	6SE20	CAMMIC	2	6	7NE100	FESOCT	1	3	7W100	TRAOCC	1	2
3NE20	BOUGRA	3	2	3W20	VERBRA	1	2	6SE20	CIROCH	1	6	7NE100	HESCOM	2	3	7W20	AGRSCRI	8	2
3NE20	ELYCAN	1	2	3W50	ARTFRI	1	2	6SE20	CONARV	1	6	7NE100	LAPOCC	1	3	7W20	AGRSTO	1	2
3NE20	ERINAU	2	2	3W50	BASSCO	1	2	6SE20	ELYELY	5	6	7NE100	PLAPAT	2	3	7W20	ARIPUR	1	2
3NE20	HESCOM	1	2	3W50	BOUDAC	7	2	6SE20	HETVIL	1	6	7NE100	PSOTEN	1	3	7W20	BOUGRA	1	2
3NE20	PHYFEN	1	2	3W50	BOUGRA	6	2	6SE20	OXYSER	1	6	7NE100	RATCOL	1	3	7W20	ELYELY	2	2
3NE20	PLAPAT	1	2	3W50	ERYASP	1	2	6SE20	POROLE	2	6	7NE100	SPHCOC	1	3	7W20	HESCOM	1	2
3NE20	PSOTEN	2	2	3W50	HETVIL	1	2	6SE20	PSOTEN	1	6	7NE100	THLARV	1	3	7W20	OPUPOL	1	2
3NE20	SONOLE	1	2	3W50	LYGUN	1	2	6SE20	TAROFF	1	6	7NE100	TRADUB	1	3	7W20	PASSMI	1	2
3NE20	SPHCOC	1	2	3W50	MIRLIN	1	2	6SE20	TRADUB	1	6	7NE100	VERBRA	1	3	7W20	PLAPAT	1	2
3NE20	THLARV	1	2	3W50	OPUPOL	1	2	6SE20	VERBRA	1	6	7NE20	AMABLI	1	3	7W20	PSOTEN	2	2
3NE20	VICAME	1	2	3W50	PSOTEN	1	2	6SE50	ARTFRI	4	6	7NE20	ARIPUR	1	3	7W20	THLARV	1	2
3NE50	ARTFRI	1	1	3W50	SONOLE	1	2	6SE50	ASTER	1	6	7NE20	BOUDAC	8	3	7W50	AGRSCRI	5	2
3NE50	BASSCO	1	1	3W50	SPHCOC	1	2	6SE50	BASSCO	1	6	7NE20	CHELP	1	3	7W50	ARIPUR	1	2
3NE50	BOUDAC	8	1	6NE100	ARTFRI	1	2	6SE50	DALCAN	2	6	7NE20	CONCAN	1	3	7W50	BOUDAC	5	2
3NE50	BOUGRA	5	1	6NE100	BASSCO	1	2	6SE50	ELYCAN	1	6	7NE20	HESCOM	1	3	7W50	BOUGRA	1	2
3NE50	ELYCAN	1	1	6NE100	BOUDAC	5	2	6SE50	ELYELY	5	6	7NE20	MEDLUP	1	3	7W50	CARROS	1	2
3NE50	ERINAU	1	1	6NE100	BOUGRA	8	2	6SE50	ERILON	1	6	7NE20	OPUPOL	1	3	7W50	CIRCAN	1	2
3NE50	HESSPA	2	1	6NE100	ELYCAN	1	2	6SE50	ERINAU	2	6	7NE20	PASSMI	1	3	7W50	CRYCIN	1	2
3NE50	PACTRI	1	1	6NE100	ERINAU	2	2	6SE50	ESCIV	1	6	7NE20	PSOTEN	2	3	7W50	ELYELY	3	2
3NE50	PENALB	1	1	6NE100	ERYASP	1	2	6SE50	HETVIL	1	6	7NE20	SECCER	1	3	7W50	FESOCT	1	2
3NE50	PHYFEN	1	1	6NE100	ESCIV	1	2	6SE50	MIRLIN	1	6	7NE50	AGRSCRI	4	3	7W50	HESCOM	1	2
3NE50	PLAPAT	1	1	6NE100	LICHEN	1	2	6SE50	OPUPOL	2	6	7NE50	BOUGRA	5	3	7W50	OPUPOL	1	2
3NE50	SPHCOC	1	1	6NE100	MIRLIN	1	2	6SE50	PHYFEN	1	6	7NE50	CONCAN	1	3	7W50	PASSMI	3	2
3NE50	TRADUB	1	1	6NE100	OPUPOL	1	2	6SE50	PLAPAT	3	6	7NE50	ELYELY	2	3	7W50	PLAPAT	1	2
3NE50	VICAME	1	1	6NE100	PSOTEN	1	2	6SE50	PSOTEN	3	6	7NE50	FESOCT	1	3	7W50	PSOTEN	3	2
3SE100	BOUDAC	6	1	6NE100	SPHCOC	1	2	6SE50	SPHCOC	1	6	7NE50	LYGUN	1	3	7W50	RATCOL	1	2
3SE100	BOUGRA	6	1	6NE100	VERBRA	1	2	6SE50	TRADUB	1	6	7NE50	OPUPOL	1	3	8NE100	AMBTRI	1	3
3SE100	ERINAU	1	1	6NE100	VICAME	1	2	6W100	AGRDES	5	2	7NE50	PASSMI	4	3	8NE100	ARTFRI	1	3
3SE100	ERYASP	1	1	6NE20	AMBPSI	1	7	6W100	BASSCO	1	2	7NE50	PLAPAT	1	3	8NE100	BOUDAC	6	3
3SE100	HETVIL	1	1	6NE20	CIRFLD	3	7	6W100	BOUDAC	2	2	7NE50	PSOTEN	5	3	8NE100	BOUGRA	6	3
3SE100	MIRLIN	1	1	6NE20	CIRUND	2	7	6W100	BOUGRA	7	2	7NE50	THLARV	1	3	8NE100	CIRCAN	1	3
3SE100	SONOLE	1	1	6NE20	DESSOP	1	7	6W100	CIROCH	1	2	7SE100	AGRSCRI	5	3	8NE100	ELYELY	2	3
3SE20	AGRDES	6	2	6NE20	ELYCAN	1	7	6W100	CONCAN	1	2	7SE100	BOUDAC	5	3	8NE100	GUTSAR	2	3
3SE20	AMBPSI	1	2	6NE20	EUPMAR	1	7	6W100	ELYCAN	1	2	7SE100	BOUGRA	3	3	8NE100	HESCOM	2	3
3SE20	ARTFRI	1	2	6NE20	HELANN	1	7	6W100	ELYELY	5	2	7SE100	CONCAN	1	3	8NE100	LYGUN	1	3
3SE20	ASTLOT	1	2	6NE20	PANCAP	1	7	6W100	ERINAU	2	2	7SE100	DESSOP	1	3	8NE100	OPUPOL	1	3
3SE20	BOUDAC	6	2	6NE20	PSOTEN	1	7	6W100	HETVIL	1	2	7SE100	ERYASP	1	3	8NE100	PASSMI	3	3
3SE20	BOUGRA	3	2	6NE20	SALTRA	2	7	6W100	MIRLIN	1	2	7SE100	HESCOM	4	3	8NE100	PENALB	1	3
3SE20	CHAGLY	1	2	6NE20	SALTRA	2	7	6W100	OENSUF	1	2	7SE100	MEDLUP	1	3	8NE100	PSOTEN	1	3

Table 6. Continued.

Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare	Plot	Species Code	Cover	Bare
8NE100	SOPNUT	1	3	8SE100	PASSMI	1	2	8W100	FESOCT	1	3	9NE20	CONCAN	1	3	9SE50	BOUDAC	3	3
8NE100	SPHCOC	2	3	8SE100	PSOTEN	1	2	8W100	LYGIUN	1	3	9NE20	ELYELY	2	3	9SE50	BOUGRA	8	3
8NE20	AGRCRI	5	7	8SE100	SPHCOC	2	2	8W100	OPUPOL	3	3	9NE20	ERYASP	1	3	9SE50	ELYELY	2	3
8NE20	AMAALB	1	7	8SE20	AGRCRI	1	4	8W100	PASSMI	5	3	9NE20	LITINC	1	3	9SE50	ERYASP	1	3
8NE20	BOUDAC	1	7	8SE20	AMAALB	1	4	8W100	SOLROS	2	3	9NE20	OPUPOL	1	3	9SE50	FESOCT	1	3
8NE20	BOUGRA	1	7	8SE20	BROTEC	1	4	8W100	SOPNUT	1	3	9NE20	PASSMI	1	3	9SE50	LYGIUN	1	3
8NE20	CHAGLY	1	7	8SE20	CHAGLY	6	4	8W100	SPHCOC	1	3	9NE20	PLAPAT	1	3	9SE50	OPUPOL	5	3
8NE20	CHEBER	1	7	8SE20	CHELEP	1	4	8W20	AGRCRI	1	5	9NE20	SOPNUT	1	3	9SE50	PLAPAT	1	3
8NE20	CHOTEN	1	7	8SE20	CHOTEN	1	4	8W20	AMAALB	1	5	9NE20	SPHCOC	2	3	9SE50	SENSPA	1	3
8NE20	CIRCAN	2	7	8SE20	CIRCAN	1	4	8W20	ARTFRI	2	5	9NE50	BOUDAC	7	3	9SE50	SOPNUT	2	3
8NE20	GUTSAR	2	7	8SE20	CONARV	1	4	8W20	BOUDAC	7	5	9NE50	BOUGRA	5	3	9SE50	SPHCOC	1	3
8NE20	HESCOM	1	7	8SE20	ELYELY	1	4	8W20	BOUGRA	1	5	9NE50	CIRSCO	1	3	9SE50	VERBRA	1	3
8NE20	HETVIL	2	7	8SE20	HETVIL	1	4	8W20	CARSIM	1	5	9NE50	CONCAN	1	3	9W100	ANTPAR	1	4
8NE20	PASSMI	1	7	8SE20	MEDLUP	3	4	8W20	FESOCT	1	5	9NE50	ELYELY	3	3	9W100	BOUDAC	5	4
8NE20	PSOTEN	1	7	8SE20	PASSMI	4	4	8W20	PASSMI	2	5	9NE50	ERYASP	1	3	9W100	BOUGRA	6	4
8NE20	SPHCOC	1	7	8SE20	SCHPAN	1	4	8W20	SOPNUT	1	5	9NE50	OPUPOL	1	3	9W100	ELYELY	1	4
8NE50	AGRCRI	5	3	8SE20	SOPNUT	1	4	8W20	SPHCOC	1	5	9NE50	PASSMI	3	3	9W100	LYGIUN	1	4
8NE50	AMAALB	2	3	8SE20	SPHCOC	3	4	8W50	ARTFRI	1	2	9NE50	PLAPAT	1	3	9W100	OPUPOL	1	4
8NE50	ARTFRI	1	3	8SE20	SPOCRY	1	4	8W50	BOUDAC	6	2	9NE50	SOPNUT	1	3	9W100	PASSMI	1	4
8NE50	BOUDAC	5	3	8SE20	TRADUB	2	4	8W50	BOUGRA	6	2	9NE50	SPHCOC	2	3	9W100	PLAPAT	1	4
8NE50	BOUGRA	4	3	8SE50	ARTFIL	1	3	8W50	ELYELY	2	2	9SE100	BOUDAC	3	3	9W100	SPHCOC	1	4
8NE50	FESOCT	1	3	8SE50	ARTFRI	1	3	8W50	HESCOM	1	2	9SE100	BOUGRA	5	3	9W20	BOUDAC	5	3
8NE50	HESCOM	1	3	8SE50	BOUDAC	3	3	8W50	LICHEN	1	2	9SE100	CHELEP	1	3	9W20	BOUGRA	7	3
8NE50	LEPDEN	1	3	8SE50	BOUGRA	5	3	8W50	OPUPOL	3	2	9SE100	ELYELY	1	3	9W20	CONCAN	1	3
8NE50	LYGIUN	2	3	8SE50	CIRFLO	1	3	8W50	PENALB	1	2	9SE100	ERINAU	1	3	9W20	ELYELY	2	3
8NE50	OPUPOL	2	3	8SE50	ELYELY	1	3	8W50	SOPNUT	1	2	9SE100	OENSUF	2	3	9W20	OPUPOL	1	3
8NE50	PLAPAT	1	3	8SE50	FESOCT	1	3	8W50	SPHCOC	1	2	9SE100	OPUPOL	4	3	9W20	SOPNUT	1	3
8NE50	SOLROS	1	3	8SE50	GUTSAR	2	3	9NE100	BOUDAC	1	4	9SE100	PASSMI	7	3	9W20	SPHCOC	1	3
8NE50	SOPNUT	1	3	8SE50	HESCOM	1	3	9NE100	BOUGRA	4	4	9SE100	PLAPAT	1	3	9W50	BOUDAC	6	4
8NE50	SPHCOC	2	3	8SE50	LITINC	1	3	9NE100	CHELEP	1	4	9SE100	SPHCOC	1	3	9W50	BOUGRA	6	4
8NE50	TRADUB	1	3	8SE50	OPUPOL	3	3	9NE100	FESOCT	1	4	9SE20	ANTPAR	1	1	9W50	CHELEP	1	4
8SE100	ARTFIL	1	2	8SE50	PLAPAT	1	3	9NE100	LITINC	1	4	9SE20	BOUDAC	2	1	9W50	ELYELY	1	4
8SE100	ARTFRI	1	2	8SE50	SOLROS	2	3	9NE100	OENSUF	1	4	9SE20	BOUGRA	8	1	9W50	ERYASP	1	4
8SE100	BOUDAC	6	2	8SE50	SOPNUT	1	3	9NE100	OPUPOL	3	4	9SE20	CARSIM	2	1	9W50	OPUPOL	1	4
8SE100	BOUGRA	6	2	8SE50	SPHCOC	1	3	9NE100	PASSMI	7	4	9SE20	CHELEP	1	1	9W50	PICOPP	1	4
8SE100	CARSIM	1	2	8W100	ARTFIL	1	3	9NE100	SOPNUT	1	4	9SE20	CONCAN	1	1	9W50	PLAPAT	1	4
8SE100	ESCVIV	1	2	8W100	ARTFRI	1	3	9NE100	SPHCOC	1	4	9SE20	ELYELY	3	1	9W50	SOPNUT	1	4
8SE100	GUTSAR	3	2	8W100	ASTER	1	3	9NE100	SPOAIR	1	4	9SE20	LAPOCC	1	1	9W50	SPHCOC	2	4
8SE100	HESCOM	2	2	8W100	BOUDAC	6	3	9NE20	BOUDAC	6	3	9SE20	OPUPOL	1	1				
8SE100	LYGIUN	1	2	8W100	BOUGRA	5	3	9NE20	BOUGRA	6	3	9SE20	PLAPAT	1	1				
8SE100	OPUPOL	2	2	8W100	ELYELY	2	3	9NE20	CIRSCO	1	3	9SE20	SPHCOC	1	1				

Table 7

*Species Codes and Functional Characteristics*

Species	Species code	Family	Duration	Habit	PP	Status	Invasive
<i>Abronia fragrans</i> Nutt. ex Hook.	ABRFRA	NYC	perennial	forb herb	C3	native	no
<i>Abronia fragrans</i> Nutt. ex Hook.	AGRCRI	POA	perennial	graminoid	C3	introduced	no
<i>Agropyron desertorum</i> (Fisch. ex Link) Schult.	AGRDES	POA	perennial	graminoid	C3	introduced	no
<i>Agrostis stolonifera</i> L.	AGRSTO	POA	perennial	graminoid	C3	introduced	no
<i>Amaranthus albus</i> L.	AMAAALB	AMA	annual	forb herb	C4	introduced	no
<i>Amaranthus blitoides</i> S. Watson	AMABLI	AMA	annual	forb herb	C3	native	no
<i>Amaranthus powellii</i> S. Watson	AMAPOW	AMA	annual	forb herb	C3	native	no
<i>Amaranthus spinosus</i> L.	AMASPI	AMA	annual	forb herb	C4	introduced	no
<i>Ambrosia artemisiifolia</i> L.	AMBART	AST	annual	forb herb	C3	introduced	no
<i>Ambrosia psilostachya</i> DC.	AMBPSI	AST	annual perennial	forb herb	C3	native	no
<i>Ambrosia tomentosa</i> Nutt.	AMBTOM	AST	perennial	forb herb	C3	native	no
<i>Ambrosia trifida</i> L.	AMBTRI	AST	annual	forb herb subshrub	C3	native	no
<i>Antennaria parvifolia</i> Nutt.	ANTPAR	AST	perennial	forb herb	C3	native	no
<i>Aristida purpurea</i> Nutt.	ARIPUR	POA	annual perennial	graminoid	C4	native	no
<i>Artemisia absinthium</i> L.	ARTABS	AST	perennial	forb herb subshrub	C3	introduced	yes
<i>Artemisia dracunculua</i> L.	ARTDRA	AST	perennial	forb herb subshrub	C3	native	no
<i>Artemisia filifolia</i> Torr.	ARTFIL	AST	perennial	shrub subshrub	C3	native	no
<i>Artemisia frigida</i> Willd.	ARTFRI	AST	perennial	subshrub	C3	native	no
Unknown aster	ASTER	AST	NA	NA	NA	NA	NA
<i>Astragalus gracilis</i> Nutt.	ASTGRA	FAB	perennial	forb herb	C3	native	no
<i>Astragalus lotiflorus</i> Hook.	ASTLOT	FAB	perennial	forb herb	C3	native	no
<i>Astragalus mollissimus</i> Torr.	ASTMOL	FAB	perennial	forb herb	C3	native	no
<i>Astragalus pectinatus</i> (Douglas ex Hook.) Douglas ex G. Don	ASTPEC	FAB	perennial	forb herb	C3	native	no
<i>Bassia scoparia</i> (L.) A.J. Scott	BASSCO	AMA	annual	forb herb	C4	introduced	no
<i>Bouteloua curtipendula</i> (Michx.) Torr.	BOUCUR	POA	perennial	graminoid	C4	native	no
<i>Bouteloua dactyloides</i> (Nutt.) J.T. Columbus	BOUDAC	POA	perennial	graminoid	C4	native	no
<i>Bouteloua gracilis</i> (Willd. ex Kunth) Lag. ex Griffiths	BOUGRA	POA	perennial	graminoid	C4	native	no
<i>Brickellia eupatorioides</i> (L.) Shinners var. <i>corymbulosa</i> (Torr. & A. Gray) Shinners	BRIEUP	AST	perennial	forb herb subshrub	C3	native	no
<i>Bromus inermis</i> Leyss.	BROINE	POA	perennial	graminoid	C3	introduced	no
<i>Bromus tectorum</i> L.	BROTEC	POA	annual	graminoid	C3	introduced	yes
<i>Calylophus berlandieri</i> Spach subsp. <i>Berlandieri</i>	CALBER	ONA	perennial	forb herb subshrub	C3	native	no
<i>Camelina microcarpa</i> Andr. ex DC.	CAMMIC	BRA	annual biennial	forb herb	C3	introduced	no
<i>Carex rossii</i>	CARROS	CYP	perennial	graminoid	C3	native	no
<i>Carex simulata</i> Mack	CARSIM	CYP	perennial	graminoid	C3	native	no
<i>Chamaesyce glyptosperma</i> (Engelm.) Small	CHAGLY	EUP	annual	forb herb	C4	native	no
<i>Chenopodium berlandieri</i> Moq.	CHEBER	AMA	annual	forb herb	C3	native	no
<i>Chenopodium leptophyllum</i> (Moq.) Nutt. ex S. Watson	CHELEP	AMA	annual	forb herb	C3	native	no
<i>Chorispora tenella</i> (Pall.) DC.	CHOTEN	BRA	annual	forb herb	C3	introduced	no
<i>Cirsium canescens</i> Nutt.	CIRCAN	AST	biennial	forb herb	C3	native	no
<i>Cirsium flodmanii</i> (Rydb.) Arthur	CIRFLO	AST	perennial	forb herb	C3	native	no
<i>Cirsium ochrocentrum</i> A. Gray	CIROCH	AST	biennial perennial	forb herb	C3	native	no
<i>Cirsium scopulorum</i> (Greene) Cockerell ex Daniels	CIRSCO	AST	biennial	forb herb	C3	native	no
<i>Cirsium species</i>	CIRSPP	AST	NA	NA	NA	NA	NA
<i>Cirsium undulatum</i> (Nutt.) Spreng.	CIRUND	AST	biennial perennial	forb herb	C3	native	no
<i>Cleome serrulata</i> Pursh	CLESER	CAP	annual	forb herb	C4	native	no
<i>Convolvulus arvensis</i> L.	CONARV	CON	perennial	forb herb vine	C3	introduced	yes
<i>Conyza canadensis</i> (L.)	CONCAN	AST	annual biennial	forb herb	C3	native	no
<i>Cryptantha cinerea</i> (Greene) Cronquist	CRYCIN	BOR	perennial	forb herb subshrub	C3	native	no
<i>Cryptantha minima</i> Rydb.	CRYMIN	BOR	annual	forb herb	C3	native	no
<i>Dalea candida</i> Michx. ex Willd.	DALCAN	FAB	perennial	forb herb	C3	native	no
<i>Descurainia pinnata</i> (Walter) Britton ssp. <i>nelsonii</i> (Rydb.) Detling	DESPIN	BRA	annual biennial	forb herb	C3	native	no
<i>Descurainia sophia</i> (L.) Webb ex Prantl	DESSOP	BRA	annual biennial	forb herb	C3	introduced	no
<i>Digitaria sanguinalis</i>	DIGSAN	POA	annual	graminoid	C4	introduced	no
<i>Echinocereus viridiflorus</i> Engelman var. <i>macrorrhiza</i>	ECHVIR	CAC	perennial	shrub	CAM	native	no
<i>Elymus canadensis</i> L.	ELYCAN	POA	perennial	graminoid	C3	native	no
<i>Elymus elymoides</i> (Raf.) Swezey	ELYELY	POA	perennial	graminoid	C3	native	no
<i>Erigeron canus</i> A. Gray	ERICAN	AST	perennial	forb herb	C3	native	no

*Notes:* Species code is a six letter code for each species using the first 3 letters of genus and species. Family is the three letter code name. Duration categories were taken from the USDA plants database (USDA 2014b) and can include more than one duration if the species is phenotypically plastic. Habit is growth habit also taken from the USDA plants database. PP is the photosynthetic pathway (C<sub>3</sub>, C<sub>4</sub>, or CAM). Invasive is whether the species is considered invasive in Colorado (yes or no), also determined by the USDA plants database. NA = unknown.

Table 7. Continued.

Species	Species code	Family	Duration	Habit	PP	Status	Invasive
<i>Erigeron lonchophyllus</i> Hook.	ERILON	AST	annual biennial	forb herb	C3	native	no
<i>Ericameria nauseosa</i>	ERINAU	AST	perennial	shrub subshrub	C3	native	no
<i>Erysimum asperum</i> (Nutt.) DC.	ERYASP	BRA	biennial perennial	forb herb	C3	native	no
<i>Escobaria vivipara</i> (Nutt.) Buxbaum var. <i>vivipara</i>	ESCIVV	CAC	perennial	shrub	CAM	native	no
<i>Euphorbia marginata</i> Pursh	EUPMAR	EUP	annual	forb herb	C3	native	no
<i>Evolvulus nuttallianus</i> Schult.	EVONUT	CNV	perennial	forb herb subshrub	C3	native	no
<i>Festuca octoflora</i>	FESQOT	POA	annual	graminoid	C3	native	no
<i>Grindelia squarrosa</i> (Pursh) Dunal	GRISQU	AST	annual biennial perennial	forb herb	C3	native	no
<i>Gutierrezia sarothrae</i>	GUTSAR	AST	perennial	forb herb shrub subshrub	C3	native	no
<i>Helianthus annuus</i> L.	HELANN	AST	annual	forb herb	C3	native	no
<i>Helianthus petiolaris</i> Nutt.	HELPEP	AST	annual	forb herb	C3	native	no
<i>Hesperostipa comata</i> (Trin. & Rupr.)	HESCOM	POA	perennial	graminoid	C3	native	no
<i>Hesperostipa spartea</i> (Trin.) Barkworth	HESSPA	POA	perennial	graminoid	C3	native	no
<i>Heterotheca villosa</i> (Pursh) Shinnery	HETVIL	AST	perennial	forb herb subshrub	C3	native	no
<i>Hordeum jubatum</i>	HORJUB	POA	perennial	graminoid	C3	native	no
<i>Hordeum vulgare</i> L.	HORVUL	POA	annual	graminoid	C3	introduced	no
<i>Hymenopappus filifolius</i> Hook.	HYMFIL	AST	perennial	forb herb subshrub	C3	native	no
<i>Hypochaeris radicata</i>	HYPRAD	AST	perennial	forb herb	C3	introduced	no
<i>Iva axillaris</i> Pursh	IVAAXI	AST	perennial	forb herb subshrub	C3	native	no
<i>Krascheninnikovia lanata</i> (Pursh) A. Meeuse & Smit	KRALAN	AMA	perennial	shrub subshrub	C3	native	no
<i>Lactuca serriola</i>	LACSER	AST	annual biennial	forb herb	C3	introduced	no
<i>Lappula occidentalis</i>	LAPOCC	BOR	annual biennial	forb herb	C3	native	no
<i>Lepidium densiflorum</i> Schrad.	LEPDEN	BRA	annual biennial	forb herb	C3	introduced	no
<i>Liatis punctata</i> Hook	LIAPUN	AST	perennial	forb herb	C3	native	no
LICHEN	LICHEN	NA	NA	NA	NA	NA	NA
<i>Linum puberulum</i> (Engelm.) A. Heller	LINPUB	LIN	annual	forb herb	C3	native	no
<i>Linum rigidum</i> Pursh	LINRIG	LIN	annual perennial	forb herb	C3	native	no
<i>Lithospermum incisum</i> Lehm.	LITINC	BOR	perennial	forb herb	C3	native	no
<i>Lupinus argenteus</i> or <i>Lupinus plattensis</i> S. Watson	LUPARG	FAB	perennial	forb herb subshrub	C3	native	no
<i>Lupinus pusillus</i> Pursh	LUPPUS	FAB	perennial	forb herb subshrub	C3	native	no
<i>Lygodesmia juncea</i> (Pursh) D. Don ex Hook.	LYGIUN	AST	perennial	forb herb	C3	native	no
<i>Machaeranthera pinnatifida</i> (Hook.) Shinnery	MACPIN	AST	perennial	forb herb subshrub	C3	native	no
<i>Machaeranthera tanacetifolia</i> (Kunth) Nees	MACTAN	AST	annual biennial	forb herb	C3	native	no
<i>medicago lupulina</i> L.	MEDLUP	FAB	annual perennial	forb herb	C3	introduced	no
<i>Melilotus officinalis</i> (L.) Lam.	MELOFF	FAB	annual biennial perennial	forb herb	C3	introduced	no
<i>Mirabilis linearis</i> (Pursh) Heimerl	MIRLIN	NYC	perennial	forb herb subshrub	C3	native	no
<i>Oenothera coronopifolia</i>	OENCOR	ONA	perennial	forb herb	C3	native	no
<i>Oenothera suffrutescens</i> (Ser.) W.L. Wagner & Hoch	OENSUF	ONA	perennial	forb herb subshrub	C3	native	no
<i>Opuntia polyacantha</i> Haw.	OPUPOL	CAC	perennial	shrub	CAM	native	no
<i>Oxytropis sericea</i>	OXYSER	FAB	perennial	forb herb	C3	native	no
<i>Packera plattensis</i> (Nutt.) W.A. Weber & Á. Löve	PACPLA	AST	biennial perennial	forb herb	C3	native	no
<i>Packera tridenticulata</i> (Rydb.) W.A. Weber & Á. Löve	PACTRI	AST	perennial	forb herb subshrub	C3	native	no
<i>Panicum capillare</i>	PANCAP	POA	annual	graminoid	C4	native	no
<i>Panicum virgatum</i> L.	PANVIR	POA	perennial	graminoid	C4	native	no
<i>Pascopyrum smithii</i> (Rydb.) Á. Löve	PASSMI	POA	perennial	graminoid	C3	native	no
<i>penstemon albidus</i>	PENALB	SCR	perennial	forb herb	C3	native	no
<i>Physaria fendleri</i>	PHYFEN	BRA	perennial	forb herb	C3	native	no
<i>Picradeniopsis oppositifolia</i> (Nutt.) Rydb. ex Britton	PICOPP	AST	perennial	forb herb subshrub	C3	native	no
<i>Plantago patagonica</i> Jacq.	PLAPAT	PLA	annual	forb herb	C3	native	no
<i>Polygonum aviculare</i> L.	POLAVI	PLG	annual perennial	forb herb	C3	introduced	no
<i>Portulaca oleracea</i> L.	POROLE	POR	annual	forb herb	CAM	introduced	no
<i>Psoralidium tenuiflorum</i> (Pursh) Rydb.	PSOTEN	FAB	perennial	forb herb	C3	native	no
<i>Quinclula lobata</i>	QUILOB	SOL	perennial	forb herb	C3	native	no
<i>Ratibida columnifera</i> (Nutt.) Wooton & Standl. Show All Show Tabs	RATCOL	AST	perennial	forb herb	C3	native	no
<i>Salsola tragus</i> L.	SALTRA	AMA	annual	forb herb	C4	introduced	no
<i>Schedonnardus paniculatus</i> (Nutt.) Trel.	SCHPAN	POA	perennial	graminoid	C4	native	no
<i>Secale cereale</i> L.	SECCER	POA	annual	graminoid	C3	introduced	no
<i>Senecio spartioides</i> Torrey & Gray	SENSPA	AST	perennial	forb herb subshrub	C3	native	no
<i>Setaria pumila</i> (Poir.) Roem. & Schult.	SETPUM	POA	annual	graminoid	C4	introduced	no
<i>Sisymbrium altissimum</i> L.	SISALT	BRA	annual biennial	forb herb	C3	introduced	no
<i>Solidago canadensis</i>	SOLCAN	AST	perennial	forb herb	C3	native	no
<i>Solanum rostratum</i> Dunal	SOLROS	SOL	annual	forb herb	C3	native	no
<i>Solanum triflorum</i>	SOLTRI	SOL	annual	forb herb	C3	native	no
<i>Sonchus oleraceus</i> L.	SONOLE	AST	annual	forb herb	C3	introduced	no
<i>Sophora nuttalliana</i> B.L. Turner	SOPNUT	FAB	perennial	forb herb	C3	native	no
<i>Sphaeralcea coccinea</i> (Nutt.) Rydb.	SPHCOC	MAL	biennial perennial	forb herb subshrub	C3	native	no
<i>Sporobolus airoides</i> (Torr.) Torr.	SPOAIR	POA	perennial	graminoid	C4	native	no
<i>Sporobolus cryptandrus</i> (Torr.) A. Gray	SPOCRY	POA	perennial	graminoid	C4	native	no
Unknown succulent	SUCULNT	NA	NA	shrub	CAM	NA	NA
<i>Taraxacum officinale</i>	TAROFF	AST	perennial	forb herb	C3	native	no
<i>Thelesperma megapotamicum</i> (Spreng.) Kuntze	THEMEG	AST	perennial	forb herb	C3	native	no
<i>Thlaspi arvense</i> L.	THLARV	BRA	annual	forb herb	C3	introduced	no
<i>Tragopogon dubius</i>	TRADUB	AST	annual biennial	forb herb	C3	introduced	no
<i>Tradescantia occidentalis</i> (Britton) Smyth	TRAOCC	CMM	perennial	forb herb	C3	native	no
<i>Trifolium repens</i> L.	TRIREP	FAB	perennial	forb herb	C3	introduced	no
<i>Verbena bracteata</i> Cav. ex Lag. & Rodr.	VERBRA	VRB	annual biennial perennial	forb herb	C3	native	no
<i>Vicia americana</i>	VICAME	FAB	perennial	forb herb vine	C3	native	no
<i>Xanthium strumarium</i> L. var. <i>canadense</i> (Mill.) Torr. & A. Gray	XANSTR	AST	annual	forb herb	C3	native	no