Bio-Agtive™ Emissions Technology

Carbon Farming Practice

Bio-Agtive Emissions Technology

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Final Report - Spring 2012

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Description of Bio-Agtive™ Technology

Bio-Agtive™ Emissions Technology was developed by Mr. Gary Lewis, the owner of N/C Quest Inc. The development of the Bio-Agtive™ Emissions Technology started in 2000 by studying and applying plant physiology on his farm near Pincher Creek, Alberta, Canada (Olin Creek Ranch). The Bio-Agtive™ Technology is the application of pure science based on "Mineral Nutrition of Higher Plants, Second Edition" by Horst Marschner and many other scientific studies. These studies back up the theory of using internal combustion emissions as minerial nutrition to plants, soils and as a carbon source. With further experimentation on Olin Creek Ranch the Bio-Agtive™ Irrigation method was developed (2001) and patented. By 2005 the second patented technology was first experimented on a JD8960 during spring seeding with oats and barley. The Bio-Agtive™ process showed positive results and the company N/C Quest Inc was formed to collaborate with farmers all over the world to help:

1. Farm Family Economics
2. Food Security
3. Agriculture Emissions Reduction and Energy Consumption
4. Responsible Farming Practices with Bio-Agtive™ Support

The N/C Quest Technology has nemours patents pending and international patents:

N/C Quest Inc. Patents

Canadian Patent No. 2,504,133 FERTILISING SYSTEM AND METHOD BY EXTRACTING NITROGEN COMPOUNDS FROM COMBUSTION EXHAUST GASES 2002

Canadian Patent No. 2,509,172 EXHAUST EMISSIONS RECYCLING SYSTEM 2005

United States Patent No. US 7735437 FERTILIZING SYSTEM AND METHOD BY EXTRACTING NITROGEN COMPOUNDS FROM COMBUSTION EXHAUST GASES 2002

United States Patent No. US 8,061,283 B2 METHOD OF RECYCLING EXHAUST EMISSIONS 2005

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Executive Summary

Bio-Agtive™ Emissions Technology is a patented method for introducing cooled exhaust from diesel engines into air tanks and seed drills and eventually into the soil during the direct seeding of agricultural crops and/or tillage of ground. Results from a field experiment in Manitoba, Canada summarized by Dr. Loraine Bailey P.Ag, FCSA, FASA, FAIC Eco-Agronomy Consultant show that plant growth and grain yields of cereals seeded with exhaust are not significantly different from those seeded with recommended fertilizer rates of nitrogen, phosphorus, potassium, and sulfur (NPKS) significantly better than the check (no inputs) (Bailey et al., 2008, 2009). Results of experiments conducted by independent consultants have shown that carbon (C), nitrogen (N), sulfur (S), and oxygen (O) content of the exhaust can vary widely depending on the tractor, fuel type, fuel additive, and towing load. Note that all these nutrients can potentially be utilized by plants and soil organisms for mineral nutrition. It is highly possible that micro amounts of N and S on the seed and in the seed furrow could combine with moisture in the organic matter and act as available nutrients for the soil biota, and growing plant. However, it is not well understood how introducing exhaust emissions into the soil can contribute to enhanced plant growth and yield and what kinds of compounds are emitted at different engine conditions and fuels.

Dr. Karl Ritz (Cranfield University, Great Britain) and Dr. Jill Clapperton suggest that it is most likely that the BioAgtive Emissions technology primes the soil microbiology. NO in nanogram (10^{-9}) quantities can stimulate the metabolism of ammonia oxidizing bacteria, and act as an antibiotic to many plant pathogens. For example, if 10% of the emissions were adsorbed to the organic matter, or dissolved in the soil solution there would be the same amount and composition of essential elements as required for a Soil Solution Equivalent Medium (Angle et al., 1991, Applied Environmental Microbiology 57: 3674-3676.) for soil bacterial growth. After all, microorganisms only need micro amounts of nutrients to grow. Elements such as C, N, O, H , S, and P are required in gram quantities; K, Ca, Mg, Fe are required in milligram quantities (10^{-3}), and Mn, Zn, Co, Ni, Mo, Cu are required in microgram (10^{-6}) to nanogram (10^{-9}) quantities in a liter of soil solution to stimulate the growth of soil bacteria. Exhaust analysis suggests it is possible for exhaust to provide micro and nano gram quantities of these cations.

This paper reports the results of the project conducted by Montana State University-Northern Bio-Energy Center in collaboration with , Bio-Agtive of Montana LLC , and N/C Quest Inc. The objectives of the project are: (1) to examine the possibility that different fuels can be used to add key fertilizer ingredients and micronutrients to the soil from tractor exhaust emissions using the Bio-Agtive™ Emission Technology, (2) to use our understanding of the chemical composition of the emissions using farm scale equipment to test the ability of the exhaust emissions to augment or replace fertilizer applications to the fields, and (3) to determine which bio-derived and petro-diesel fuels work best for stimulating microbiological activity in the soil, and thereby maximizing the availability of essential crop nutrients.

MSU-Northern Bio-Energy Center used a CAT 3176 diesel mounted on a 3100 Taylor Dynamometer and the exhaust emissions were monitored using an AVL SESAM FTIR Emission Analyzer and Microsoot Analyzer to conduct the emission testing. Emission results showed that (NO_x) decrease as the load increases. The results also provided evidence that biofuels, including biodiesel and straight vegetable oil, do not always produce more NO_x emissions than petro-diesel. Other factors, particularly engine load can increase or decrease the formation of NO_x. For example, biodiesel produces more NO_x emission than petro-diesel at low engine loads but at high engine loads, biodiesel has lower NO_x emissions than petro-diesel. Other minor emissions such as formaldehyde, formic acid, hydrogen cyanide and sulfur dioxide were trapped in the water scrubber as verified by the decreased pH of the water collected and the results of the emission tests. It is probable that the condensate produced during cooling of the exhaust emissions in the Bio-Agtive™ Emission Technology may contain these other minor emissions and concentrations of some metals and anions that are plant and microbial nutrients.

Field tests and analysis of the Bio-Agtive Emission Technology from two different fields were conducted. Tests include microbial biomass and activity, nutrient and chemical content analysis of the soil, protein content and actual crop yields. Due to unforeseen circumstances, MSU-Northern Bio-Energy Center was also responsible for conducting the field testing and analysis. Note that the Bio-Energy Center has no expertise on soil and plant tissue research. Thus, the interpretation of the field results was only based on the numerical values of the results from different laboratories and test instruments. Field tests showed conclusive trends in the chemical and microbial content of soils and plant tissue analysis, grain quality, yield, protein among different fuel treatments tested in the project. However, a comprehensive examination of the field results from a soil scientist is recommended to have a better interpretation of data gathered from this project. Following this report, attached is a study conducted by an independent third party.

Title **PG #**

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1.1 Objective

The primary objective of this project was to use the engine science and fuel chemistry capabilities of the MSU-Northern laboratory at Havre MT, to examine the possibility that different fuels (including biofuels from different plants) can be blended with diesel fuel as additives to add key fertilizer ingredients and micronutrients to the soil from tractor exhaust emissions using the Bio-AgtiveTM Emission Technology. MSU-Northern has a fuel injection electronic control module on a Dyno engine that can simulate field loads, engine timing, and heat generated, and then analyze the chemical components and mineral elements in the exhaust emissions under the various control conditions. This presently is a state-of-the-art facility within the Northwest region. Field tests were also conducted to confirm whether the findings in the laboratory agree with the results in actual field trials.

1.2 Testing and Results

This report covers the exhaust emission monitoring performed at MSU-Northern Bio-Energy Center using a CAT 3176 diesel engine mounted on a 3100 Taylor Dynamometer (Figure 1). The exhaust emissions were monitored using an AVL SESAM-FTIR Emission Analyzer equipped with an FID detector and a Paramagnetic detector for total hydrocarbon and oxygen monitoring, respectively (Figure 2).

Figure 1. Taylor 3100 Engine Dynamometer (A) and 3176 CAT Diesel (B) Engine used in the performance testing.

Bio-Agtive™ Emissions Technology

Emissions Testing at MSU-Northern

Table 1. Engine exhaust gas species monitored during emission monitoring using AVL SESAM-FTIR Emission Analyzer.

Oxides of Nitrogen (NOx)	Ethene(C_2H_4)
Water (H_2O)	Ethane(C_2H_6)
Carbon dioxide (CO_2)	Aromatics (AHC)
Carbon monoxide (CO)	Methane (CH_4)
Oxygen (O_2)[1]	Propane (C_3H_8)
Total hydrocarbon (THC)[2]	Formaldehyde (HCHO)
Nitrogen monoxide (NO)	Sulfur Dioxide (SO_2)
Nitrogen dioxide (NO_2)	Carbonyl sulfide (COS)
Nitrous oxide (N_2O)	Octane (C_8H_{18})
Ammonia (NH_3)	Isocyanic Acid (HNCO)
Propene (C_3H_6)	Cyanide (HCN)
Butadiene (C_4H_6)	Formic Acid (HCOOH)
Ethyne (C_2H_2)	Soot[3]

Legends: [1]Measured by Paramagnetic detector
[2]Measure by FID detector
[3]Measure by AVL Microsoot Analyzer

Figure 2. The AVL SESAM-FTIR Emission Analyzer (A) and the AVL Microsoot Analyzer (B) used during the test.

The diesel engine was fueled with various fuels including diesel No. 2, neat biodiesel derived from camelina, canola and safflower (100% biodiesel or B100) and straight vegetable oil (SVO; canola).

Testing for SVO was conducted using a second, auxiliary fuel tank- solely for SVO, which is similar to commercially available SVO conversion kits. Such in a typical vehicle running on SVO, the engine starts using conventional diesel fuel while heating up the SVO tank. Once the SVO reaches a temperature around 80oC, the fuel supply is shifted to the second tank containing the SVO and the diesel fuel supply is shut-off. Few minutes prior to stopping the engine from running, the fuel is shifted again to diesel fuel to purge the fuel system of any remaining vegetable oil.

In a typical test, the engine was run at varying load (power and torque) while maintaining engine speed at constant rpm (Table 2). To mimic the actual operation of the N/C Quest technology, the exhaust emission was cooled down using stainless steel tubing (0.42 inch inside diameter; 20 ft long) prior to the actual measurement of the gas species. This mimics the condenser typically used by N/C Quest. However in doing so the water in the exhaust gas also condenses during cooling, which is harmful to the emission analyzer. Hence, a Gas Scrubber (Figure 3) was used during the tests, which serves two functions:

1. Removes condensed moisture, which is harmful to the emission analyzer, from the gas stream prior to measurement.
2. Provides a more realistic composition of the exhaust emissions introduced into the soil, thus mimicking in-vivo operation.

All water samples collected after the tests were sent to Analytical Sciences Laboratory at the University of Idaho for analysis including metal, anion, pH and diesel and oil contents.

Summarized in Table 2 is the experimental matrix showing the engine parameters and other conditions during the tests. The engine load, which was controlled using the dynamometer was maintained at different torques while maintaining a certain engine speed (rpm). Each test was conducted for 30 minutes in the absence and presence of the Gas Scrubber using diesel No. 2 fuel, biodiesel from different feedstock (camelina, canola and safflower), and canola SVO. This report covers the six runs that were performed at the Bio-Energy Center.

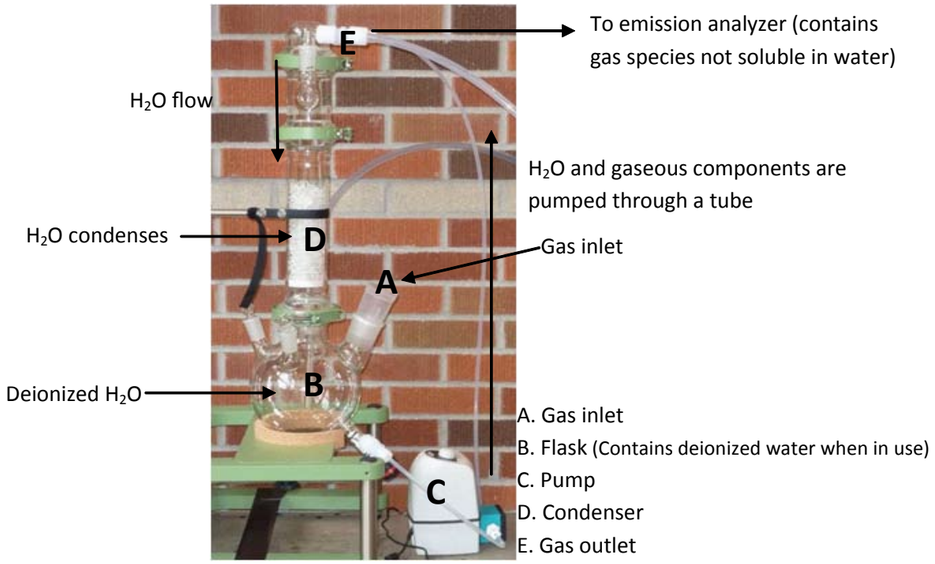


Figure 3. The Gas Scrubber used during emission monitoring.

The Gas Scrubber: The exhaust gas is introduced into the FLASK (B) containing 1.5L of deionized water through the GAS INLET (A). As the gas is bubbled into the water, water soluble gas species are incorporated into the water phase. Through PUMP (C), the contents of the FLASK (B) are introduced at the top of the CONDENSER (D). At this point the water soluble species condense together with water, which are reintroduced into the FLASK (B). The non-water soluble gas species on the other hand are pumped out from the system through the GAS OUTLET (E) and to the emission analyzer for measurement.

Table 2. Experimental matrix.

Run	Trial	Engine Speed (RPM)	Torque (ft/lb)	Power (HP)	Time (mins)	Scrubber
1	1	constant	Low	Low	30	No
2	1	constant	Low	Low	30	Yes
3	1	constant	High	High	30	No
4	1	constant	High	High	30	Yes
5	1	constant	Medium	Medium	30	No
6	1	constant	Medium	Medium	30	Yes

Bubbling the exhaust gas through deionized water ("water trap") using the scrubber resulted in increased acidity to give a pH of 3.3 to 3.6 from pH 5.0 of the control deionized water. The levels of metals and anions in the control (deionized water) were all very low if not negligible (data not shown). Hence, the presence of metals and anions in the "water trap" could be attributed to the use of the scrubber during testing. The concentrations of some metals and anions in the "water trap" were affected by the engine load. For instance using diesel, higher engine load resulted in the increased levels of both calcium and magnesium by 41 to 51% and 44 to 87% respectively, compared to runs with lower engine loads (Figures 4, 5 and 6). The levels of copper and zinc on the other hand were generally highest during Run 6.

The lowest engine load gave the lowest anion levels such as chloride, nitrite and sulfate but with the highest nitrate. Increasing engine load in terms of power and torque had led to the increase in chloride, nitrite and sulfate with accompanying decrease in nitrate. In all cases, bromide and phos-

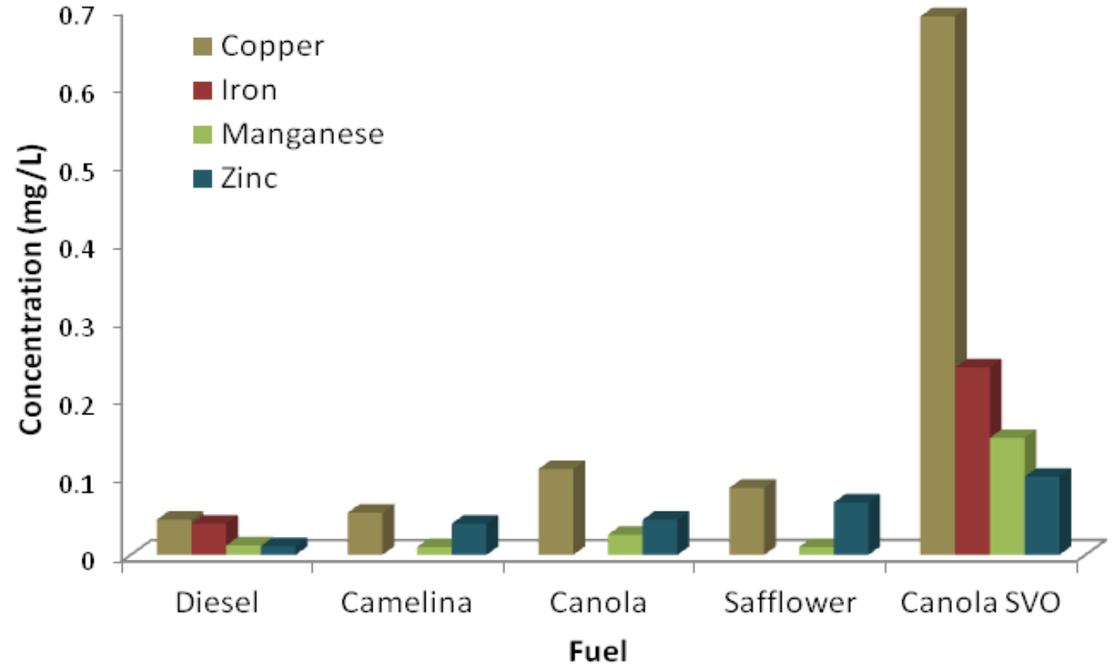


Figure 4.a. Metal content of the "water trap" obtained after bubbling with exhaust gas emissions at engine conditions Run 2.

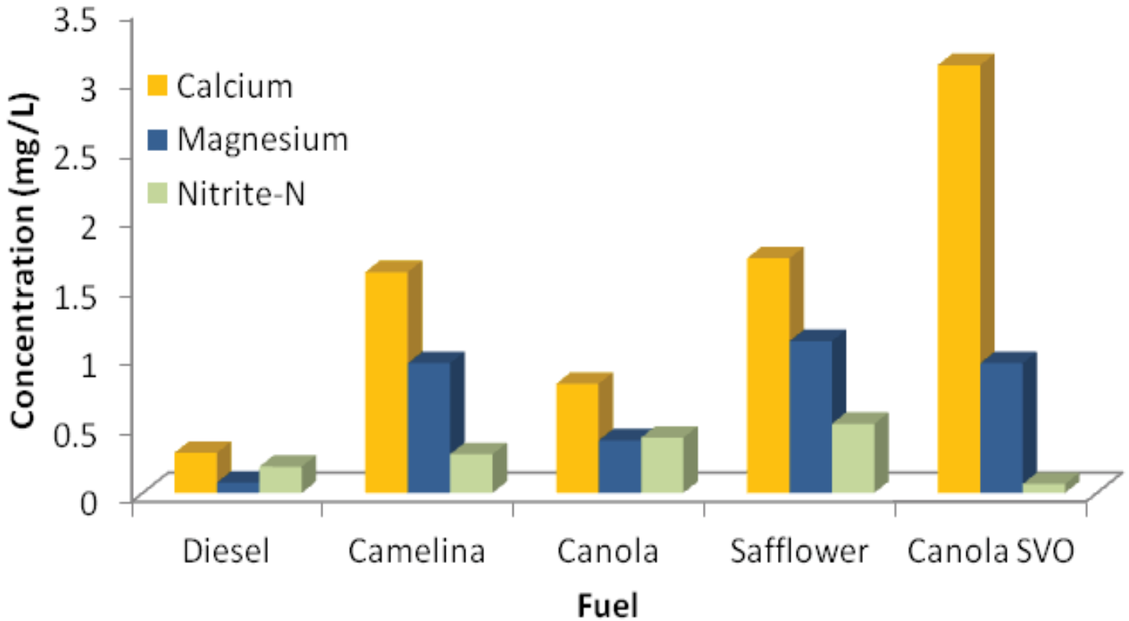


Figure 4.b. Metal and anion content of the "water trap" obtained after bubbling with exhaust gas emissions at engine conditions Run 2.

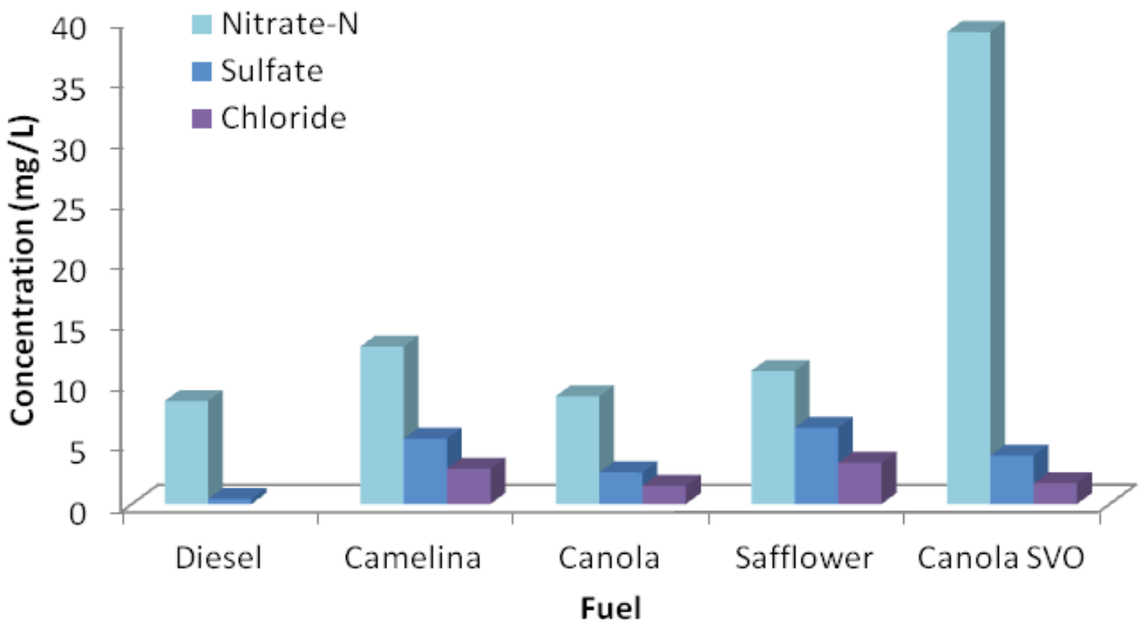


Figure 4.c. Anion content of the "water trap" obtained after bubbling with exhaust gas emissions at engine conditions Run 2.

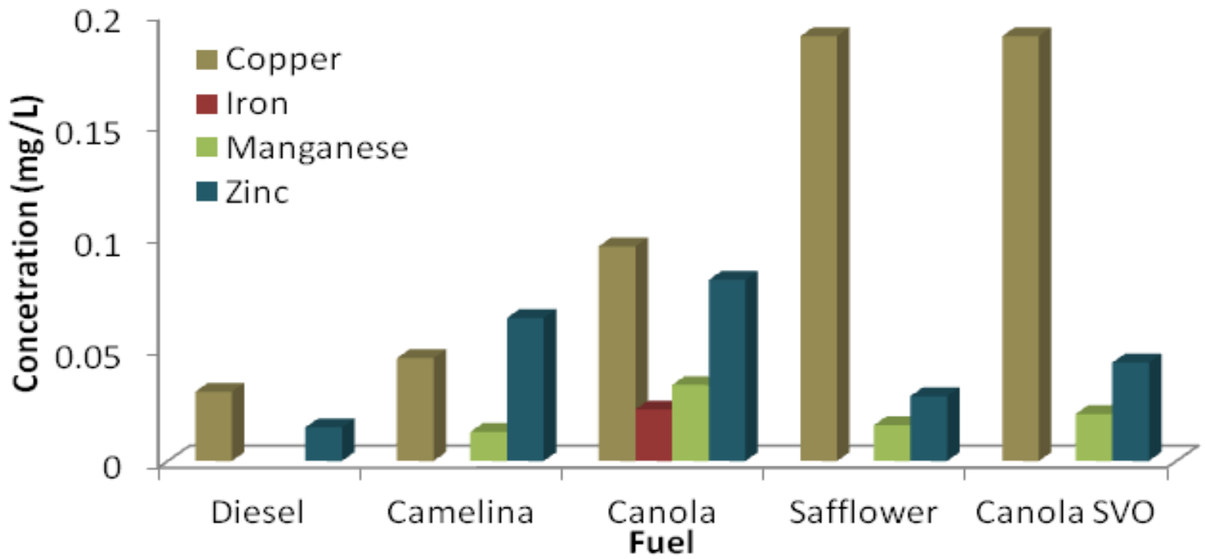


Figure 5.a. Metal content of the "water trap" obtained after bubbling with exhaust gas emissions at engine conditions Run 4.

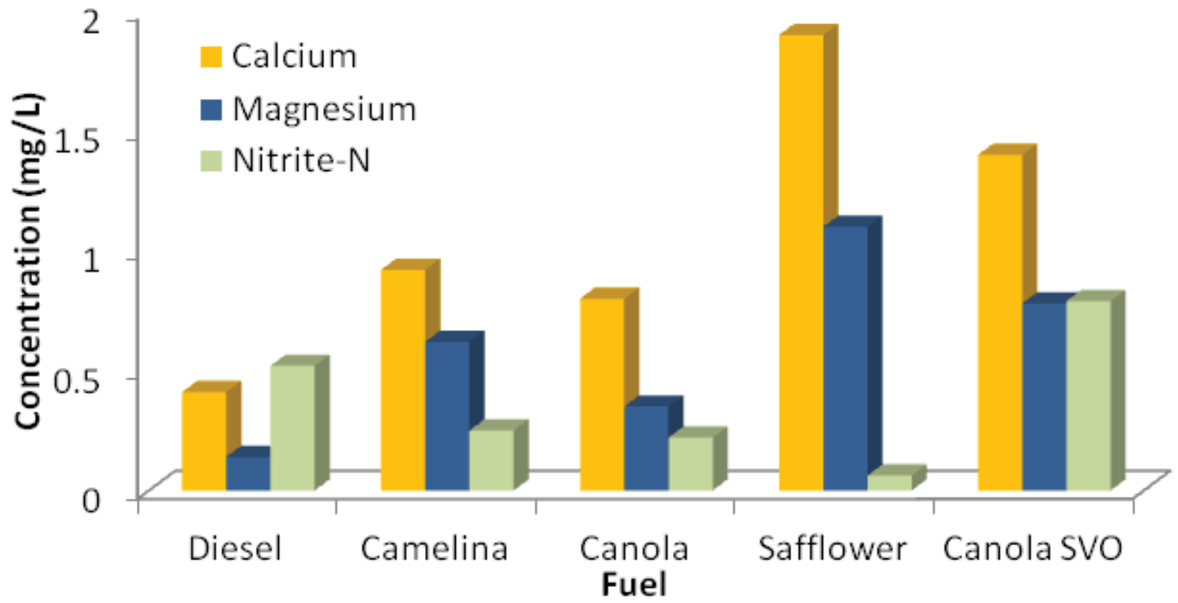


Figure 5.b. Metal and anion content of the "water trap" obtained after bubbling with exhaust gas emissions at engine conditions Run 4.

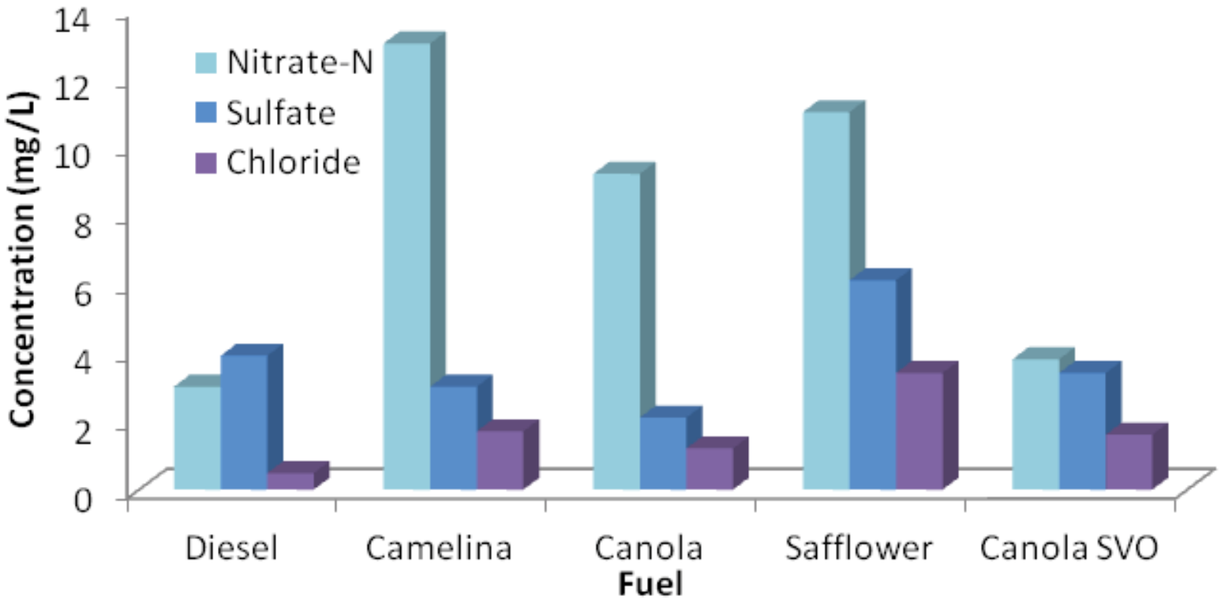


Figure 5.c. Anion content of the "water trap" obtained after bubbling with exhaust gas emissions at engine conditions Run 4.

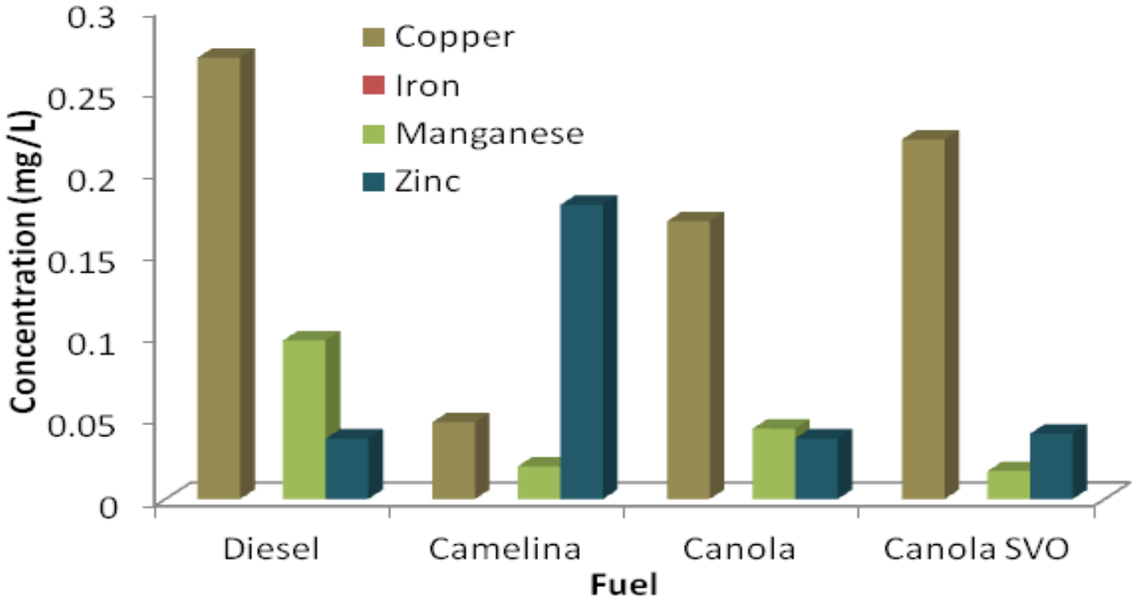


Figure 6.a. Metal content of the "water trap" obtained after bubbling with exhaust gas emissions at engine conditions Run 6.

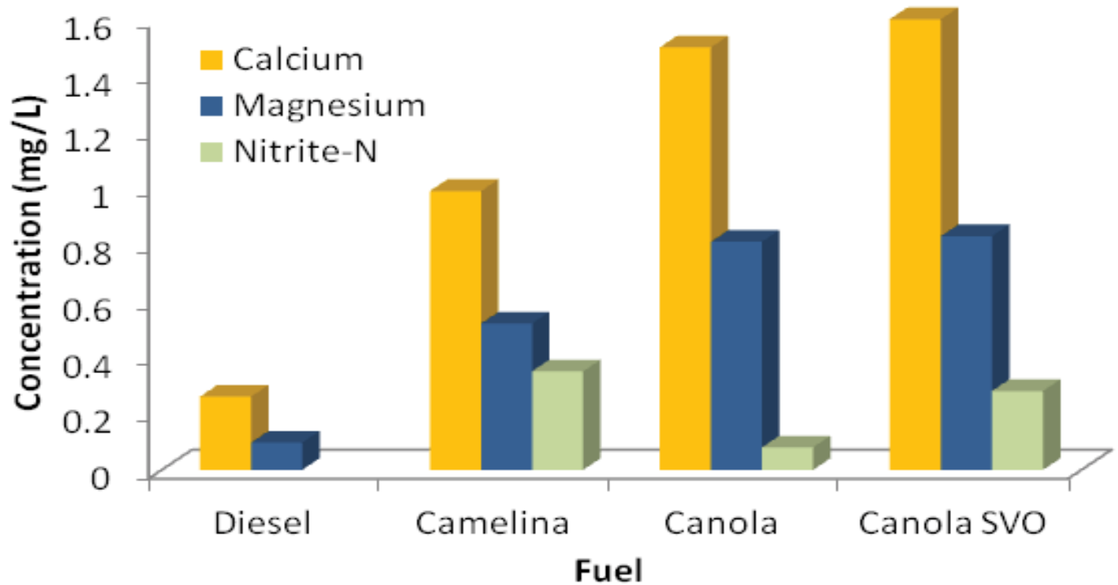


Figure 6.b. Metal and anion content of the "water trap" obtained after bubbling with exhaust gas emissions at engine conditions Run 6.

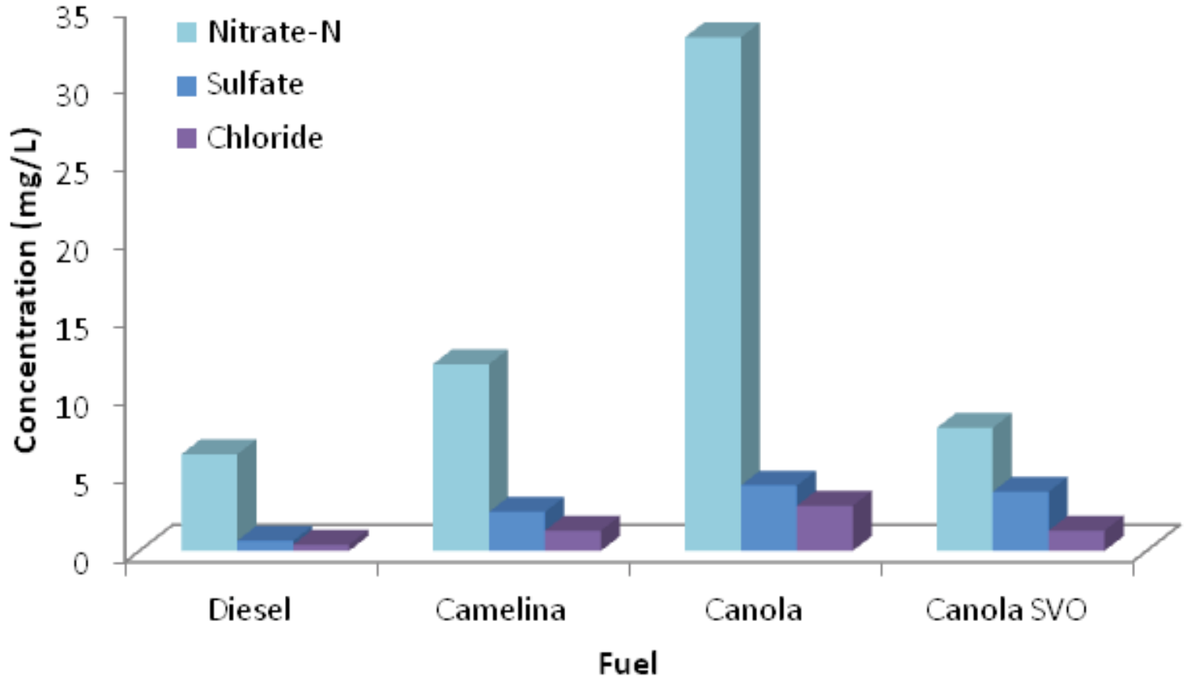


Figure 6.c. Anion content of the "water trap" obtained after bubbling with exhaust gas emissions at engine conditions Run 6.

Actual field tests were also conducted to determine metal and anion components found in the scrubber is also present in the condensate produced in the Bio-Agtive condenser. A water trap was installed in the Bio-Agtive to collect the condensate in the Bio-Agtive™ condenser during actual seeding process. Table 3 summarizes the results of the actual field trials in comparison with laboratory tests. In general, most of the components identified present in the water trap collected in the laboratory were also found in the condensate in actual field tests.

Table 3. Comparison of metal and anion content of the condensate collected in the field and "water trap" in the lab.

Metal/Anion Content	TREATMENT					
	Water Trap[1]	Control	30 sec	2 min	2 min	1 min (in situ)
Copper	0.031	<0.02	<0.02	<0.02	<0.02	<0.02
Iron	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Manganese	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Zinc	0.015	<0.01	<0.01	0.01	0.014	0.014
Calcium	0.041	<0.05	0.17	0.31	0.45	0.12
Magnesium	0.14	<0.02	0.036	0.054	0.12	<0.02
Nitrite-N	0.52	ND	ND	ND	ND	ND
Sulfate	0.87	N.D	N.D	N.D	N.D	N.D
Chloride	0.48	<0.2	<0.2	<0.2	<0.2	<0.2

Note: 1 – Metal and anion content of the "water trap" obtained from Run 4 in the lab.

Germination tests of seeds treated by the Bio-Agtive™ system while spring seeding were also conducted (see Appendix 1). Replicating the conditions used in the laboratory tests in actual field trials was also performed. Using a full-scale farm equipment, different fuels were tested in replicated field trials, data logging of emissions to verify the emissions sequestration at seeding time by analyzing the (air fuel ratio) concentrations at four locations: (1) engine turbo, (2) Bio-Agtive™ condenser after the injection fan, and (3) oxygen sampling after the seed furrow tines and (4) behind the packer wheels. The results of these tests are summarized in Appendix 2.

1.2.1. Major Emissions

a) Carbon dioxide emission

Figure 7 shows the carbon dioxide emissions at different engine loads. In most of the fuels tested, carbon dioxide emission depends on the engine load. Step 1 which has the lowest engine load, generally resulted in the lowest carbon dioxide emission compared to other steps. Based on these observations, it is possible that less fuel was consumed at lower engine loads which resulted in less carbon dioxide formation.

b) Carbon monoxide emission

In contrast to carbon dioxide emissions, carbon monoxide emission is at the highest amount at the lowest engine load (Step 1) as shown in Figure 8. It is noted that the differences between steps are in the range of 30-50 ppm and not in percentages. The results also suggest that using biodiesel produces less carbon monoxide emission compared to diesel.

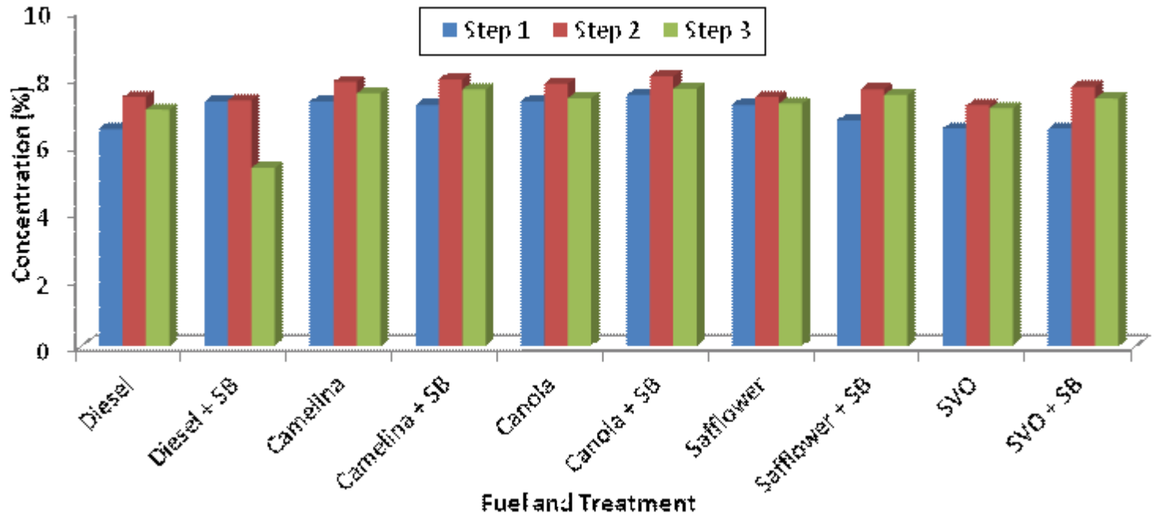


Figure 7. Carbon dioxide emissions of control and treated fuel samples at different engine loads. (Notes: + SB – the exhaust was bubbled through a water trap using the scrubber; Treatment Step 1: Low engine load; Treatment Step 2: High engine load; Treatment Step 2: Medium engine load)

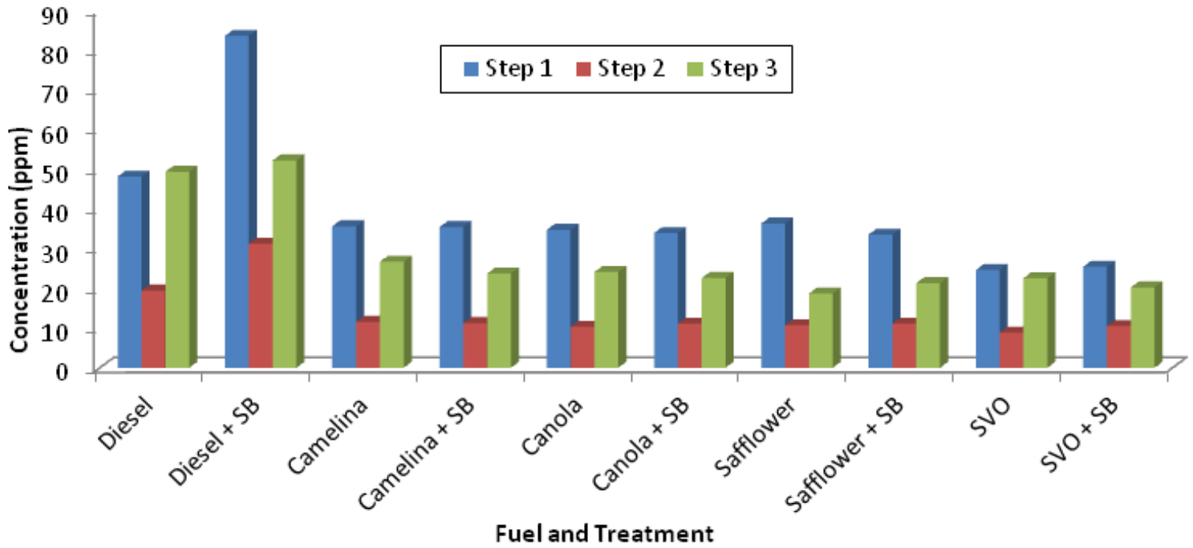


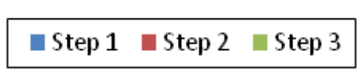
Figure 8. Carbon monoxide emissions of control and treated fuel samples at different engine loads. (Notes: + SB – the exhaust was bubbled through a water trap using the scrubber; Treatment Step 1: Low engine load; Treatment Step 2: High engine load; Treatment Step 2: Medium engine load)

c) Nitrogen oxides (NOx) emission

Similar to carbon monoxide emission results, NOX emissions were higher at lower engine loads (Figure 9). It can also be observed that at low engine loads, biodiesel produces more NOx compared to diesel. However at Steps 2 and 3 (with high and medium engine loads), NOx emissions for treatments that used biodiesel were the same and sometimes lower than the treatments that used diesel. Therefore, it could be deduced that the amount of NOx produced in a diesel engine does not completely depend on the type of fuel used. Moreover, biodiesel, in general, does not always produce more NOx than diesel. Rather, other factors such as engine load and conditions can be attributed to the increase of NOx formation in diesel engines. It was also observed that the majority of the NOX emissions were nitrogen oxide (NO) (Figures 10-12).

d) Soot

Figure 13 shows that biodiesel significantly produces less soot emissions compared to diesel. The presence of aromatics and impurities in diesel causes the formation of soot during combustion. Biodiesel does not contain any of these compounds.



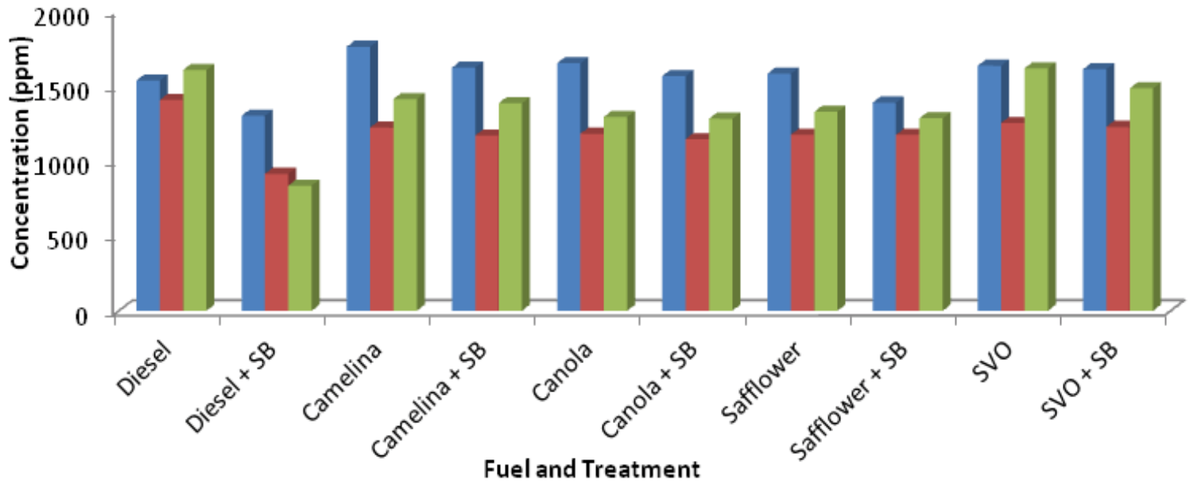


Figure 9. Nitrogen oxides emissions of control and treated fuel samples at different engine loads. (Notes: + SB – the exhaust was bubbled through a water trap using the scrubber; Treatment Step 1: Low engine load; Treatment Step 2: High engine load; Treatment Step 2: Medium engine load)

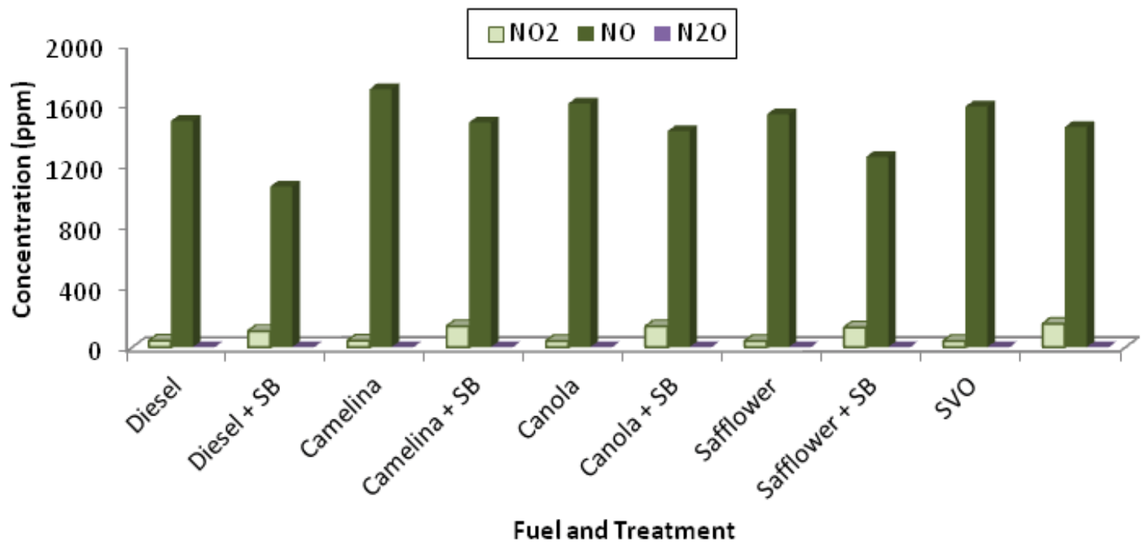


Figure 10. Nitrogen dioxide, nitrogen oxide, and nitrous oxide emissions of control and treated samples at low engine load.

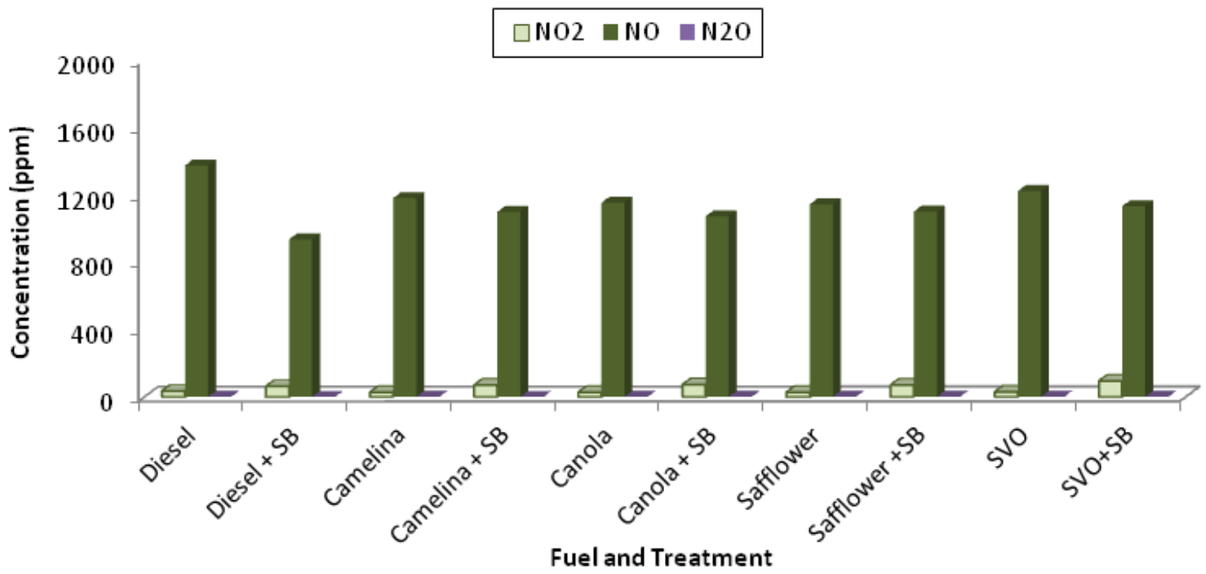


Figure 11. Nitrogen dioxide, nitrogen oxide, and nitrous oxide emissions of control and treated samples at high engine load.

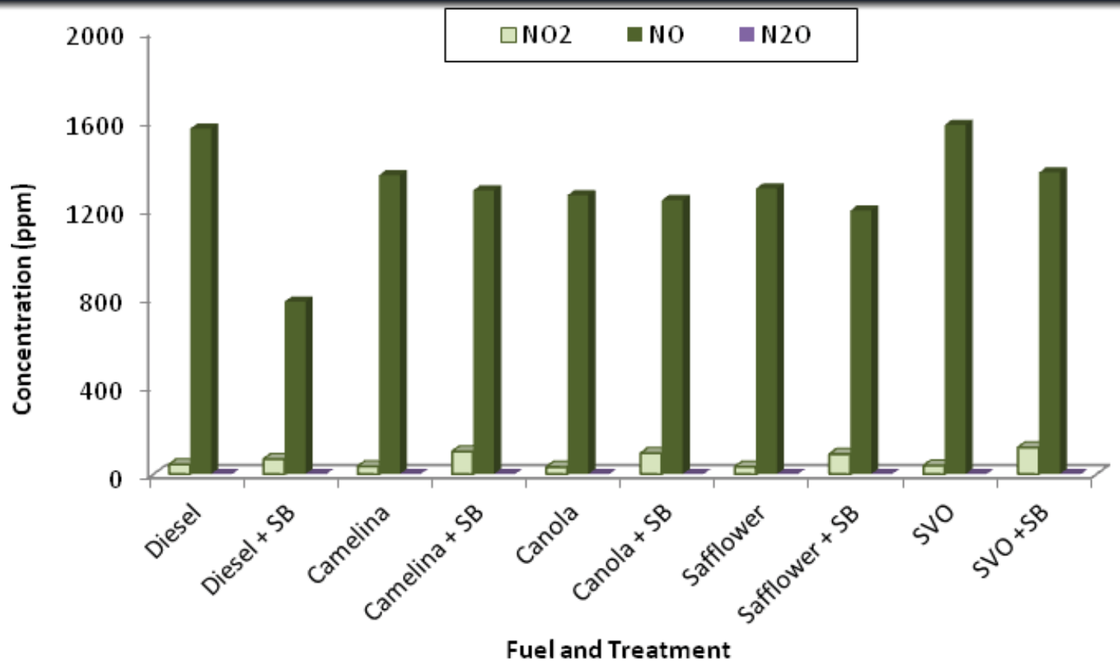


Figure 12. Nitrogen dioxide, nitrogen oxide, and nitrous oxide emissions of control and treated samples at medium engine load.

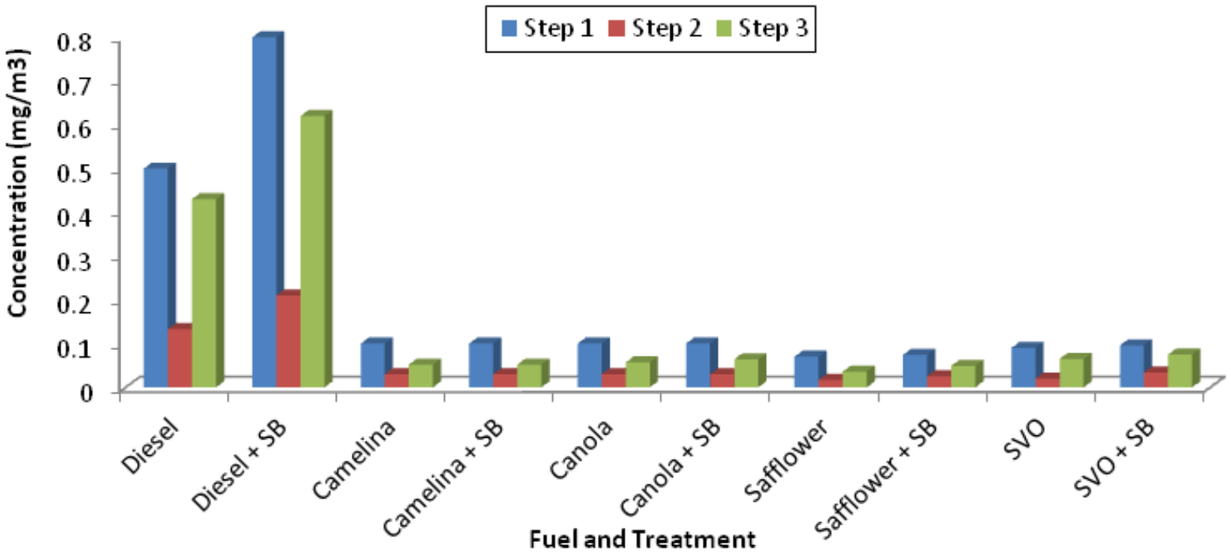


Figure 13: Soot emissions of control and treated fuel samples at different engine loads. (Notes: + SB – the exhaust was bubbled through a water trap using the scrubber; Treatment Step 1: Low engine load; Treatment Step 2: High engine load; Treatment Step 2: Medium engine load)

1.2.2. Minor Emissions

The minor emissions that were measured during the tests include formaldehyde (HCHO), formic acid (HCOOH), hydrogen cyanide (HCN) and sulfur dioxide (SO2). Comparing the emissions of treatments with and without the scrubber at the exhaust, it can be observed that the measured minor emissions were lower at treatments with the scrubber (Figures 14, 15 and 16). This suggests that these emissions were dissolved in the water in the scrubber. Sulfur dioxide and formic acid, when dissolved, can lower the pH of water. In the experiment, the water collected from the scrubber was acidic (pH of 3.3-3.6) which confirms that these minor emissions were removed by scrubber. Based on these observations, it is possible that using the Bio-AgtiveTM Emission Technology, the condensate produced during cooling of the exhaust emissions might contain these compounds that can have a biocidal effect on seeds (see Appendix 1). It was also observed that emissions contain an average of 0.016 ppm of ammonia (NH3) (Figure 17).

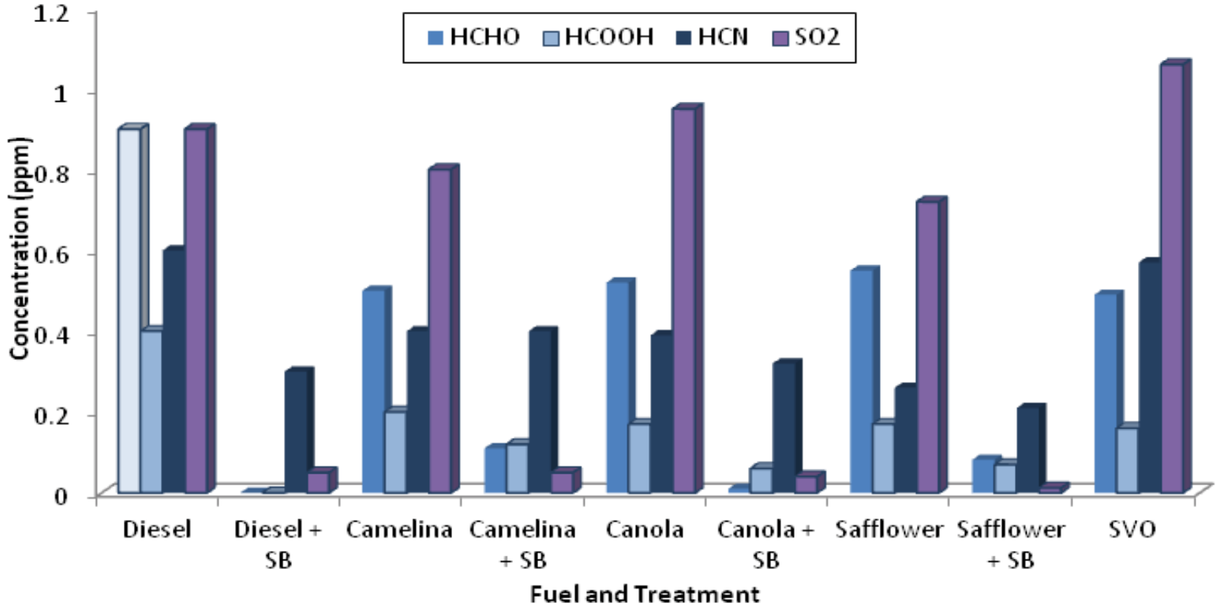


Figure 14. Minor emissions of control and treated samples at low engine load.

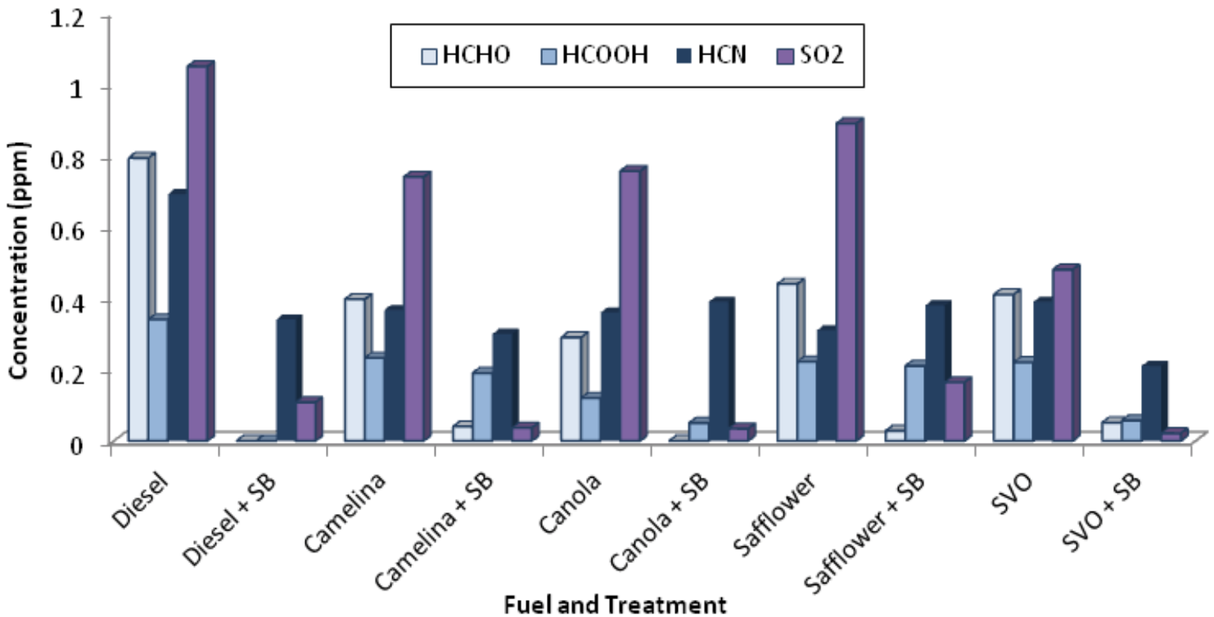


Figure 15. Minor emissions of control and treated samples at high engine load.

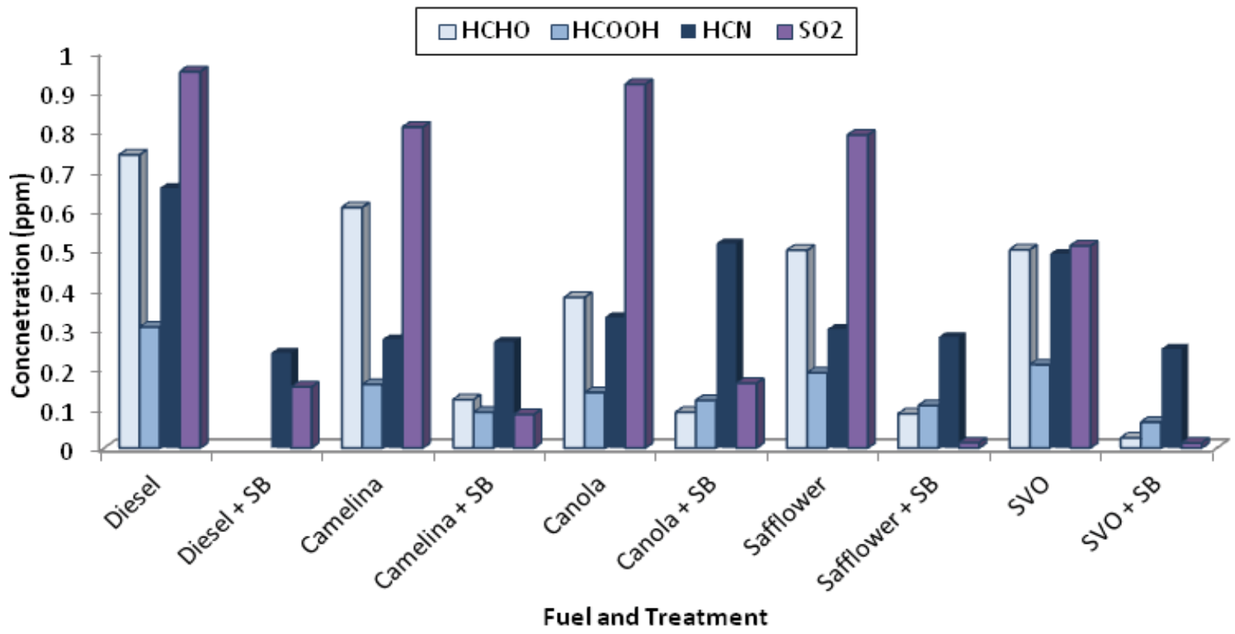


Figure 16. Minor emissions of control and treated fuel samples at medium engine load.

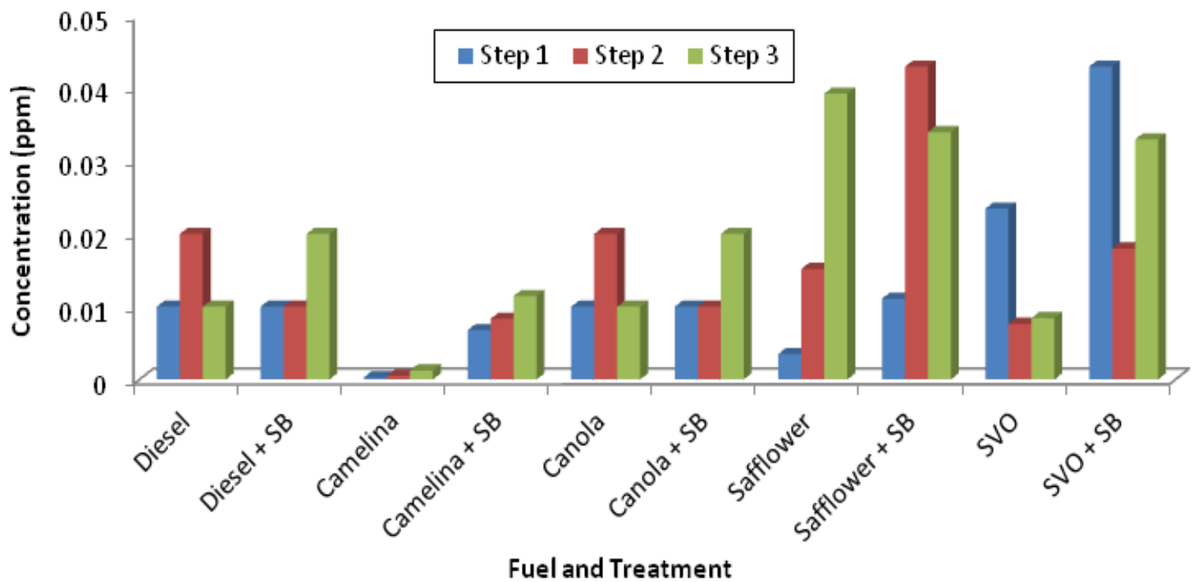


Figure 17. Ammonia emissions of control and treated fuel samples at different engine loads. (Notes: + SB – the exhaust was bubbled through a water trap using the scrubber; Treatment Step 1: Low engine load; Treatment Step 2: High engine load; Treatment Step 2: Medium engine load)

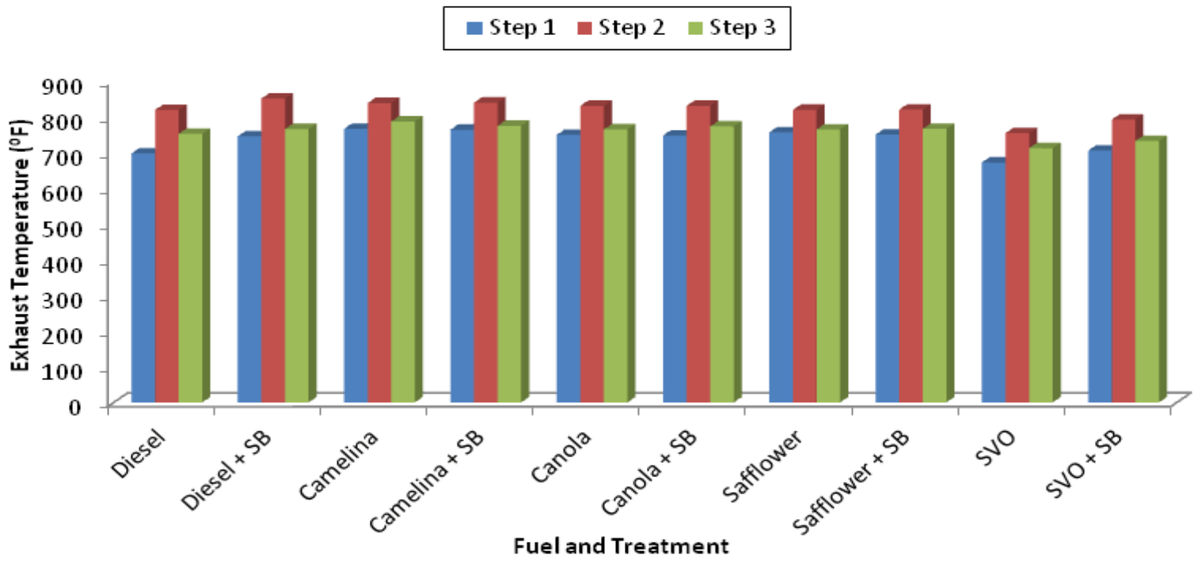


Figure 18. Exhaust temperature of control and treated fuel samples at different engine loads. (Notes: + SB – the exhaust was bubbled through a water trap using the scrubber; Treatment Step 1: Low engine load; Treatment Step 2: High engine load; Treatment Step 2: Medium engine load)

1.2.3. Exhaust temperature

The exhaust temperatures were the same for both diesel and biodiesel as shown in Figure 18. However when straight vegetable oil (SVO; canola) was used, the exhaust temperature was relatively lower regardless of the engine load and power used in the test.

2. Micronutrient Analysis in Soils and Plant Tissues

2.1 Objective

The second objective of this project was to use our understanding of the chemical composition of the emissions in the field, on a farm, using farm scale equipment to test the ability of the exhaust emissions to augment or replace starter fertilizer and/or other fertilizer applications to the fields. We want to know if it is possible to supply essential micronutrients in fuels through exhaust emissions, instead of using costly customized fertilizers, and to what extent the effects of exhaust emissions have on biological, chemical and physical properties of soil.

2.2 Background

In order to better understand the micronutrients released by the exhaust emissions via the Bio-AgtiveTM Emission Technology in an actual field, the following tests, namely, routine soil analysis, plant tissue analysis, PLFA, soil respiration, and GC/MS were conducted. Two different fields were tested with the Bio-Agtive Emission Technology. Since there were differences in conditions between fields like soil type, time of seeding, and farming practices, the results and discussion were sectioned into two case studies, namely, Field 1 and Field 2.

2.3 Routine Soil and Plant Tissue Analysis

CASE STUDY 1: FIELD 1

2.3.1 Sample Plot Layout of Field 1

Field 1 contained four treatments and a replicate per each treatment equaling eight plots total (Figure 19). The treatments were the following: (a) control (no exhaust or fertilizer used during air seeding), (b) traditional fertilizer (11-52-0 -NPK) applied at 50 lb/acre, (c) BAET using diesel No.2, and (d) BAET using B100 camelina biodiesel. All plots were air seeded at a rate of 60 lb/acre. Each plot was 2,400 feet in length and 50 feet wide. There were buffer or "warm-up" areas at the beginning and end of each plot in order to reduce engine ramp-up and engine throttle-down interference. Four samples for routine soil property and plant tissue analysis each were taken per each treatment plot to total eight samples per treatment.

Figure 19. Sample plot layout for Field 1

2.3.2. Sampling of Field 1: Routine Soil Analysis

Two sample sets per each field were taken for routine soil analysis: one before seeding and one after harvest. Samples were taken from the furrow and collected a depth of six inches. The samples were then placed in bags provided by A & L Great Lakes Labs, Indiana and kept refrigerated for a period until they were shipped. Properties tested for included: organic matter, phosphorus, potassium, magnesium, calcium, sulfur, zinc, manganese, iron, copper, boron, nitrate, soil pH, cation exchange capacity, and percent base saturation of K, Mg, Ca, and H.

2.3.3. Sampling of Field 1: Plant Tissue Analysis

One sample set per each field was taken for plant tissue analysis. Field 1's sample was taken 44 days after seeding. Tissue samples included the top 3-5 inches of the wheat plant and were refrigerated until shipping. Properties tested included: nitrogen, sulfur, phosphorus, potassium, magnesium, calcium, sodium, boron, zinc, manganese, iron, copper, and aluminum.

2.3.4 Field 1: Soil Properties and Plant Tissue Analysis Results

The results from the routine soil analysis are summarized in Tables 4, and 5. The organic matter average was around 3% and pH 8 which suggests the soil is a Calcareous soil type. Using GPS, field results of before seeding and after harvest showed natural soil fertility changes and influenced changes by the treatments. In all plot treatments, soil pH decreased and organic matter increased after harvest. These changes suggest a natural change in the soil from the crop growing. Nitrogen and phosphorus were the most influenced by the emissions in the soil tests, tissue tests, grain nutrient tests and protein yield results. Available phosphorus increased from 17 ± 8 ppm to 32 ± 8.7 ppm-P in plots treated with BAET diesel. In plots with BAET Diesel treatment, the nitrogen in the soil (in terms of nitrates) increased by 7.8 ± 4.0 ppm or an average of 15 lbs in the soil after harvest.

Table 4. Routine soil analysis results at Field 1 before seeding.

SOIL PROPERTIES	TREATMENT			
	Control	Fertilizer	BAET Diesel	BAET Biodiesel
Organic Matter(%)	2.6 ± 0.2	2.9 ± 0.4	2.8 ±0.1	2.6 ±0.4
Available Phosphorus (ppm-P)	18 ± 8	22 ± 10	17 ± 8	26 ± 13
Potassium (ppm)	531 ± 148	539 ± 117	536 ± 110	582 ± 72
Magnesium (ppm)	1168 ± 120	1215 ± 146	1240 ± 219	1203 ± 149
Calcium (ppm)	4356± 1707	4712 ±1277	5206 ± 1135	4769 ± 1737
Soil pH	7.8 ± 0.4	8.0 ± 0.4	8.1 ± 0.4	7.9 ± 0.4
Cation Exchange Capacity (meq/100g)	33.0 ± 7.7	35.1 ± 5.4	37.7 ± 4.2	35.35 ± 7.6
Sulfur(ppm)	30 ± 50	7.5 ± 2	7.2 ± 2.7	6 ± 1.5
Zinc (ppm)	1.8 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.8 ± 0.3
Manganese (ppm)	61 ± 6	57 ± 4	56 ± 4	59 ± 5
Iron (ppm)	2 ± 1	2 ± 2	1 ± 0.5	2 ± 2
Copper (ppm)	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.2	2.0 ± 0.1
Boron (ppm)	1.7 ± 0.3	1.7 ± 0.4	1.8 ± 0.5	1.7 ± 0.4
Nitrate (ppm)	6 ± 1	6 ± 2	5 ± 2	5 ± 1
Percent Base Saturation				
%K	4.8 ± 1.7	4.1 ± 1.4	3.7 ± 1.0	4.5 ± 1.4
%Mg	31.2 ± 8.8	30.3 ± 8.4	28.0 ± 7.4	30.0 ± 9.7
%Ca	64.0 ± 10.3	66.1 ± 8.3	68.3 ± 8.1	65.5 ± 10.8

Table 5. Routine soil analysis results at Field 1 after harvesting.

SOIL PROPERTIES	TREATMENT			
	Control	Fertilizer	BAET Diesel	BAET Biodiesel
Organic Matter(%)	4.0±2.2	3.0±0.7	3.0±0.2	3.2±0.4
Available Phosphorus (ppm-P)	28.3±8.6	34.6±12.2	32±8.7	33±7.6
Potassium (ppm)	634.4±111.6	591.4±145.9	652.6±95	689.4±95.7
Magnesium (ppm)	966.4±139.8	1055±131.2	1029.4±157.4	993.1±125.6
Calcium (ppm)	4000±1293.9	3493.8±1936.9	3406.3±1712	3406.3±1839.7
Soil pH	7.7±0.4	7.6±0.5	7.6±0.5	7.5±0.6
Cation Exchange Capacity (meq/100g)	29.7±5.3	27.8±8.9	27.4±7.4	27.2±8.2
Sulfur(ppm)	6.7±1.1	6.1±1.4	6.5±1.4	6.5±1.2
Zinc (ppm)	1.8±0.2	1.9±0.2	1.9±0.1	2±0.2
Manganese(ppm)	54.1±4.7	54±5.3	54.1+4.4	54.1±3.3
Iron (ppm)	1±0.0	1±0.0	1±0.0	1±0.0
Copper (ppm)	2±0.1	2±0.2	2±0.1	1.9±0.1
Boron (ppm)	1.7±0.4	1.6±0.5	1.7±0.5	1.6±0.5
Nitrate (ppm)	9.7±3	8.6±4.8	12.8±3.5	9.3±3.4
Percent Base Saturation				
%K	5.7±1.9	6.1±2.7	6.4±1.7	7.1±2.5
%Mg	28.4±8.4	34.6±11.4	33.9±11.7	33.2±11.4
%Ca	65.8±10.2	59.2±13.5	59.2±13.4	58.9±14.5

Table 6 summarizes the results from the plant tissue analysis. Nitrogen, Phosphorus, Potassium, Sulfur (NPKS) is the lowest in the fertilizer plant tissues compared to the other treatments. The increase in calcium to phosphorus ratio, selenium, iron and zinc in the plant tissues may suggests that plots with BAET Diesel produced quality grain. It is noted that iron, selenium and zinc is highest in BAET diesel treatment (Table 6).

Table 6. Plant tissue analysis of spring wheat 44 days after seeding at Field 1.

NUTRIENT	TREATMENT			
	Control	Fertilizer	BAET Diesel	BAET Biodiesel
Nitrogen (%)	3.51±0.27	3.28±0.14	3.39±0.47	3.54±0.28
Sulfur (%)	0.30±0.02	0.27±0.01	0.28±0.03	0.29±0.01
Phosphorus (%)	0.45±0.05	0.37±0.03	0.42±0.05	0.40±0.05
Potassium (%)	3.89±0.30	3.65±0.18	3.90±0.34	4.01±0.06
Magnesium (%)	0.23±0.03	0.23±0.05	0.23±0.05	0.20±0.03
Calcium (%)	0.32±0.04	0.31±0.08	0.31±0.06	0.25±0.02
Sodium (%)	0.03±0.01	0.03±0.02	0.04±0.02	0.04±0.03
Boron (ppm)	7.75±1.26	8.50±1.73	8.25±1.71	8.00±1.41
Zinc (ppm)	20.25±1.71	18.50±1.29	22.25±1.71	21.75±0.96
Manganese (ppm)	68.75±11.81	57.75±7.97	65.00±5.72	57.50±3.79
Iron (ppm)	161.25±12.95	139.25±23.06	179.75±30.19	147.50±11.15
Copper (ppm)	10.25±0.50	10.00±1.41	10.75±1.71	10.00±0.82
Aluminum (ppm)	160.25±19.05	153.00±18.71	202.25±78.65	130.25±15.65

CASE STUDY 2: FIELD 2

2.3.5 Sample Plot Layout of Field 2

Field 2 contained two treatments with one replicate per each treatment to equal two plots. The treatments were the following: (a) control (no exhaust treatment or fertilizer) and (b) BAET using No. 2 diesel. Each plot was 60 feet wide and 120 feet long with buffers at the beginning and end of each plot. Three samples for routine soil properties and plant tissue analysis were taken per each treatment to equal six samples total. Figure 20 illustrates Field 2's layout.

It should be noted that Field 2 was a transitional organic field in which conventional no-till practices were not applied. Trial plot area was tilled with a disc to accommodate a previously established barley crop (Figure 21). The intentions were to seed with organic vegetable oil as the fuel. Due to operability problems, the SVO test plot was not seeded. However, a portion of the warm-up area was seeded with SVO. Also due to unseasonably late rains, seeding was delayed until end of June. Given with the unseasonable late rains the decision was to go ahead with the trial as far as we could to give insights in an organic farming practice.

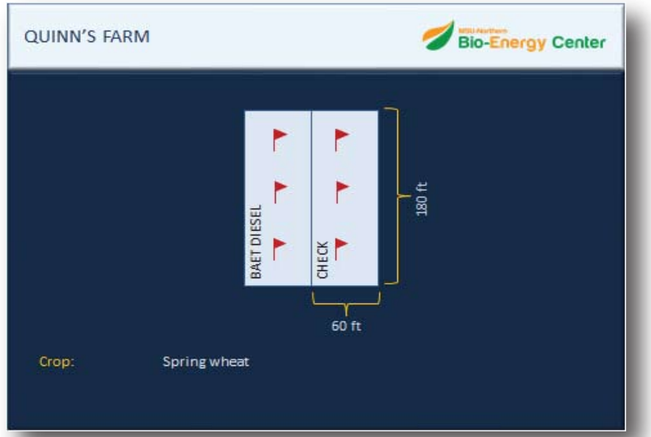


Figure 20. Sample plot layout of Field 2.

Figure 21. Late June seeding at Field 2.

2.3.6. Sampling of Field 2: Routine Soil Analysis

The same protocol as discussed in section 2.3.2 was used for Field 2.

2.3.7. Sampling of Field 2: Plant Tissue Analysis

One sample set per each field was taken for plant tissue analysis. Field 2's sample was taken 49 days after seeding. Similar testing protocol as discussed in section 2.3.3 was used.

2.3.8 Field 2: Soil Properties and Plant Tissue Analysis Results

Field 2 is an organic farm and has a lower organic matter of 1.5% and lower pH (pH of around 7) than Field 1 (Table 7). Nonetheless, the same natural changes as in Field 1 like pH going down and organic matter going up at end of season were observed. The results also showed that nitrogen and phosphorus increased the highest in plots treated with BAET Diesel (Table 8). Results of the plant tissue tests showed that the nitrogen and potassium were higher in BAET Diesel compared to other treatments (Table 9).

Table 7. Routine soil analysis results at Field 2 before seeding.

SOIL PROPERTIES	TREATMENT		
	Control	BAET Diesel	BAET SVO
Organic Matter(%)	1.4±0.1	1.6±0.2	1.6±0.2
Available Phosphorus (ppm-P)	11.6±0.1	11.00±1.7	16.0±1.0
Potassium (ppm)	295±39.1	294.7±9.0	350.7±11.1
Magnesium (ppm)	485±72.1	530.0±39.1	465.0±36.1
Calcium (ppm)	1583.4±333.0	1216.7±160.7	1066.7±115.5
Soil pH	7.3±0.6	6.7±0.3	6.4±0.1
Cation Exchange Capacity (meq/100g)	12.8±1.0	12.07±0.4	11.4±0.6
Sulfur(ppm)	4.0±1.0	3.7±0.6	4.4±0.58
Zinc (ppm)	1.9±0.3	1.7±0.1	1.8±0.0
Manganese(ppm)	57.0±3.6	52.7±5.1	43.0±3.6
Iron (ppm)	3.0±2.0	3.4±1.5	7.4±2.1
Copper (ppm)	1.4±0.1	1.3±0.1	1.3±0.1
Boron (ppm)	0.4±0.2	0.4±0.1	0.3±0.1
Nitrate (ppm)	1.7±0.6	2.0±0.0	2.0±0.0
Percent Base Saturation			
%K	6.0±1.1	6.30±0.2	7.9±0.6
%Mg	31.7±6.3	36.7±2.0	34.3±2.7
%Ca	61.3±8.4	50.4±5.6	47.1±3.2
%H	3.0±0.0	10.1±0.2	10.7±0.6

Table 8. Routine soil analysis results at Field 2 after harvesting.

SOIL PROPERTIES	TREATMENT	
	CONTROL	BAET DIESEL
Organic Matter(%)	2.2±0.2	2.3±0.2
Available Phosphorus (ppm-P)	11.0±1.0	13.0±1.0
Potassium (ppm)	281.3±19.4	285.3±12.7
Magnesium (ppm)	448.3±68.3	473.3±10.4
Calcium (ppm)	1583.3±621.2	1083.3±189.3
Soil pH	7.2±0.7	6.6±0.4
Cation Exchange Capacity (meq/100g)	12.9±2.0	10.9±0.2
Sulfur(ppm)	10.3±2.9	8.0±2.6
Zinc (ppm)	1.6±0.1	1.6±0.1
Manganese(ppm)	53.7±4.2	47.7±3.5
Iron (ppm)	4.3±3.1	4.7±3.5
Copper (ppm)	1.4±0.1	1.3±0.0
Boron (ppm)	0.5±0.2	0.3±0.1
Nitrate (ppm)	7.3±2.5	10.0±3.0
Percent Base Saturation		
%K	5.7±1.2	6.7±0.3
%Mg	29.9±8.5	36.2±1.4
%Ca	60.0±13.5	49.6±7.7

Table 9. Plant tissue analysis of spring wheat 49 days after seeding at Field 2.

Nutrient	Treatment	
	Control	BAET Diesel
Nitrogen (%)	2.88±0.19	3.73±0.20
Sulfur (%)	0.21±0.04	0.21±0.02
Phosphorus (%)	0.33±0.01	0.33±0.03
Potassium (%)	1.70±0.28	2.06±0.27
Magnesium (%)	0.23±0.04	0.19±0.02
Calcium (%)	0.35±0.20	0.17±0.04
Sodium (%)	0.06±0.02	0.02±0.01
Boron (ppm)	36.33±10.02	19.00±2.65
Zinc (ppm)	34.67±4.51	33.33±1.53
Manganese (ppm)	51.33±23.18	37.00±2.65
Iron (ppm)	101.00±23.39	80.00±12.12
Copper (ppm)	16.00±2.65	22.00±5.00
Aluminum (ppm)	361.67±24.70	552.67±133.61

2.4. Composition Analysis of Soils using Solid-Liquid Extraction

This part of study investigated whether trace amounts of unburned hydrocarbons and other organic compounds in the exhaust emissions were introduced into the soil.

2.4.1 Sample Collection and Solid-Liquid Extraction

Soil samples were collected at the site before and immediately after seeding. This was to establish a baseline for all of the treatments and to compare that baseline to soil conditions after using the Bio-Agtive Technology. The GPS location of each sample plot was recorded to ensure consistency in the position of the sample plots. A portion of the soil samples collected was then soaked in hexane, mixed using a sonicator, and sealed and kept at 5 C (40 F) for a week. A control with no soil was also prepared. The soil particulates were removed using Whatman filters (Grade 114). The liquid was then injected to gas chromatography-mass spectrometry (GC-MS) for analysis.

In a separate test, selected soil samples were extracted for 3 hours using soxhlet extraction. One hundred milliliters of acetone-hexane (1:1 by volume) and 10 g of soil were used in the extraction process. Two milliliters of the extraction solvent were collected and analyzed. The remaining solvent was transferred to a beaker and heated to 50-60 C (122-140 F) until only 10% of the original volume remained in the beaker. The remaining liquid solution was then analyzed using GC-MS. By boiling off 90% of the extraction solvent, any heavy compounds extracted during soxhlet extraction were concentrated.

2.4.2 Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

The liquid samples after extraction were injected into an Agilent 7890A gas chromatograph equipped with an Agilent 5975C inert mass spectrometer with Triple-Axis Detector and HP-5MS gas chromatograph column. The inlet temperature and injection split ratio were set to 250 C and 250:1, respectively. The carrier gas was helium at a flow rate of 1.5 mL/min. The temperature of the oven was initially at 50 C, held for 1 min, increased to 275 C at a rate of 30 C/min and maintained for 2 min. In order to analyze any heavier compounds, the liquid samples were also analyzed using GC-MS by using a different method. The split ratio was changed to 100:1 and the oven final oven temperature was set to 300 C and held for 30 minutes.

The GC/MS results showed that no hydrocarbons and organic compounds were extracted from the soil before and after seeding with the BAET. The chromatogram only showed peaks of the solvent used in the extraction process. This suggests that any compounds introduced by the BAET system did not retain in the soil or the amount was too small for the GC-MS to detect.

2.5 Microbial Profiling and Biomass Concentration of Soil Samples

2.5.1 CASE STUDY 1: Field 1 Results

The biomass content is represented as cells per gram (cell equivalence). The cell equivalence is based on the total amount of phospholipid fatty acids (PLFA) extracted from a given soil sample. Comparing the soil samples of Field 1 before seeding and immediately after seeding, there is an increase in the amount of cells in the soil treated with the Bio-AgtiveTM Emission Technology (Figure 22 and 23).

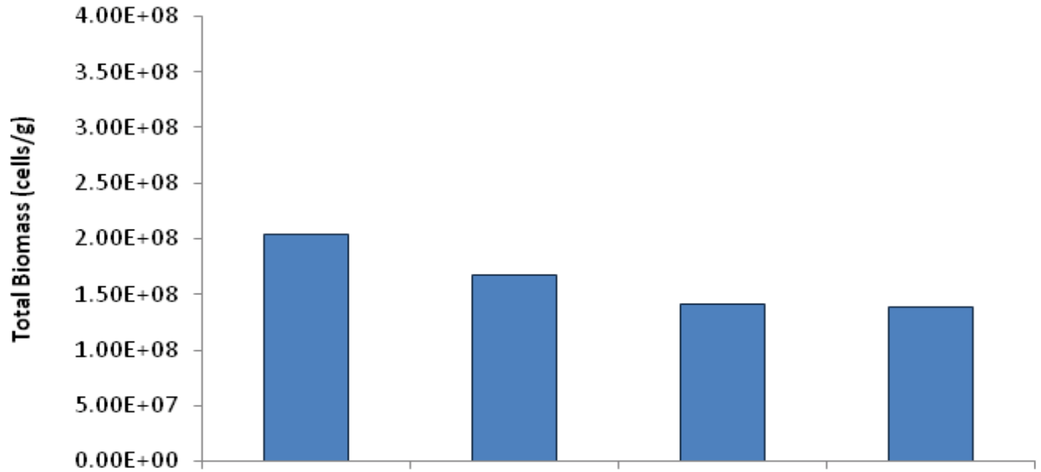


Figure 22. Results showing the total biomass content of the soil samples collected before seeding at Field 1.

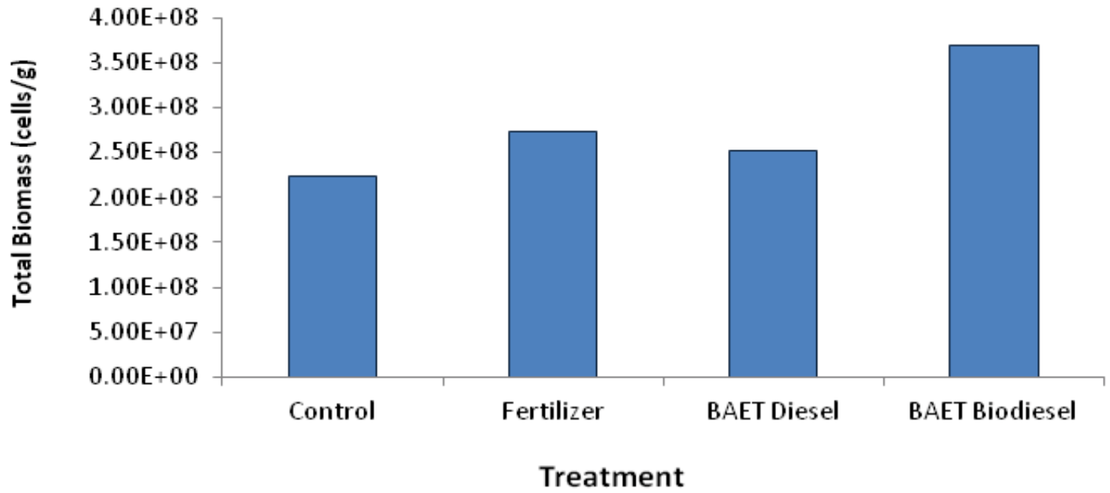


Figure 23. Results showing the total biomass content of the soil samples collected right after seeding at Field 1.

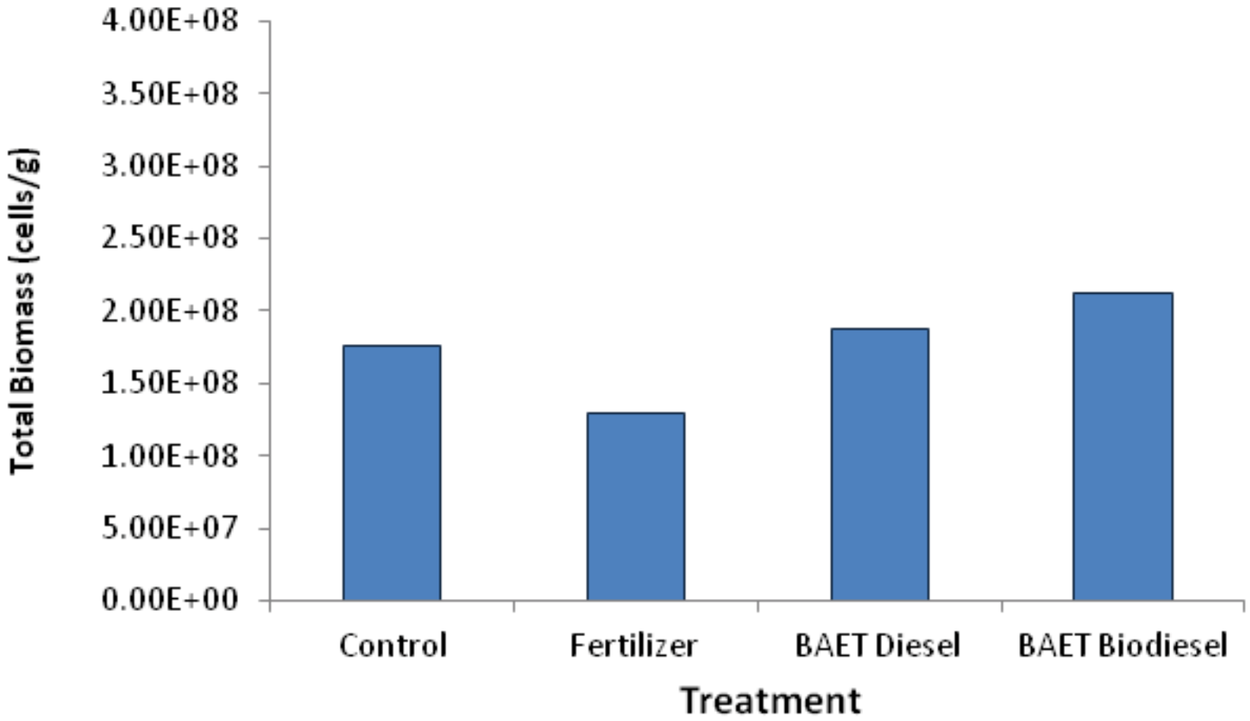


Figure 24. Results showing the total biomass content of the soil samples collected after harvesting at Field 1.

The relative percentages of total PLFA structural groups in the soil samples were also analyzed. The structural groups were assigned according to PLFA chemical structure, which is related to its fatty acid biosynthesis. Results show significant changes in the microbial profile in the soil before and after seeding (Figures 26 and 27). There was a noticeably increase in the percentage of eukaryotes. It was also observed that the proteobacteria (monos) population was greatly affected by the type of fuel used. For plots treated with BAET Diesel, proteobacteria increased in percentage while other treatments showed a decreasing trend. Proteobacteria consists of free living nitrogen fixing bacterial and its increase agrees with the increase in nitrogen content in the soil (Tables 4 and 5).

Figure 25. Plant tissue collection at Field 1.

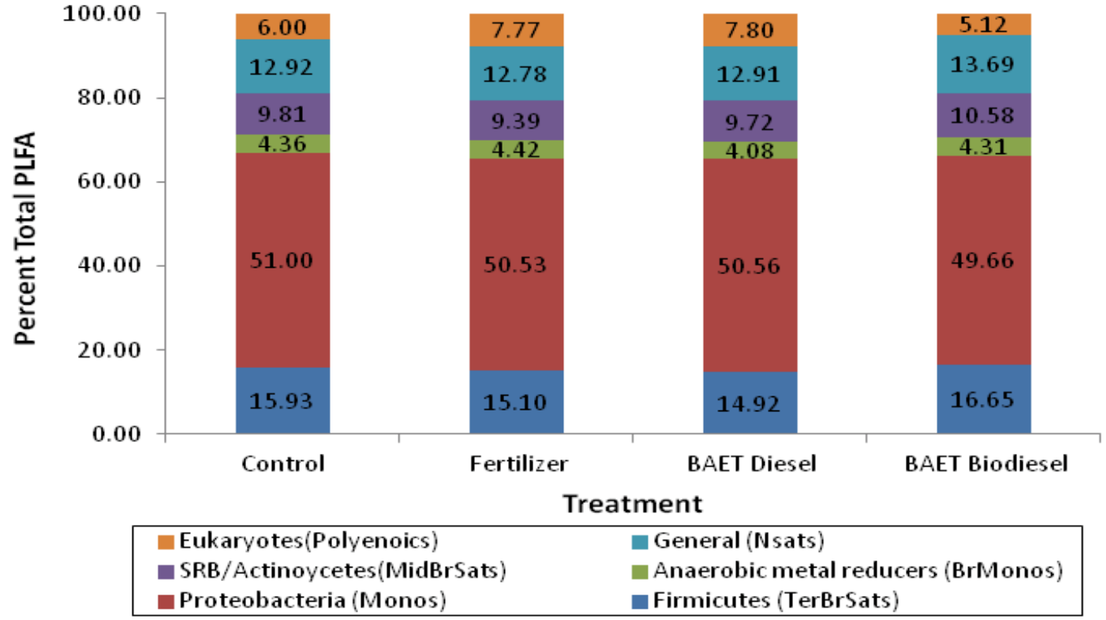


Figure 26. Results of the percentage of total PLFA for the soil samples collected before seeding at Field 1.

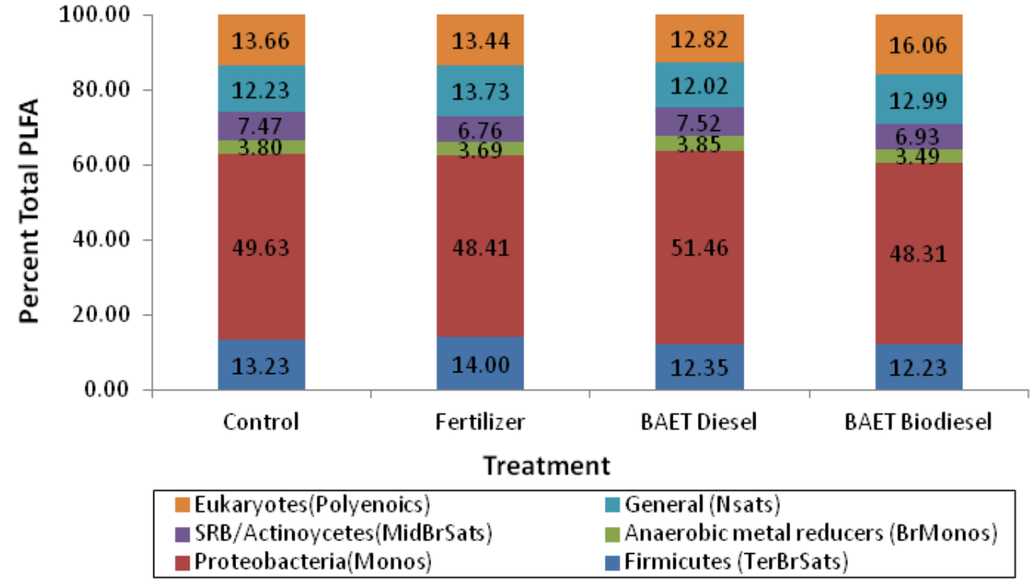


Figure 27. Results showing the percentage of total PLFA for the soil samples collected right after seeding at Field 1.

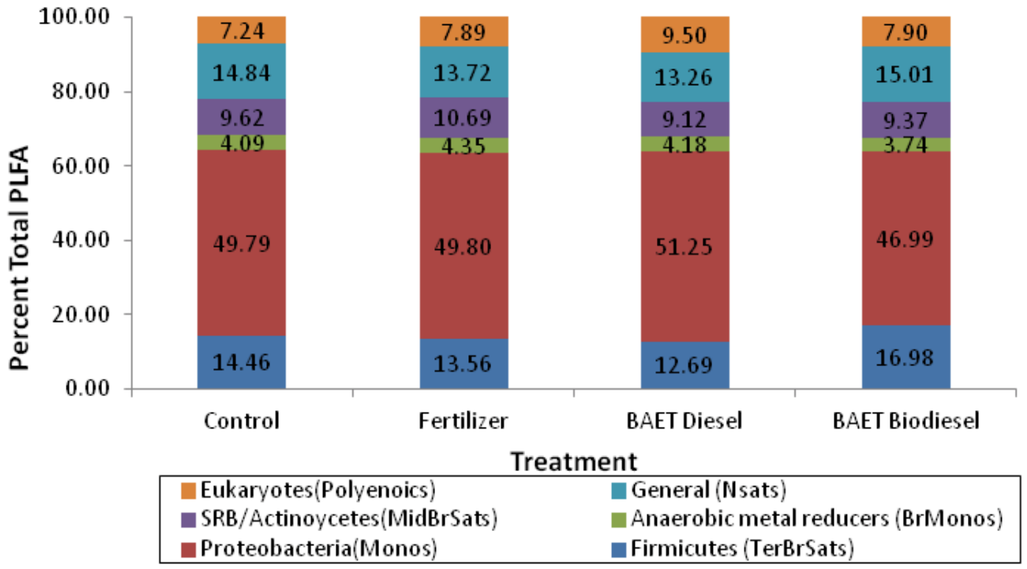


Figure 28. Results showing the percentage of total PLFA for the soil samples collected after harvesting at Field 1.

2.5.1 CASE STUDY 2: Field 2 Results

Comparing the soil samples of Field 2 before seeding and immediately after seeding, there is an increase in the amount of cells in the soil treated with the Bio-AgtiveTM Emission Technology (Figure 29 and 30).

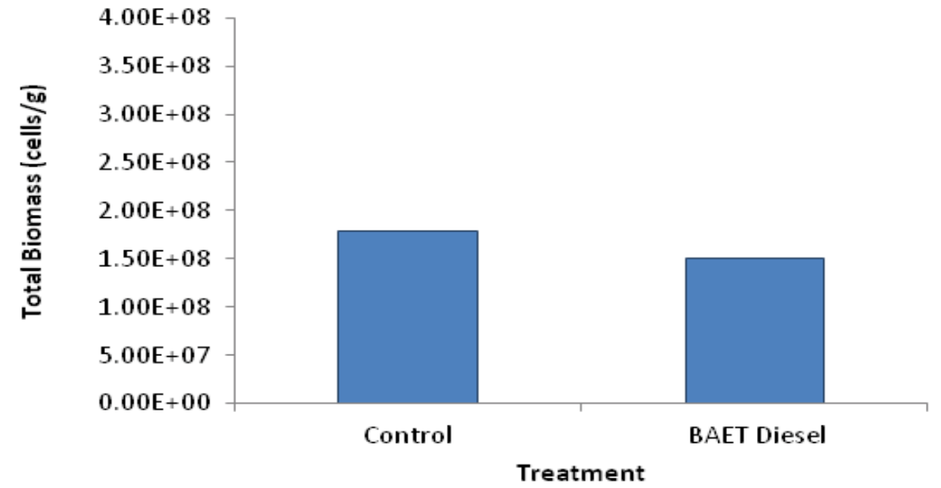


Figure 29. Results showing the total biomass content of the soil samples collected before seeding at

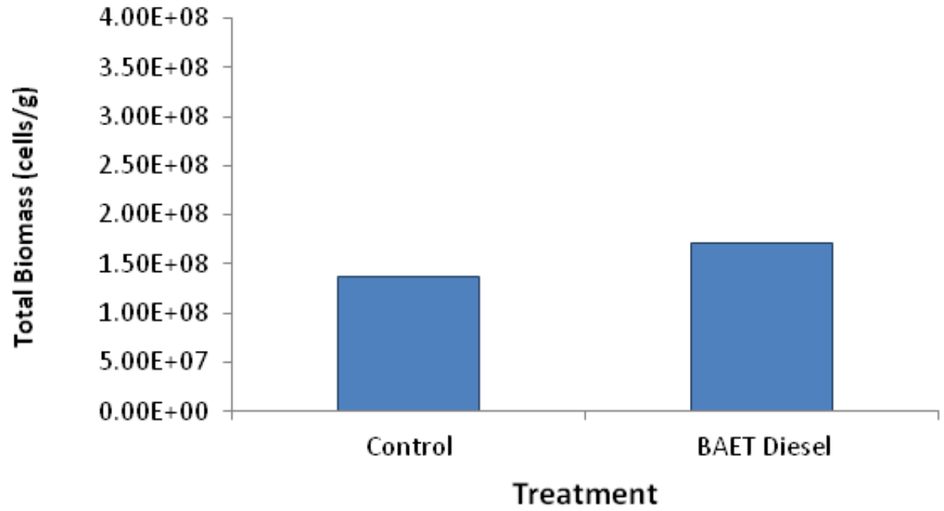


Figure 30. Results showing the total biomass content of the soil samples collected right after seeding at Field 2.

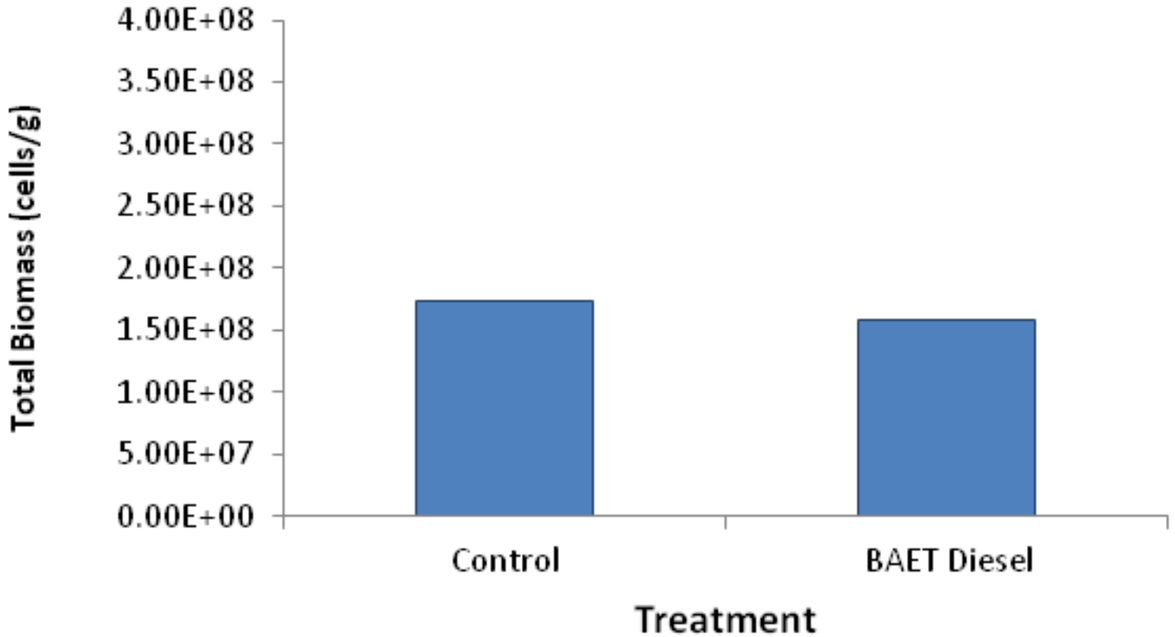


Figure 31. Results showing the total biomass content of the soil samples collected after harvesting at Field 2.

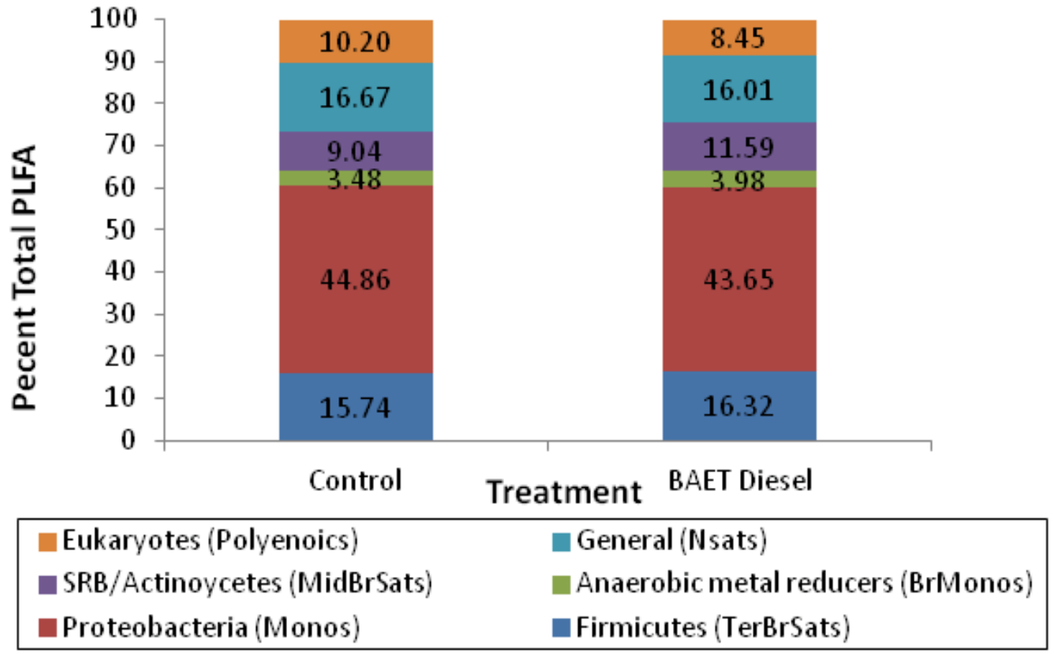


Figure 32. Results showing the percentage of total PLFA for the soil samples collected before seeding at Field 2.

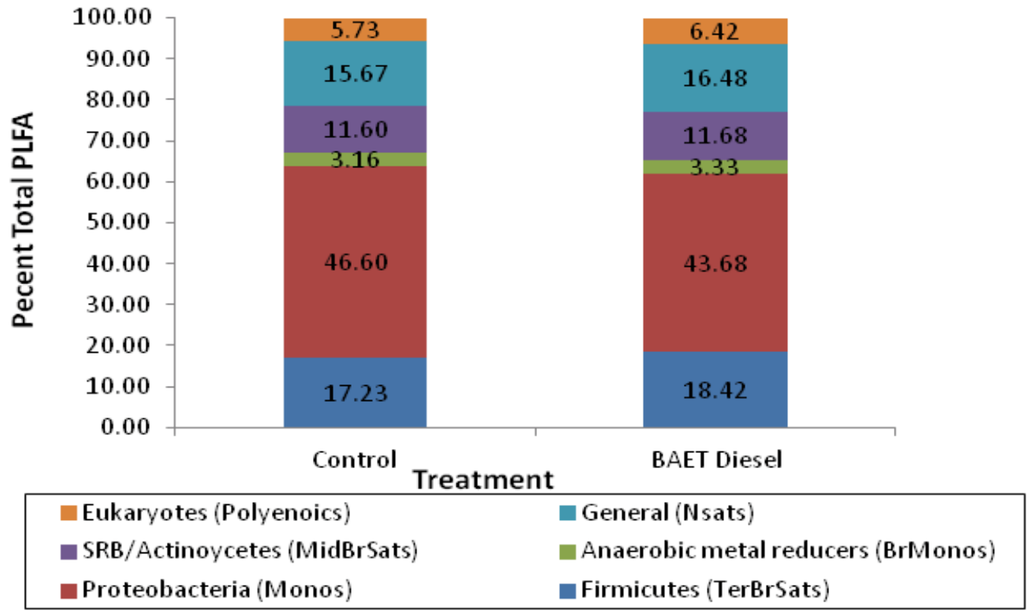


Figure 33. Results showing the percentage of total PLFA for the soil samples collected right after seeding at Field 2.

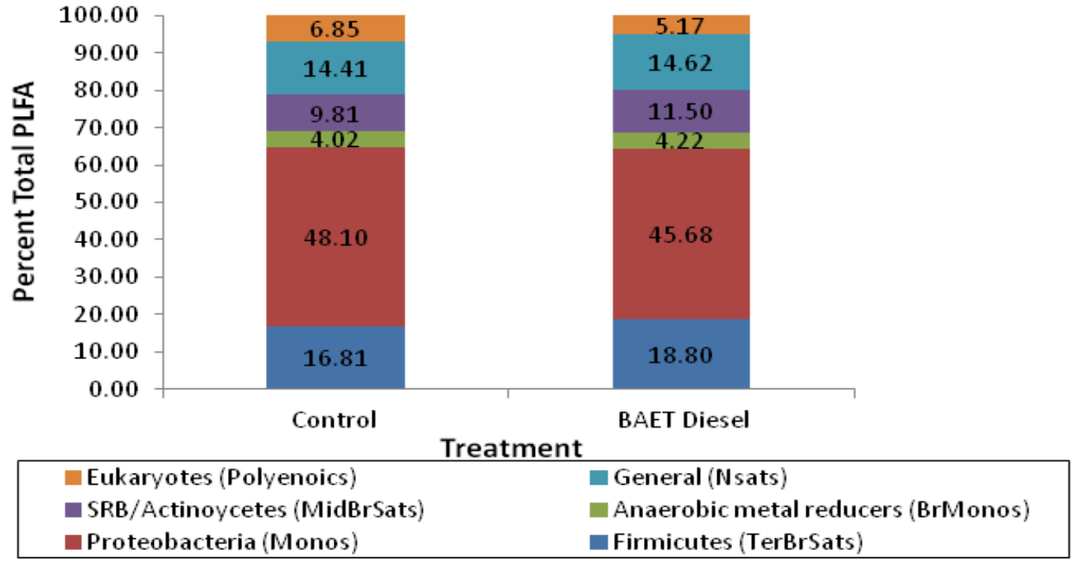


Figure 34. Results showing the percentage of total PLFA for the soil samples collected after harvesting at Field 2.

2.6 Soil Respiration Analysis

The part of this study was to measure the soil biological CO2 respiration. The Haney-Brinton Solvita test was used to estimate the amount of the CO2 pulse which is directly related to the microbial biomass and nutrient delivery potential.

2.6.1 Haney-Brinton Solvita test for soil biomass and C-N Mineralization

Soil samples were collected at the site before and immediately after seeding and after harvesting from both Fields 1 and 2. The GPS location of each sample plot was recorded to ensure consistency in the position of the sample plots. Each plot strip was treated either with fertilizer, BAET diesel or BAET biodiesel. The samples were stored in the laboratory freezer. The samples were first dried in a convection oven at 40-500C for at least 24 hours or until completely dry. The soil was then grounded and sieved through a 2mm screen. Forty grams of the pre-dried and sieved soil sample was weighed in the plastic beaker which was enclosed with the fiber filter at the bottom. The plastic beaker was then placed in the glass jar. Twenty five milliliters of the deionized water was added to the glass jar, and then the Solvita soil probe was placed into the glass jar alongside the plastic beaker (Figure 35). The lid was tightly screwed and the start time was recorded. Note that at the start of the test, the gel should be color #0 (bright blue). The jar was stored at the room temperature for 23-25 hours. At 24 hours, the color of the probe was read by inserting it into the Digital Color Reader. The color and CO2-C was reported for each soil sample. The appropriate interpretation of the results is shown in Table 10.

Table 10. Soil condition and soil care based on the Haney-Brinton respiration test

Test Result ppm CO_2-C	N-Mineralization Potential	Biomass
>100	High N-Potential soil. May provide sufficient N for entire crop.	Soil is well supplied with organic matter. Biomass >2,500 ppm.
61-100	Moderately high. This soil has limited need for supplemental N.	Ideal state of biological activity and adequate organic matter level.
31-60	Moderate level. Supplemental N is most likely indicated.	Requires new applications of the stable organic matter. Biomass 1,000 ppm
6-30	Moderate-Low-will not provide sufficient N for most crops.	Low in organic structure and microbial activity.
0-5	Little biological activity; requires significant fertilization.	Biomass<100 ppm. Put into intensive green manure or other long-term cover crops.

Figure 35: Soil respiration test

2.6.2 FIELD 1: Results of the Soil Respiration Tests

The results of CO2 concentration for the soil samples collected before and right after seeding at Field 1 are shown in Figure 37. BAET Biodiesel has a higher respiration after seeding. Similarly, the carbon dioxide respiration results of the soils after harvesting were indicating BAET Biodiesel as the highest soil respiration .This result is analogous to the PLFA tests (based on total biomass) indicating that the BAET Biodiesel feeds the soil more mineral nutrition also indicated by the water trap condensate test.

Figure 36. Soil respiration tests using Bio-Agtive SEED (Sensing Emission Escaping Detector).

It was also observed that before the test field was tilled with a disc, the respiration was high at 19.9% O_2 which is equal to 1% CO_2. Detailed discussion of this experiment is on Appendix 2.

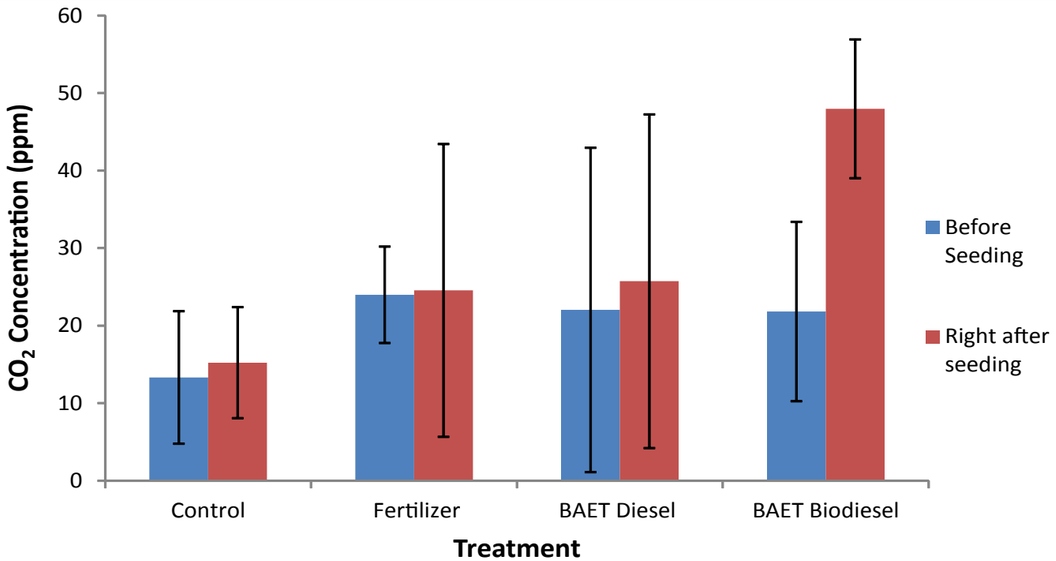


Figure 37. Carbon dioxide concentration results for the soil samples collected before and immediately after seeding at Field 1.

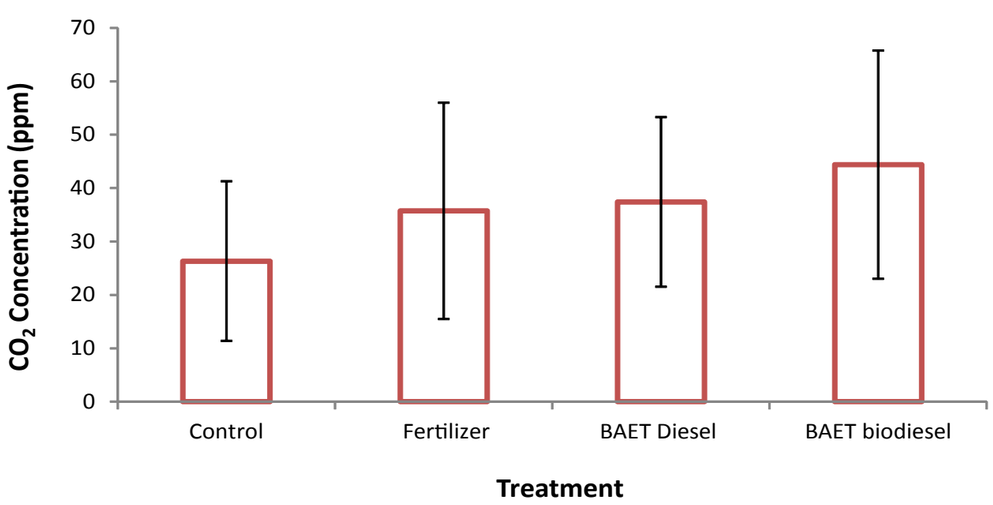


Figure 38. Carbon dioxide concentration for the soil samples collected after harvesting at Field 1.

2.6.3 FIELD 2: Results of the Soil Respiration Tests

The results of CO_2 concentration for the soil samples collected before and immediately after seeding at Field 2 were reported in the Figure 39.

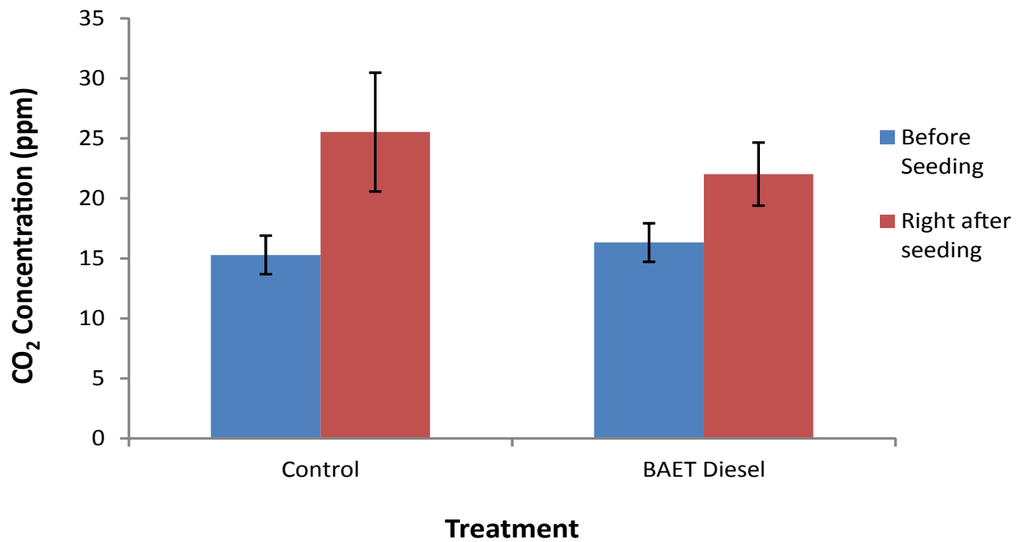


Figure 39. Carbon dioxide concentration results for soil samples collected before and immediately after seeding at Field 2.

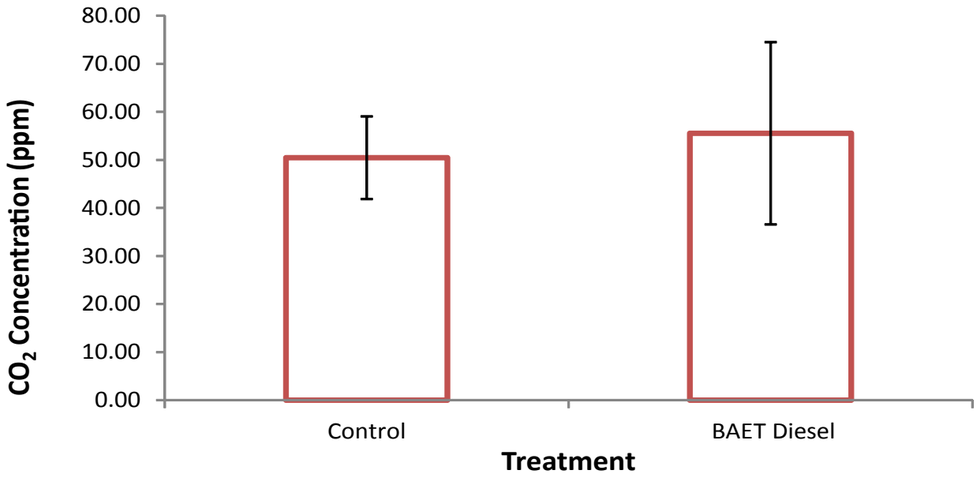


Figure 40. Carbon dioxide concentration results for the soil samples collected after harvesting at Field 2.

1. Nutrient and Yield Analysis of Harvested Crops

3.1 Objective

The third objective was to determine which biofuels or blends of biofuels and diesel fuels work best for supplying crop nutrients, or stimulating the soil to provide crop nutrients.

3.2 Nutrient Analysis of Spring Wheat Grains

3.2.1 CASE STUDY 1: Field 1 Results
Nutrient content of the wheat grain between different treatments and control does show significant difference. The Bio-Agtive™ diesel has the highest nutrient values, yield and protein compared to control and fertilizer. Based on the results of the tests, diesel tends to increase the activity of free living nitrogen fixing bacteria in the soil (proteobacteria) which correlates to the slight increase in nitrates in the soil, nitrogen in plant tissues, and protein content of the grains. Biodiesel tends to increase biological activity, notably fungal (eukaryotes), in the soil as illustrated by the PLFA, soil respiration (Figure 37) and emission tests.

Table 11. Nutrient content of wheat grain harvested from Field 1.

NUTRIENT	TREATMENT			
	Control	Fertilizer	BAET Diesel	BAET Biodiesel
Calcium (%)	0.032	0.032	0.039	0.032
Copper (µg/g or ppm)	<5.0	<5.0	5.5	<5.0
Iron (µg/g or ppm)	75	67	76	75
Potassium (%)	0.43	0.44	0.45	0.44
Magnesium (%)	0.15	0.15	0.16	0.15
Manganese (µg/g or ppm)	51	50	52	54
Sodium (%)	0.0044	0.0041	0.0045	0.0038
Phosphorus (%)	0.38	0.36	0.36	0.39
Sulfur (%)	0.11	0.098	0.14	0.12
Selenium (µg/g or ppm)	1.1	1.5	1.8	1.6
Zinc (µg/g or ppm)	29	30	41	30

Results of the test plots showed increase in wheat yields for treatments where the BAET system was used. Direct parallel conclusions can be drawn through the complete experiment and tests, from the mineral content of the Bio-Agtive condensate and water trap setup in the lab, soil fertility tests, plant tissue tests, soil respiration, and PLFA that supports the increase in wheat yields and quality of grain. There was no decrease in nutrient content and bushel weight, more protein, less shrunk kernels, and less defects (Table 12). Also, there was a decrease in phosphorus and iron in the plant tissues collected in fertilizer treated plots, it could be because that the fertilizer makes phosphorus and iron in the soil unavailable to the plants.

The quality of the soil was also affected by the BAET system. Field trials and treatments were conducted on a field that has had emissions only (no fertilizer) for 6 years and continues cropping wheat. The decline of soil fertility with the application of ammonium phosphate was observed from the study (Figure 41).

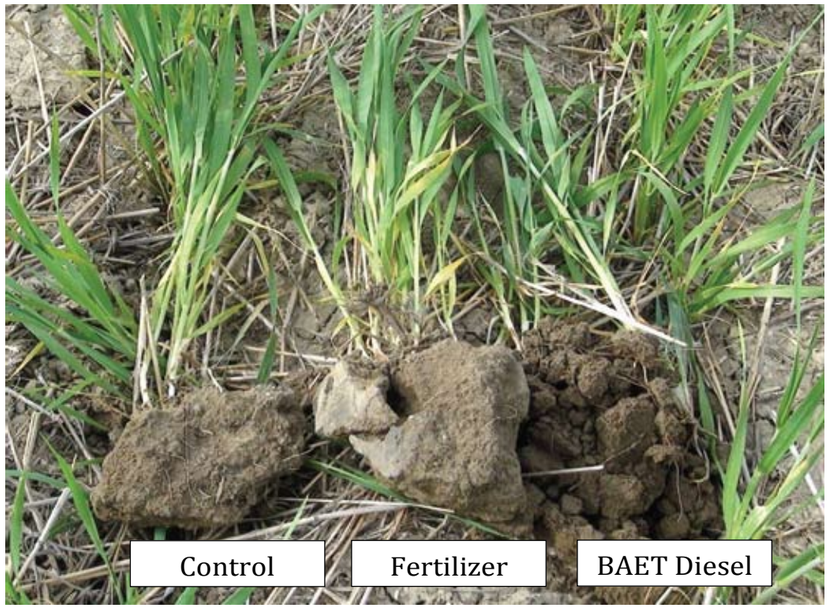


Figure 41. Soil characteristics of different treatments.

Table 12. Yield of wheat from Field 1 at different treatments.

Treatment/ Plot	Yield (bushel/acre)	Test weight per bushel (lb/bu)	Weight of other classes %	Foreign material %	Defects %	Moisture %	Damaged Kernels total %	Shrunken and broken kernels %	Protein %
Control	16.04	62.2	6	0	1.8	10	0	1.8	12
Fertilizer	15.08	61.6	9	0	1.8	9.9	0	1.8	11.7
BAET Diesel	16.9	61.8	1.5	0	0.8	10	0	0.8	13.3
BAET Biodiesel	16.62	62.2	3	0	1.3	10.4	0	1.3	11.6

3.2.1 Case Study 2: FIELD 2
The results of the nutrient content and yield results are summarized in Tables 12 and 13. It should be noted that Field 2 was a transitional organic field in which conventional no-till practices were not applied and seeding was delayed until end of June due to unforeseen circumstances as discussed in section 2.3.5.

Table 13. Nutrient content of wheat grain harvested from Field 2.

NUTRIENT	TREATMENT	
	Control	BAET Diesel
Calcium (%)	0.048	0.044
Copper (µg/g or ppm)	6.4	5.8
Iron (µg/g or ppm)	46	54.4
Potassium (%)	0.5	0.48
Magnesium (%)	0.2	0.17
Manganese (µg/g or ppm)	37	34
Sodium (%)	0.0027	0.003
Phosphorus (%)	0.46	0.43
Selenium (µg/g or ppm)	0.98	0.80
Zinc (µg/g or ppm)	41.7	38.7

Table 14. Yield of wheat from Field 2 at different treatments.

Treatment/Plot	Yield[1] (bushel/acre)	Moisture, %	Test weight lbs/bushel	TKW[2] grams	Protein[3], %
Control	6.7	13.7	55.7	29.2	14.4
BAET Diesel	8.8	14.7	54.8	29.2	14.2

Legend: 1- Volumetric yields are based on plot weights adjusted to uniform 12 percent grain moisture and 60 lbs/bu as the standard test weight for wheat; 2 - TKW is the weight of one thousand kernels; 3 – Protein values are adjusted to 12 percent grain moisture.

Figure 42. Root growth of the plant of different treatments. (Note: Roots were longer in BAET-SVO)

The results of the laboratory emissions analysis and field trials including soil, PLFA, soil respiration, plant tissue tests gave conclusive trends of the effects of the Bio-Agtive Emission Technology to soil fertility and plant growth. The results also suggest that type of fuel used could dictate response required. BAET-Diesel tends to have biocidal effect and favor the growth of free living nitrogen bacteria while BAET-Biodiesel tends to increase biological activity in the soil. Based on the results of this study, Figure 43 illustrates possible beneficial effects of BAET-Diesel, BAET-Biodiesel and BAET-SVO to soil fertility and plant growth.

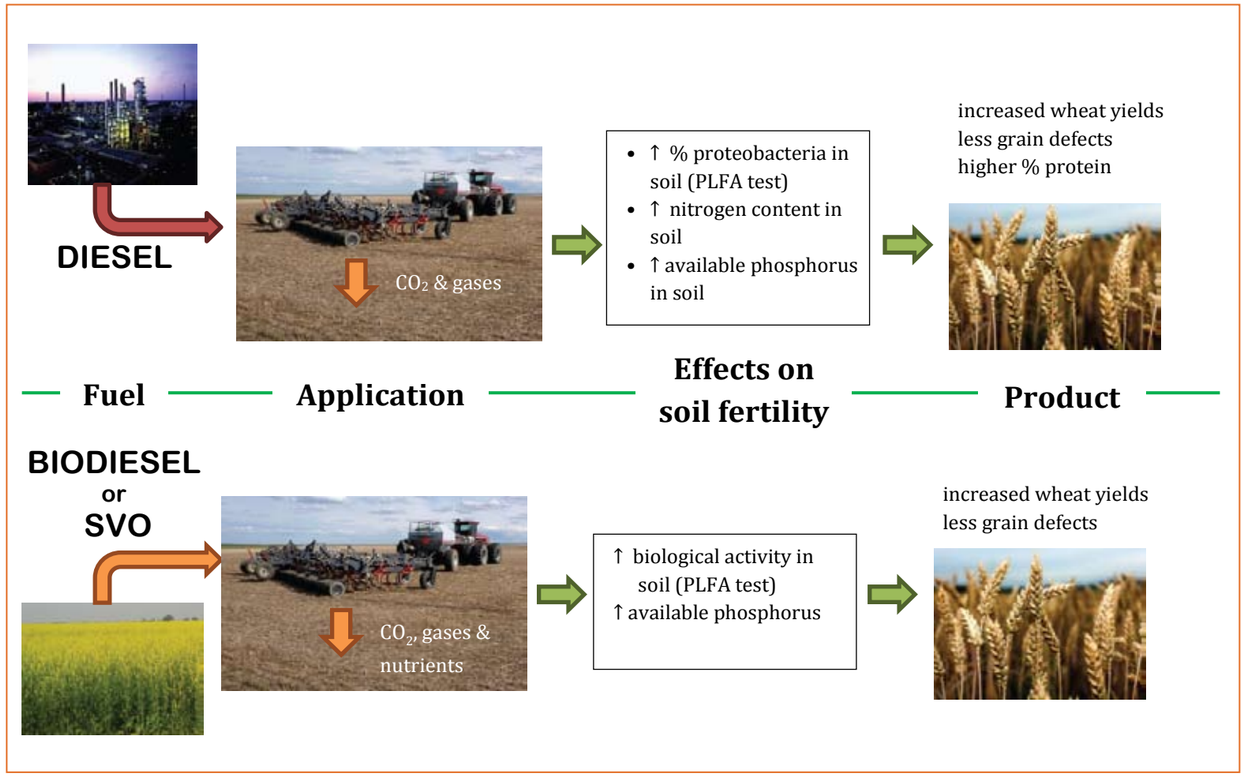


Figure 43. Schematic illustration of the effects of BAET using different fuels.

End – of – Report

Appendix #1

Disclaimer: *The attached study was conducted separately from the study completed by the Bio-Energy Center, Montana State University-Northern. All methods, results and conclusions were reported independently by Dr. M. Jill Clapperton.*

Report to Montana State University Northern Bio-Energy Center and N/C Quest

Montana Board of Commercialization and Technology Grant

Final Report: Experiments and Results performed by Earthspirit Consulting

Prepared by M. Jill Clapperton PhD
Principal Research Scientist/ Owner

Field Experiment at Fossen's Farm- Inverness MT

On April 20 2009, 6 plots were seeded to hard white spring wheat var Agawam at a rate of 60 lbs/acre with 12 in spacing. The treatments on Field 17 were repeated from the previous year when exhaust had been applied in fall when the winter wheat was seeded. Treatment 1 had the full length of the field supplied with the full recommended rate of fertilizer (60-12-0-0) making the plot width 304 ft and running the length of the field. The Check (no fertilizer and no exhaust) was one pass without fertilizer and no exhaust (57 ft wide). The Exhaust treatment (no fertilizer applied) was 2 passes wide using an air seeder modified with the Bio-Agtive™ technology (112 ft wide). Tractor was running at 5 mi/hr.

Field 20 had been in fallow 2008, and was seeded to the same treatments as Field 17. However, the fertilizer plot was 168 ft wide, exhaust was 112ft wide, and the check was 39ft wide. The size of the check plot was kept to a minimum to reduce the yield penalty within the field trial.

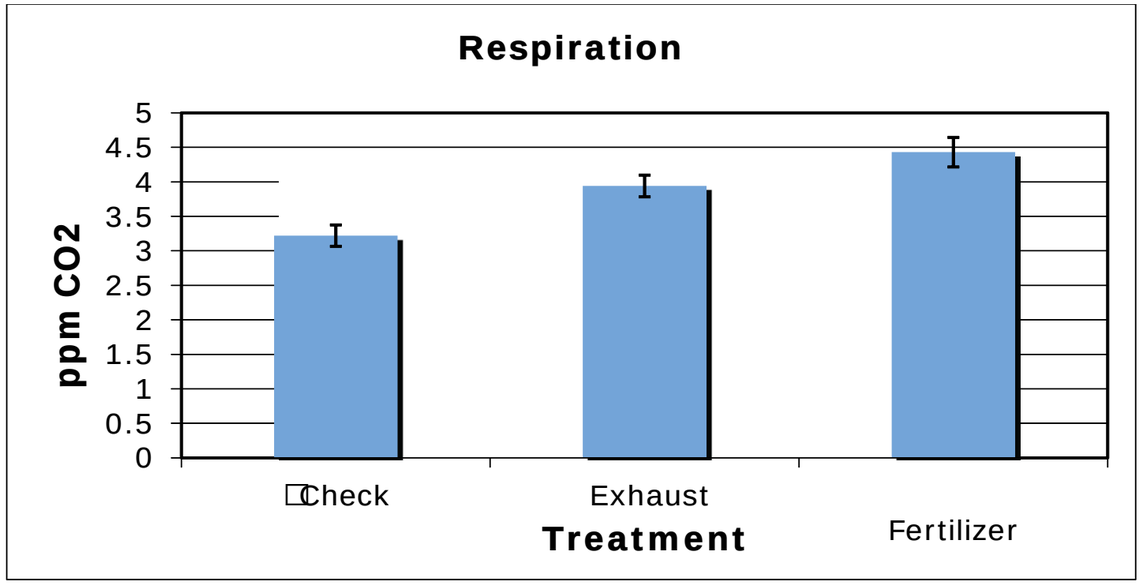
Figure 1 Showing the colour difference between the Field 17 exhaust (left side) and the check treatments in June 2009. The Check plot was showing distinct signs of n difficiency.

Statistical Analysis
All data was subjected to Bartletts Test to determine heterosedacity of variance and transformed with either square root or natural log (if indicated) before performing analysis of variance (ANOVA) (Statview 1998, SAS Institute Inc), $p<0.05$ unless otherwise indicated.

On May 22, 2009 we placed 10 PVC collars at preselected random points along a 50 m transect in the middle of each plot. The collars were inserted 1 cm into the soil to form a seal. Soil respiration would be measured in June when the plants were larger and the soil warmer.

Figure 2. A photo showing the Li Cor portable CO2 analyser used to measure soil respiration.

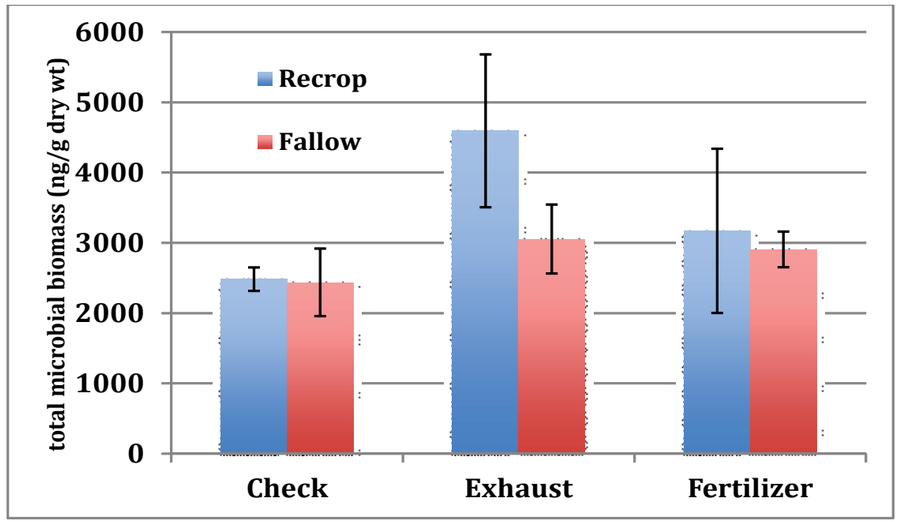
Figure 3. The mean amount of CO_2 respired from the soil in each of the three treatments (n= 5, ± s.e., $p<0.05$) in Field 20.



The soil temperature at the time of measuring the soil respiration was between 16.1°C and 18.2°C. Each reading took approximately 3 minutes and 3 replicate reading were done at each point. There were 5 points randomly selected in the field.

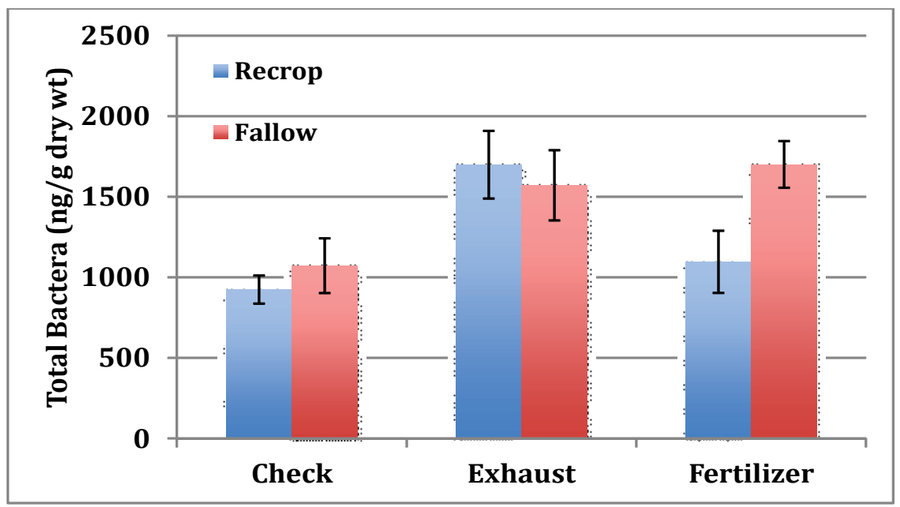
The results show that the check had the lowest soil respiration ($p<0.05$) while the exhaust and fertilizer treatments were similar. Once it was determined that soil respiration did differ between the treatments, soil sample were taken to determine the composition of the microbial community. Both Field 17 and 20 were sampled for soil microbiology.

Microbial Community Analysis
Soil samples were taken at 10 randomly selected points on a 50 m transect to the west of the center in each plot to avoid over lap with other data collecting and trampling. Soils were cored to 10 cm, and 3 replicate cores were taken at each point to form 1 conglomerate sample. The samples were placed in coolers to remain at field temperature, and then frozen at -40°C.
Analysis for phospholipid fatty acids (PLFA) were performed at the University of Montana according to the method described in (Clapperton et al. 2001). Ramsey et al (2006) compared PLFA analysis, community level physiological profiling (CLPP), and PCR (polymerase chain reaction)-based approaches to determining treatment affects on microbial community structure, and suggested that PLFA offered the most powerful approach.
The data for the PLFA analysis was transformed using natural log function to overcome heterosedacity of variance.
Figure 4. Compares the mean (n=6) total microbial biomass (ng/g dry weight of soil) between the 3 treatments (± se), fallow is Field 20, and the recrop is Field 17.



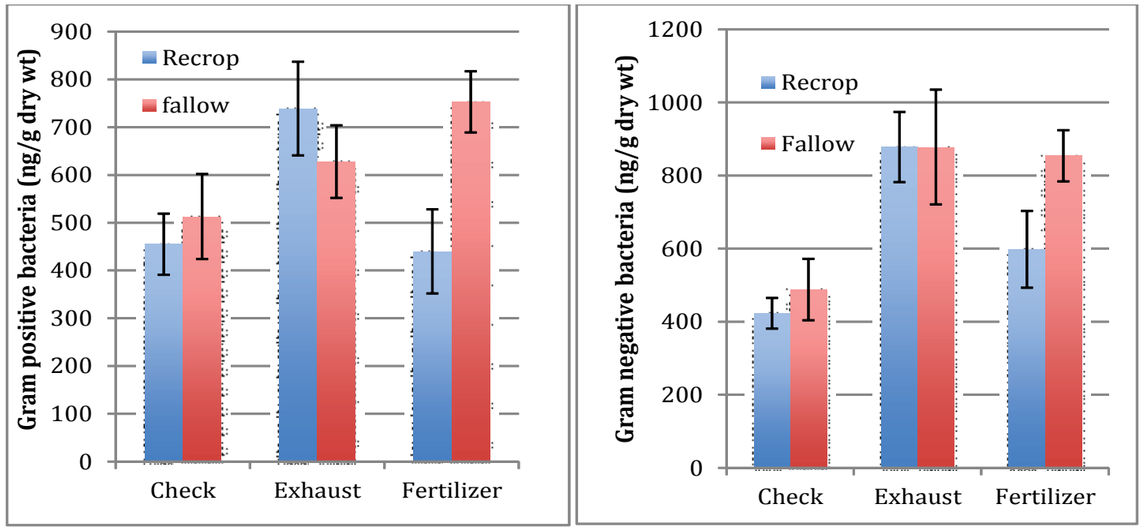
The Exhaust treatment had the highest overall microbial biomass in Field 17 (recrop) compared with the Check, and the fertilizer treatment appeared to be intermediate although the variation was too high to separate the Exhaust from the Fertilizer treatment ($p<0.05$). There were significant differences in the wheat on fallow soils (Field 20).

Figure 5. Compares the total amount of bacterial biomass between the treatments at the two field sites (± se, $p<0.01$)



The results show that the recrop exhaust treatment had the highest biomass of bacteria compared with both the fertilizer and check. Whereas, for the wheat on fallow Field 20, the exhaust and fertilizer treatments have significantly more bacteria compared with the check.

Figure 6. The amount of Gram positive and Gram negative biomass (ng/g dry weight soil) in the different treatments and both field sites.



Again, there are differences in the response of the microbial community to the treatments and field site. In the Recrop Field 17, the exhaust treatment had the most Gram positive and Gram negative bacteria compared with the Check and fertilizer treatment. In the Wheat on fallow Field 20 the Exhaust and Fertilizer treatments had significantly more Gram negative bacteria compared with the Check.

Figure 7. Comparing the amount of PLFA for actinomycetes between treatments and field sites.

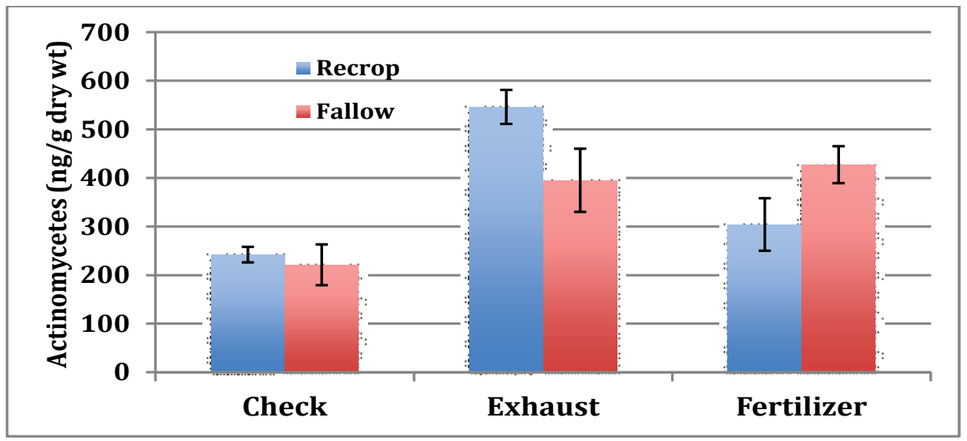
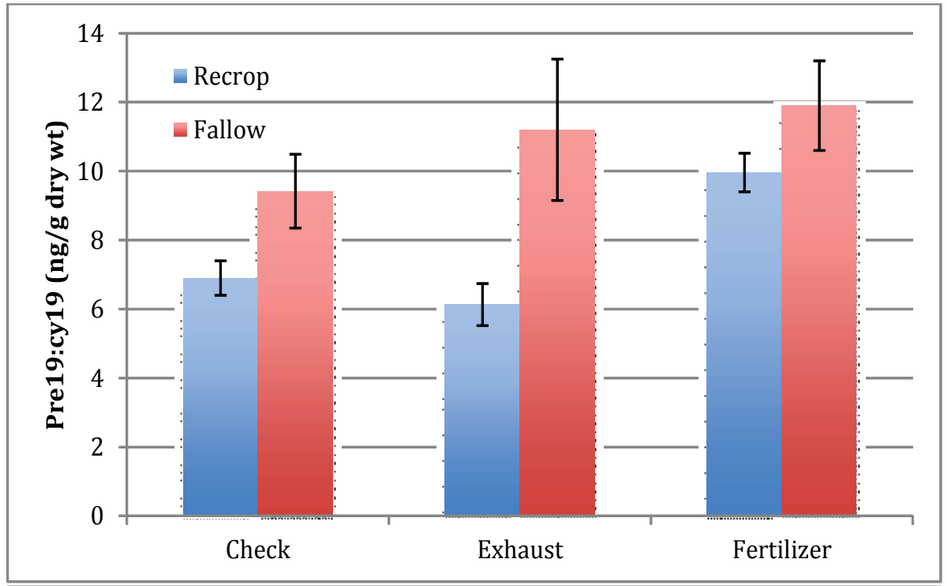
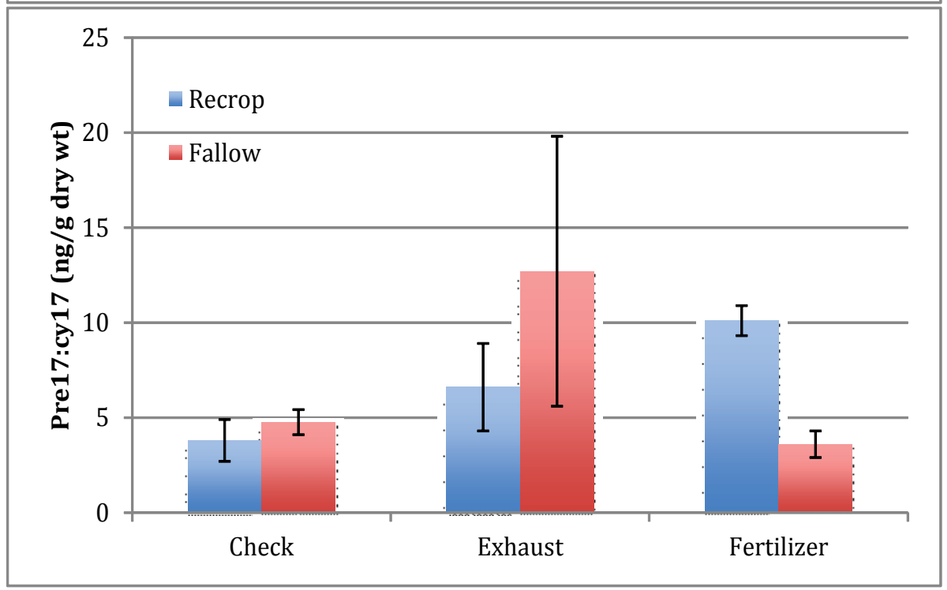


Figure 8. A comparison of the ratio of 16:1w7c (precursor) to cy17 (end product), and 18:1w7c (precursor) to cy19 (end product) which indicate the activity of the microbial community (particularly Gram negative community) (Petersen and Klug, 1994).





These results show that in the recrop Field 17 the microbial community is more active compared with the Check and Exhaust, and in the wheat on fallow Field 17 there were no significant differences among the treatments.

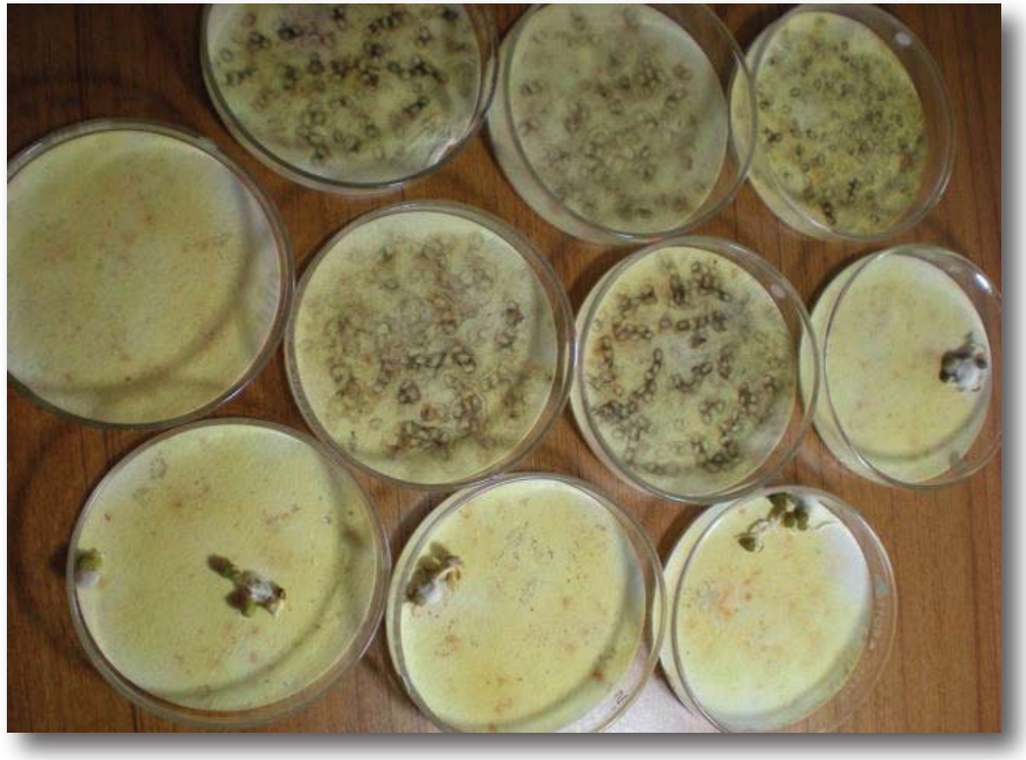
Overall the results for the PLFA analysis show that the affects of the exhaust treatment are accumulating, and the microbial community structure is altered in the second year of the exhaust treatment.

The affects of exhaust on seed germination and seed microbial community

Seed Germination

The Bio-Agtive™ emission technology runs cooled exhaust emissions into the air cart in an air seeder, this means that after a number of acres of seeding the seed is coated with the emissions at a higher temperature than without an emission system.

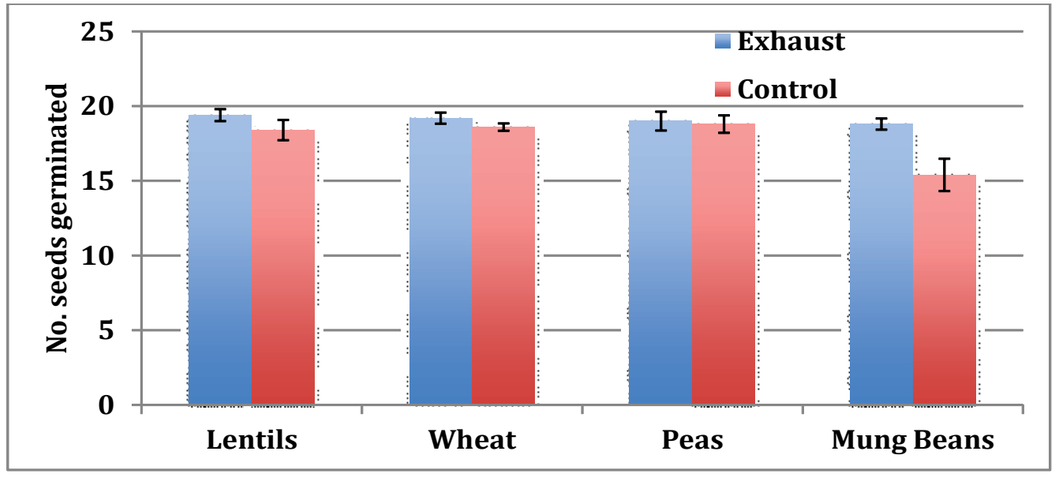
Figure 9. A photo of the emissions halo off germinated seed treated with exhaust emissions and untreated seed. There are some untreated germinated seed on the plates that are colonized by fungus.



To determine if there was an affect of the exhaust emissions on seed germination: we place 20 seeds of similar size in sterile glass petri plates on a sterile saturated filter paper. The seeds were incubated at room temperature, and water was replaced after 3 days or as necessary. There were 5 replicate plates of 20 seeds each for a total of 100 seeds. After 5 days the number of seeds not germinated on each plate were counted and recorded. This experiment was repeated twice for each group of seeds.

We asked farmers using the Bio-Agtive™ emission system to submit seed samples that had been exposed to emissions, and the same seed batch without emissions. We tested the germination on lentils, peas, wheat, and mung beans.

Figure 10. A comparison of the mean (± se) number of seeds that germinated on each of 5 plates of seed that had been exposed to exhaust in the air seed cart, or not.



The results showed that there was a statistically significant interaction between the seed species, and the exhaust treatment ($p < 0.05$), and that the germination of seed exposed to the exhaust was greater than seed without exhaust (control) ($p<0.004$). This suggests that seed from different crops might be more or less affected by the exhaust. For example, the mung bean seed had the greatest benefit from being treated by the exhaust in terms of improved germination.

It was consistently observed that there was fungi growing on the control germinated seed, and that this was rarely observed when the seed was treated with exhaust. This suggested that the exhaust and or the temperature of the exhaust emissions was affecting the microbiology of the seed coat, and perhaps seed-borne fungi.

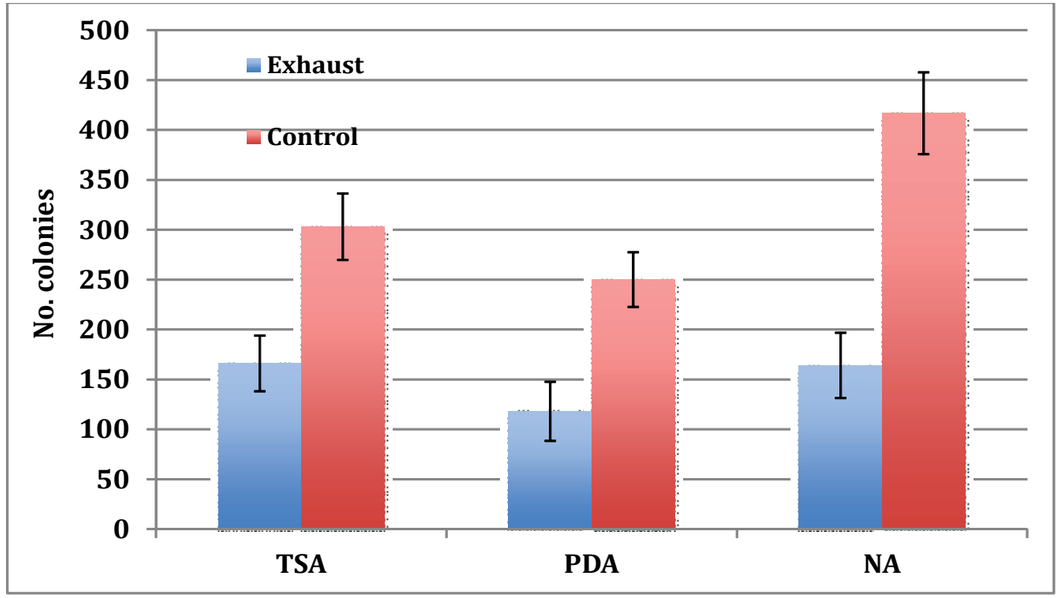
Figure 11. Shows the potato dextrose agar plates growing fungi washed from seeds that were treated with exhaust emission or not.

Microbiology of the seed

The microbial community on the seed was determined in 2 different ways: seed wash was plated onto different growth media in a dilution series, and seed wash was freeze dried and analysed for PLFA. Since all seeds of different plant species have different shapes and sizes, 10 g of seed was weighed into a sterile flask (instead of counting 100 seeds) with 100ml of sterile water, and swirled on a rotary shaker for 15 minutes. The wash was decanted into another sterile flask and a 10-fold dilution series was prepared, 1 ml of each dilution series was plated onto each of 10 plates of different growth media. The inoculated plates were incubated in sealed containers at room temperature for 3-5 days. Fungal colonies on potato dextrose agar (PDA) often took as many as 9 days to appear.

Few colonies appeared in any dilution after the first 10 fold dilution. It may have been better idea to use a buffer solution to wash the bacteria and fungi from the seed. I may have liberated more cells. Seed wash was plated onto Typticase Soy Agar (TSA), Nutrient Agar (NA), Potato Dextrose Agar (PDA), and Pseudomonas Minimal Media (PMM).

Figure 12. Shows the typical results for seed washes. This figure shows the results for Laird lentils exposed to canola-based diesel fuel or not (control) during field-scale seeding.



Overall, the number of growing bacteria (TSA and NA), and fungi (PDA) are less ($p<0.001$) on seed treated with exhaust emissions compared with the same seed that was not. The bacterial growth on PMM was sparse and therefore omitted. The plating data supported the observation that there were more fungi on the control seed.

The second method was to wash the seed and then do fatty acid methyl ester (FAME) analysis. In this case seed used in the 2011 field trials at the Henke and Quinn farms was used. This seed had been exposed to the exhaust emissions for only a short time by comparison to the other seed from the field-scale seeding operations. Indeed there was only a slight emission halo from the germinated seed. There was no significant affect of the emissions on the germination of the wheat seed (data not shown). However, the FAME analysis did show some

There were 5 different seed treatments: control, ethanol washed or surface sterilized seed, Diesel, SVO (vegetable oil), and biodiesel. 10g of seed was placed into sterile flask with 100 ml of sterile water and swirled on a rotary shaker for 15 minutes; the wash was decanted into sterile flasks, frozen and freeze dried. The freeze-dried wash was extracted, methylated, and analysed by gas chromatography (GC) (in collaboration with Ward Laboratories- Jill Clapperton performed the analysis).

FAME analysis differs from PLFA analysis in that all the lipids in the wash are analyzed and not just the PLFA. The PLFA represent living microbial biomass, whereas the FAME is all lipids (including exudates from microbes, and dead cells), however the yield of lipids is higher for FAME. Therefore, there would be enough lipid to get an accurate analysis on the GC. There were 3 replicate seed washes for each seed treatment.

Table 2. A comparison of the mean Total, Saturated, Monounsaturated, Polyunsaturated, Fungi, and bacterial FAME from the seed washes. Means followed by the same letter are not significantly different ($p<0.01$, $n=3$ ±se).

Treatment	Total FAME	Saturated	Mono	Poly	Fungi	Total bacteria
SVO	537±41 B	246±58 A	240±58 A	56±21 B	110±37 B	470±18 A
Diesel	242±49 C	134±25 B	69±25 B	39±4 B	58±14 C	184±39 B
Biodiesel	656±30 A	292±31 A	253±47 A	111±6 A	201±7 A	455±26 A
Sterile	189±21 C	112±13 B	37±21 B	17±3 B	29±4 C	160±18 B
Control	305±23 C	119±17 B	16±9 B	19±16 A	60±33 C	246±31 B

These results show that the microbial community structure is different depending in what fuel was used in the tractor and run into the seed cart. It seems likely that there would have been enough microbial biomass on the seed to perform PLFA analysis. It is likely that the FAME also showed some of the oils from the fuels.

The PLFA analysis likely would have shown that the exhaust treatments either killed the fungi, or stimulated the bacterial growth on the seed. However, the analyses that were performed showed clearly that the exhaust does affect the microflora on the seed. This could be beneficial if it acts as a seed treatment against seed and soil-borne fungi.

Conclusion

The results show that putting exhaust emissions onto the seed and into the soil does affect the soil microbiology. The data does not show if these affects are significant enough to effect changes in soil chemistry or mineralization. Given the soil PLFA results it seems likely that the affect of the emissions can build in the soil. It is possible that all the noticed affects are associated with the seed being coated by the emissions, and it would be possible to build populations of soil microbes by changing the seed and rhizosphere populations on an annual basis. It is also likely that the amount of time the seed is exposed to the exhaust and increased temperature would affect the efficacy of the anti-fungal affect of seed germination. This would have to be investigated, but does not limit this technology from moving forward in my opinion.
The use of exhaust emissions as a seed treatment against seed-borne fungi should be explored, and would extend the use of the BioAgtive™ technology.

There were some preliminary experiments to determine the amount of exhaust that was going into the soil. These will be necessary to do something similar to a mass balance of the amount of emissions coming into the seed cart, what is left on the seed, on the hoses and tank, and then how much of the emission actually contact the soil and how many stay.

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Appendix #2

Disclaimer: *The attached study was conducted separately from the study completed by the Bio-Energy Center, Montana State University-Northern. All methods, results and conclusions were reported independently by N/C Quest Inc*

Bio-Agtive™ SEED
Sensing Emissions Escaping Detector

Bio-Agtive™ Emissions Technology

Bio-Agtive™ SEED

Introduction

The Bio-Agtive™ SEED Monitor (Sensing Emissions Escaping Detector) has been developed by N/C Quest Inc, Canada to assist the operator of the Bio-Agtive System during seeding. This monitor has been in development over the past four years to help farmers and researchers understand the Bio-Agtive™ Emissions Technology a carbon farming practice, of managing CO_2 that is invisible and can only be detected with instruments that detect greenhouse gases. Maintaining the proper temperature range to prevent the risk of damaging the tractor engine, air seeder equipment and the seed is essential to the successful use of the system. The Bio-Agtive™ SEED equipment can measure, monitor and verify that the gases are being sequestered successfully back into the soil air spaces, to ensure stimulation of the seed bed. The Bio-Agtive™ SEED monitor can also be taken off the farm equipment and be used as a respiration meter to determine if the soil is giving off CO_2, a direct relation to microbial activity or organic matter decaying . The system is now wireless from the Bio-Agtive™ Condenser back to the tractor cab through Wi-Fi and displayed on iPad or iTouch data logging all channels for easy review for verification and troubleshooting. The logs can be sent back to N/C Quest by the Bio-Agtive™ website or from the users email.

Bio-Agtive SEED trials on Test Fields 1 and 2

The latest development of the Bio-Agtive™ SEED has been tested at Bio-Agtive™ Montana LLC in collaboration with Montana State University-Northern Bio-Energy Center. During seeding test at Field 1 (at Craig Henke's farm) using diesel fuel, biodiesel fuel, and fertilizer only and no emissions (control), the Bio-Agtive™ SEED monitors four wideband oxygen sensors at four different locations;

- Engine's Turbo
- After the Bio-Agtive™ System, Injection Fan
- In the Seed Furrow (Under a Rubber Hood)
- After Packer Wheel

Four temperatures were also logged; before the Bio-Agtive™ Condenser, after the Bio-Agtive™ Condenser, Seed Boot, and Ambient Air Temperature. Two fan speeds were also logged; at the air seeder fan and condenser fan during seeding.

During seeding at Field 2, Ken Yirsa's tractor and air seeder were used with the Bio-Agtive™ SEED installed for the research plot. All sensor channels were logged at the same positions on the system.

Capabilities of Bio-Agtive™ SEED

These experiments were set up to data log and verify that the exhaust is staying in the soil and not escaping during spring seeding. If the equipment is adjusted properly the operator can eliminate emissions. The data log of this trail has demonstrated that if the equipment is not adjusted correctly, the system will not be sequestrating all exhaust to the soil. When the air seeder fan is running at a higher RPM it dilutes the emissions inside the seed tubes resulting in a higher air-to-fuel ratio. An excessive injection flow (running the fan faster) causes emissions to bounce and escape out of the furrow. All other trial passes with lower air-fuel ratio and a slower injection fan speed indicated no emissions escaped from the furrow. This indicates that if the exhaust inside the seed tubes is a richer air-to-fuel ratio results to better absorption of the emissions in the soil.

Bio-Agtive™ SEED has the ability to measure CO_2 respiration from the soil before the seeder disturbs the soil and after the seeder tine passes with a press wheel detector, thus indicating the emissions are not escaping when the soil is packed. When seeding over the control and fertilizer strips with no exhaust (see Figure 2.00), the monitor picks up CO_2 respiration from the packer wheel. As an example some soil readings could be as high as 19.9% oxygen (representing 1% CO_2 or 10,000 ppm per tine) during late seeding or early fall seeding. This is due to the release of CO_2 through decarboxylation when soil temperatures are warmer. Seeding during this time isn't acceptable for "carbon farming practices" if emissions reduction is to be achieved. When temperatures are cooler, the soil carboxylases and takes in CO_2, therefore seeding earlier in the spring and later in the fall can help sequester the exhaust in an effective manner; seeding during this time is acceptable for carbon farming practices maintaining 20.9% oxygen behind the tine.

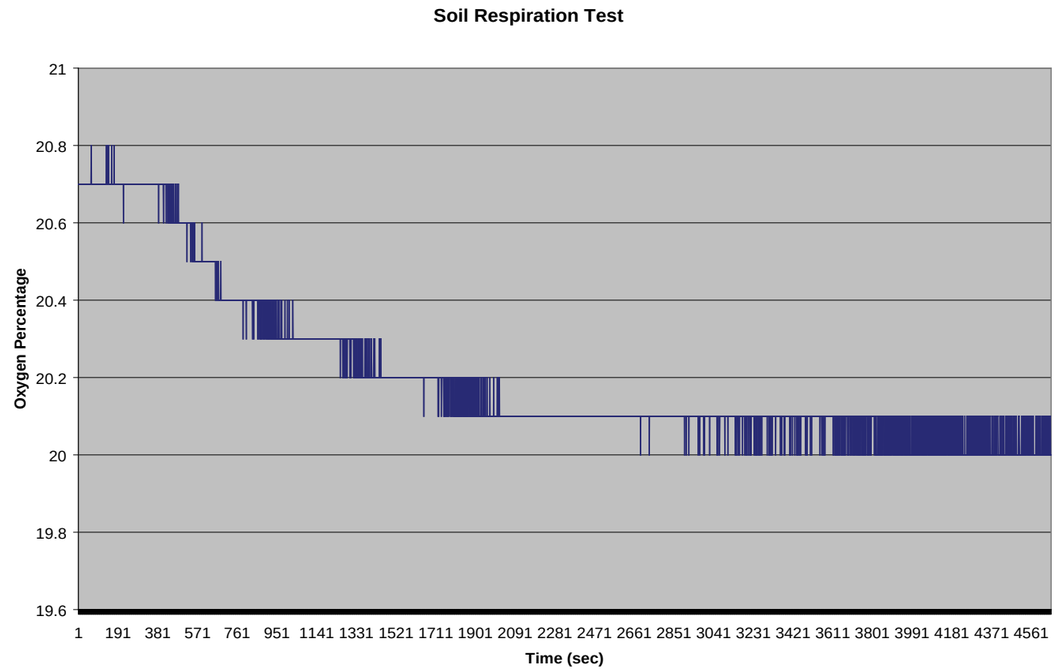


Fig 2.00 – *Field Test Strip 2, Bob Quinn field respiration test.*

developed to manage all the inputs to control the Bio-Agtive™ System and to automatically keep emissions at zero and push agriculture beyond carbon neutral. As some of the statistics shows that agriculture uses 30% of the world's energy to produce the required food and emits 22% of all greenhouse emissions.

Bio-Agtive™ SEED Operation

The air we breathe consists of 20.9 percent oxygen. When other greenhouse gases are introduced to the oxygen sensors they dilute the oxygen percentage, thus displacing oxygen. When injecting exhaust into the soil, the Bio-Agtive SEED monitor should be reading 20.9% at the tine if 100% of the tractors emissions are being sequestered.

The wideband oxygen sensors are calibrated to the current altitude with an oxygen percent of 20.9%. The atmospheric air has less than 400ppm of CO_2 gas, 79% nitrogen, 600 ppm of other gases. When emissions are present the CO_2 replaces the oxygen to a percentage. Every 0.1 percent decrease in oxygen the sensor detects is equivalent to 1000 ppm of CO_2 or greenhouse gases per tine. If the equipment measures 19.8% of oxygen, it is equivalent to 11,000 ppm of CO_2 gas per tine. The lower the oxygen percentage the higher the CO_2 level (*Fig 2.01*)

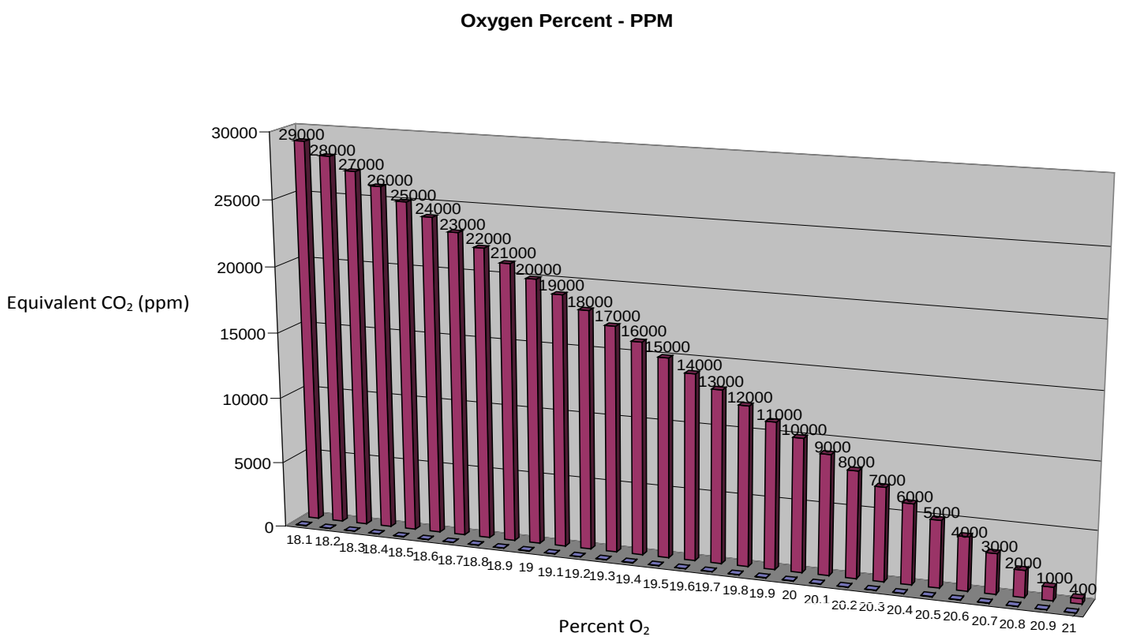
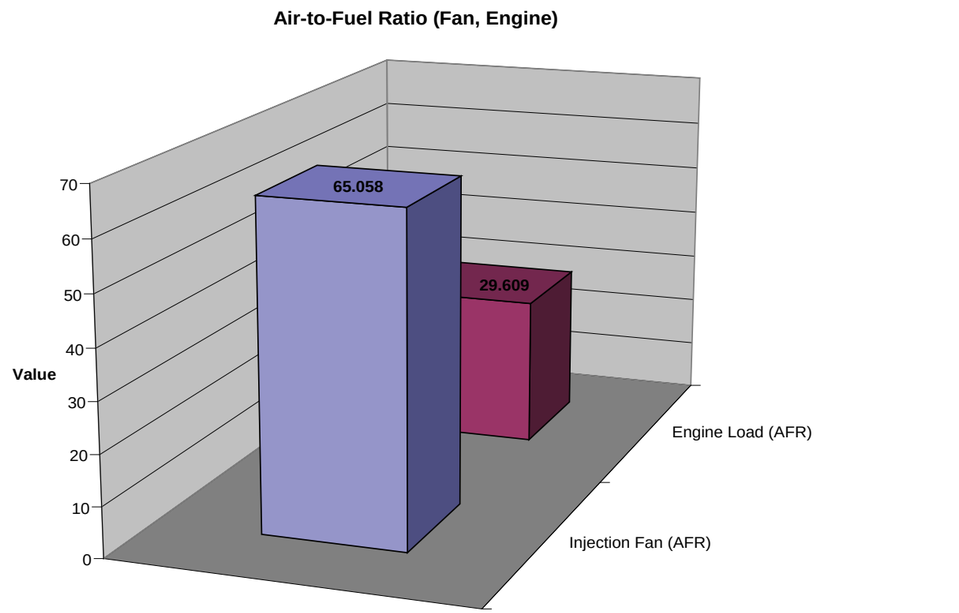


Fig 2.01 – *Relative oxygen percent to gases in parts per million.*

Understanding Air-to-Fuel Ratio (AFR) with the Bio-Agtive System

Once the oxygen sensor becomes diluted with external gases (below 18% O_2) it switches into an Air-to-Fuel Ratio measurement starting at 80.000 AFR. Measurements of an AFR such as 80.000 can be read as 80 parts of air to one part of fuel. Higher AFR measurements indicate there is more oxygen in the emission and less CO_2, resulting in a lean mixture (or diluted gases with the air seeder fan). Lower AFR measurements (medium torque setting of 30.000 AFR) signify that there is less air being mixed with more gas, resulting to a rich mixture.

The following graph (*Fig 2.02*) illustrates the **Engine Load (AFR)** is producing a strong 29.609 AFR (rich) mixture, meaning the engine is adding more fuel to the cylinders to pull more engine load. The sensor at the **Injection Fan (AFR)** is measuring a much leaner mixture of 65.058 AFR. This is because gases are being mixed with external oxygen being introduced through the seeder fan before being injected into the soil.



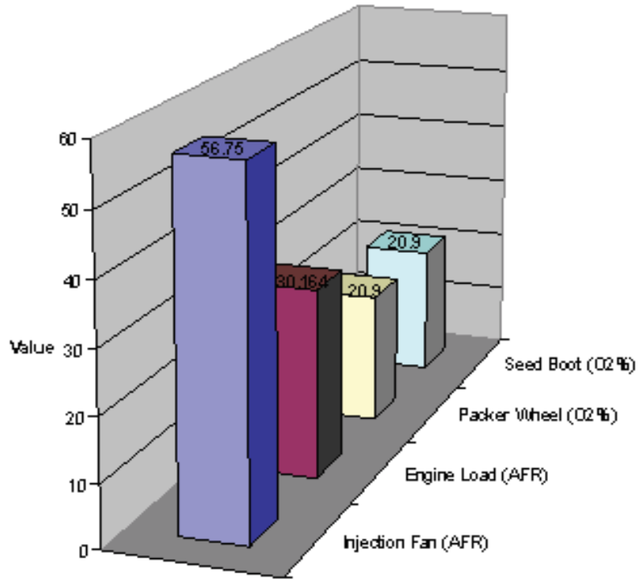
	1
Injection Fan (AFR)	65.058
Engine Load (AFR)	29.609

Fig 2.02 – *Diesel from Research Trial, Injection Fan Air-to-Fuel Ratio with Engine Load Air-to-Fuel Ratio*

Older prototypes with old technology have had to rely on the seed cart fan to draw in extra air to keep the exhaust cooled down to prevent damage to equipment and seed. This leans out the mixture of gas and with the increased pressure of the fan it can bounce exhaust from the furrow. The following tables demonstrate how this can have a big effect on the user's results and shows how the Bio-Agtive SEED monitor can help prevent this issue.

Managing Exhaust Incorporation with SEED

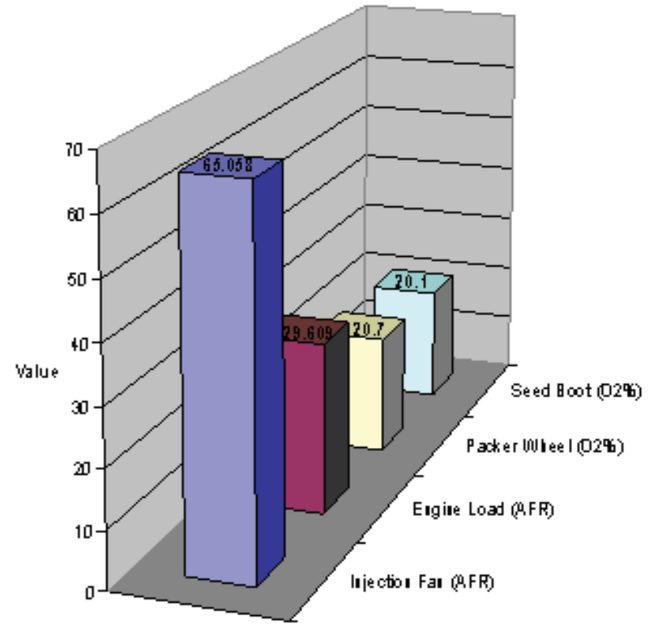
Fig 2.03 shows an example of the Bio-Agtive™ System performing correctly with proper equipment setup, running at an injection fan speed of 3000 RPM. The **Engine Load** is producing a rich mixture at 30.164 AFR from the engine to do the work needed at that time (soil, weather, weight, etc will alter Engine Load). When the exhaust is injected into the furrow with a low seed cart fan setting, it maintains a richer air to fuel ratio inside the seed tubes. The advantages are a higher CO_2 level and more effective absorption to the soil.
With this setup and adjustments (depth, speed, seed cart fan, temperature, boot type and seed rate) we are able to read and maintain oxygen levels at 20.9% both at the Packer Wheel sensor and Seed Boot sensor. This verifies all 56.750 AFR of exhaust is being absorbed to the soil.



	1
Injection Fan (AFR)	56.75
Engine Load (AFR)	30.164
Packer Wheel (O2%)	20.9
Seed Boot (O2%)	20.9

Fig 2.03 – *A well-tuned Bio-Agtive System with equipment adjusted properly and incorporating all exhaust into the soil.*

Fig 2.04 shows the result of the Bio-Agtive System adjusted poorly. Increasing the injection fan speed to 4000 RPM creates detection of exhaust escaping from the furrow resulting to the decrease in O_2 at the Seed Boot O2 sensor. The sensor is giving a reading of 20.1% oxygen behind the tine, 0.8% of the oxygen is being replaced with 9000 ppm of CO_2 per tine (Fig 2.04). The **Packer Wheel O2%** sensor displays a reading of 20.7% oxygen. The excessive volume of oxygen and exhaust are being pushed out of the soil even after the packer wheel passes by with these current settings. The **Injection Fan AFR** sensor clarifies that the gas will dilute and will increase the parts of air to fuel ratio from 56.750 AFR (Fig. 2.03) to 65.058AFR (Fig 2.04). This is because extra volume of air is being introduced from the atmosphere by the seed cart fan at 4000 rpm. Other factor settings such as depth, speed, seed cart fan, temperature, soil type and moisture, boot type and seed rate can all effect exhaust bounce.



	1
Injection Fan (AFR)	65.058
Engine Load (AFR)	29.609
Packer Wheel (O2%)	20.7
Seed Boot (O2%)	20.1

Fig 2.04 An *increase of fan speed means an increase of AFR (leaner) and exhaust bounce.*

Conclusion

Bio-Agtive™ SEED has been successful at measuring monitoring and verifying that the tractor emissions can be 100% sequestered into the soil during spring seeding. With collaboration of Montana State University-Northern Bio-Energy Center, Bio-Agtive of Montana LLC and N/C Quest Inc., Dynamometer lab testing quantifying different fuel chemistries of the emissions and condensation water testing in their state of the art facility. Full scale farm testing has answered the unknown questions that was outlined in the study objectives.