DEVELOPMENT OF A NON-DISPERSIVE INFRARED CO_2 SENSOR-BASED SYSTEM FOR ASSESSING SOIL TOXICITY USING SUBSTRATE-INDUCED RESPIRATION

By

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ABSTRACT

Soil pollution due to anthropogenic petroleum hydrocarbon (PHC) spills has become a major environmental hazard. The eco-toxicological indicators used to evaluate soil quality complement physico-chemical criteria employed in contaminated site remediation. But their cost, time consumption, sophisticated analytical methods and *in-situ* inapplicability pose a major challenge to the rapid detection and mapping of the extent of the soil contamination. This research describes a sensor based approach for measuring potential (substrate-induced) microbial respiration in PHC-contaminated and non-contaminated soil and hence, indirectly evaluates their microbial activity. A simple CO_2 sensing system was developed using an inexpensive non-dispersive infrared (NDIR) CO_2 sensor and was successfully deployed to differentiate between the control and diesel-contaminated soils in terms of CO_2 emission after glucose addition. Glucose was used as a substrate at an optimal concentration of 25 mg g^{-1}, dissolved in deionized water to adjust the soil moisture to 80% of water holding capacity (WHC) and sprayed over 20 g soil samples to trigger microbial respiration and hence, CO_2 emission. The sensor was able to distinguish glucose-induced CO_2 emission from sterile and control soil samples ($p \leq 0.0001$). Acetone was used as an organic solvent to spike soil samples with phenanthrene and was found to suppress CO_2 emission from phenanthrene-contaminated soil samples after incubation. Significant effects of diesel contamination ($p \leq 0.0001$) and soil type ($p \leq 0.0001$) on glucose-induced CO_2 emission were found. The sensing system can provide *in-situ* evaluation of soil microbial activity, an indicator of soil quality and is a promising tool for the initial screening of contaminated environmental sites and for creating high spatial density maps at a relatively low cost.

RÉSUMÉ

La pollution du sol causée par des déversements d'hydrocarbures pétroliers (PHC) anthropiques est devenue une menace environnementale majeure. Les indicateurs éco-toxicologiques, utilisés pour évaluer la qualité des sols, complémentent les critères physico-chimiques utilisés lors de l'assainissement de sites contaminés. Par contre, les coûts, les délais, les méthodes analytiques sophistiquées et l'inapplicabilité *in-situ* présentent des défis majeurs pour la détection rapide et la cartographie de l'étendue de la contamination du sol. Cette recherche présente une approche basée sur des capteurs servant à mesurer le potentiel de respiration microbienne (induite par un substrat) pour des sols contaminés et non contaminées aux HCP, et par conséquent à évaluer indirectement leur activité microbienne. Un système simple de détection du CO_2 a été développé en utilisant un capteur de CO_2 infrarouge non dispersif (NDIR) peu coûteux et est parvenu à différencier les sols contaminés au diesel de ceux de références en matière d'émissions de CO_2 après l'ajout de glucose. Ce dernier a été utilisé comme substrat à une concentration optimale de 25 mg g^{-1}, dissous dans l'eau déminéralisée, pour ajuster l'humidité du sol à 80% de sa capacité de rétention de l'eau (WHC), et pulvérisé sur des échantillons de 20 g de sol pour déclencher la respiration bactérienne et donc les émissions de CO_2. Le capteur a pu distinguer les émissions de CO_2 induites par le glucose de celles des échantillons de sol stérile et de référence ($p \leq 0.0001$). De l'acétone a été utilisée comme solvant organique pour charger de phénanthrène les échantillons de sol et s'est avérée éliminer les émissions de CO_2 de ces échantillons de sol contaminés aux phénanthrènes après l'incubation. La contamination au diesel ($p \leq 0.0001$) et le type de sol ($p \leq 0.0001$) ont démontré avoir des effets significatifs sur les émissions de CO_2 induites par le glucose. Le système de détection peut fournir une évaluation *in-situ* de l'activité microbienne du sol, un indicateur de sa qualité, et est

un outil prometteur pour effectuer l'examen préliminaire de sites environnementaux contaminés et pour créer des cartes à haute densité spatiale à des coûts relativement bas.

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CONTRIBUTIONS OF AUTHORS

The research work of this thesis has been submitted for publication in a peer-reviewed journal. The author of this thesis was responsible for the development and evaluation of NDIR-based CO_2 sensor system. The author also designed and carried out the experimental and analytical work to meet the research objectives of this thesis. She is also responsible for the preparation of the manuscript based on this thesis. Dr. Viacheslav Adamchuk, an Associate Professor in Department of Bioresource Engineering of McGill University, is the thesis supervisor. He created the idea for this research and offered scientific advice and technical guidance throughout the study. He is also responsible for editing and reviewing the prepared manuscript. Dr. Ashraf Ismail is an Associate Professor in the Department of Food Science and Agricultural Chemistry of McGill University; he constantly provided scientific suggestions for this study as a member of the author's graduate committee and helped with the development of the system with his insight on soil toxicity. Dr. Joann Whalen is an Associate Professor in Department of Natural Resource Sciences of McGill University and provided valuable recommendations, especially during the preliminary phase of this research. She inspired the idea of focusing on quantifying soil biota respiration and helped with the experimental design for the evaluation of the system.

Publication related to the thesis

1. **Kaur, J.**, Adamchuk, V.I., Whalen, J.K., and Ismail, A.A. Development of an NDIR CO_2 sensor-based system for assessing soil toxicity using substrate-induced respiration. *Sensors* (in press).

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
ABC	Automatic baseline correction
ELISA	Enzyme linked immunosorbent assay
FID	Flame ionisation detector
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
HPLC	High performance liquid chromatography
HSD	Honest significant difference
IRGA	Infrared gas analyzers
MS	Mass spectrometry
MEMS	Micro-electro-mechanical systems
NDIR	Non-dispersive infrared
PHC	Petroleum hydrocarbons
PAH	Polycyclic aromatic hydrocarbon
NASICON	Sodium (Na) super ionic conductor
SOM	Soil organic matter
SWC	Soil water content
SPE	Solid phase extraction
SIR	Substrate-induced respiration
U.S. EPA	United States Environmental Protection Agency
WHC	Water holding capacity

1. INTRODUCTION

Soil is a complex and dynamic biological system that directly and indirectly influences the quality of human life. It is essential for plant growth, nutrient cycling and maintaining the O_2/CO_2 balance of the atmosphere. It acts as the ultimate sink for most waste products. A number of chemicals have been introduced claiming to increase agricultural yield and to improve human life e.g., pesticides, metals and petroleum products. Increased use of these xenobiotic compounds has resulted in their release into soil and other environmental resources, as well as water and the atmosphere.

Research on the distribution and fate of various pollutants in the ecosystems emphasized their harmful impacts on both ecosystems and living organisms. Consequently, soil pollution is viewed with apprehension and has become a major environmental concern. Petroleum hydrocarbons (PHCs) are common environmental contaminants. They are complex mixtures of aliphatic, alicyclic and aromatic compounds and enter terrestrial ecosystems due to surface spills or leaks from pipelines or storage tanks (Potter and Simmons, 1998).

Since the recent research has been focusing the remediation and reclamation of PHC-contaminated sites, rapid, low cost and effective *in-situ* technologies are required to assess the scope and level of PHC contamination at a remediation site. Laboratory-based techniques are sensitive and accurate, but are costly and time-consuming. Moreover, they do not take into account the bioavailability (Peijnenburg et al., 2004) of the pollutant. PHCs get entrapped within a soil or sediment matrix and become increasingly resistant to desorption and less bioavailable to soil biota. Various bioindicator-based assays have also been introduced, but their synthesis is challenging. Eco-toxicological tests directly determine the possible hazards of pollutants on soil ecology. In general, acute and chronic soil toxicity tests are conducted by collecting soil samples

from the polluted site and sending them to the laboratory for testing; they offer the advantage of assessment of bioavailability of the pollutants. Alternatively, several toxicity test kits have been developed in which test organisms are exposed to the soil sample on site. Such tests actually are performed in a mobile laboratory system which has been brought to the site. This system has reagents for extracting the PHCs and the test organism vials for their detection using a battery-powered instrument. These tests are performed under standard laboratory conditions, not site conditions and, hence, should not be confused with *in-situ* tests because *in-situ* tests expose a given organism at the site under actual environmental conditions, such as temperature, pH, and light. Hence, a promising *in-situ* soil PHC toxicity evaluation technology is required for the initial biological risk assessment of a contaminated site. Site toxicity data from such an *in-situ* test would prove to be a more realistic assessment of toxicity than data from any analytical or biological laboratory tests. Ultimately, it would help to characterize the actual ecological risk of a polluted site.

An important element in the characterization of petroleum-contaminated sites is the rate of microbial activity. Soil respiration rates have been used to measure microbial activity since the latter part of the 1800s and have also been used effectively to measure soil contamination. While basal respiration only measures the "potential" soil microbial community, substrate-induced respiration (SIR) adds a labile substrate (e.g., glucose) to maximize the respiration rate and can be used to determine the total microbial activity present in the soil.

Currently, commercially available chamber systems (e.g., Li-Cor Li-8100) integrated with portable infrared gas analyzers (IRGAs) are used to measure soil CO_2 efflux *in-situ*. To improve the efficiency and lower the cost of these chamber systems, a number of simplified non-dispersive infrared-based (NDIR-based) sensors are available now (Yasuda et al., 2012). Such

systems can be modified to measure SIR responses and hence, act as a rapid tool for quantifying the eco-toxicological impact of contaminants. They provide promising tools for the initial risk assessment of contaminated environmental sites by creating high spatial density maps at a relatively low cost. Potential applications range from former industrial sites to oil and gas industry locations which need to be remediated to convert these land resources into alternative uses. Moreover, they can be applied to measure the soil "quality" of agriculture soils, in general, as they determine the "potential" microbial community present in the soil (Doran and Zeiss, 2000).

1.1. Objectives

The objectives of this study were to develop and evaluate an NDIR-based CO_2 sensor system suitable for *in-situ* deployment to measure soil CO_2 emissions in response to added glucose, based on the SIR method and to investigate its suitability for toxicity assessment of PHC-contaminated soils. In particular, the following tasks were to be achieved: 1) to design and test a CO_2 sensor installed in a closed chamber to measure potential (or induced) soil CO_2 emission**,** 2) to optimize the substrate concentration to be used for SIR experiments, 3) to investigate the applicability of the newly designed NDIR-based CO_2 sensor system to distinguish between soil samples contaminated with different levels of PHCs in terms of SIR rates.

2. REVIEW OF LITERATURE

PHCs may be introduced into the soil as a result of their accidental discharge during transportation, leakage from storage tanks or due to pipeline ruptures (Nicolotti and Egli, 1998). Crude oil spillage has a significant effect on soil properties (e.g., soil pH, hydraulic conductivity, total nitrogen (N), available phosphorus (P)) that reduces the fertility of agricultural soils (Essien and John, 2010). In addition, crude oil contaminated soils have been proven to be toxic to both flora and fauna (Dorn et al., 1998; van Gestel et al., 2001; Wong et al., 1999) and dangerous to human health (Ordinioha and Brisibe, 2013).

Diesel oil is a complex PHC derived from crude oil distillation (Air Force, 1989). It is composed of low molecular weight alkanes and polycyclic aromatic hydrocarbons (PAHs; Adam and Duncan, 1999). PAHs constitute 5-30% of diesel oil (Air Force, 1989). PAHs also exist in soil due to the incomplete combustion of coal, oil, petroleum, and wood (Saim et al., 1997), or due to petrochemical industrial activities (Jaouen-Madoulet et al., 2000). The fate of PAHs is a huge concern as they are toxic, carcinogenic and/or mutagenic priority micro-pollutants listed by the United States Environmental Protection Agency (U.S. EPA; Keith and Telliard, 1979). Thus, recent research has been focusing on the development and investigation of various chemical and biological methods for hydrocarbon remediation of contaminated sites. Development of technologies to rapidly assess the scope and level of PAH pollution at a relatively low cost and within a short time (Wang et al., 1990) provides a means of improving management decisions for site remediation and assists the hydrocarbon remediation research initiatives.

2.1. Laboratory-scale methods

Common approaches to determine the total concentration of hydrocarbon pollutants in soil focus on sophisticated, time consuming, laboratory based analytical techniques. PHCs are lipophilic in nature and show affinity towards the soil organic matter (SOM; Kleineidam et al., 2002) due to sorption (Golding et al., 2005). Sorption is a phase distribution process due to solutes which accumulate at surfaces and interphases (i.e., adsorption) of solids or are transported from one phase to another (i.e., partitioning). So, the quantification of PHCs in soil requires powerful extraction techniques to release the strongly sorbed contaminants from the soil material. The steps followed for the analytical determination of PAH pollutants in soil are as follows:

2.1.1. Pretreatment, which increases both the homogeneity of the soil and the extractability of the analytes in the soil. The sample pretreatment includes sieving, air-drying, grinding (Wischmann et al., 1996) and/or acidification (Bergknut et al., 2004) of soil samples.

2.1.2. Extraction, which releases the contaminants from the soil matrix and quantitatively transfers them to an organic solvent. The extraction solvent has a major effect on the extraction process. Dichloromethane and toluene are preferred due to their medium polarity or mixtures of polar and non-polar solvents are also used. Hexane, acetone/hexane (1:1 v/v), dichloromethane-acetone (1:1 v/v), dichloromethane-ethanol (1:1 v/v), n-butanol (Hatzinger and Alexander, 1995), methanol (Codina et al., 1994), are also used. Extraction techniques include ultrasonic extraction (Eiceman et al., 1980), soxhlet extraction (Arment, 1999), pressurized fluid extraction (Saim and Dean, 1998), pressurized hot water extraction, microwave assisted extraction (Lopez-Avila et al., 1994) and supercritical fluid extraction (Hartonen et al., 2000).

2.1.3. Clean up, to remove the co-extracted compounds that could interfere with subsequent analysis. The fractionation of PHCs can be performed by adsorption chromatography, open column chromatography (Saponaro et al., 2002), solid phase extraction (SPE; Meyer et al., 1999) or high performance liquid chromatography (HPLC)(Brooks et al., 1998).

2.1.4. Instrumental analysis, which separates, identifies and quantifies the individual analytes in the sample. Both the HPLC (Berset et al., 1999) and gas chromatography techniques such as GC/FID (U.S. EPA, 1996a), GC/MS (U.S. EPA, 1996b) and GC/FTIR (U.S. EPA, 1994a) are considered to be equally valid approaches to analyze PAHs, though GC/MS is preferred over the others (Disdier et al., 1999).

The advantages of these methods include their high accuracy and sensitivity towards the PHCs. However, these methods involve lengthy extraction processes for target contaminants, large volumes of extraction solvents and require instruments with high infrastructure and operating costs (Sporring et al., 2005). Moreover, the toxic effects of a pollutant in the soil depend, not only on its chemical properties and the quantity present, but also upon the amount that is bioavailable, to be absorbed or up taken by the biota (Peijnenburg et al., 2004).

2.2. Bioavailability

Bioavailability plays an important role in the fate of organic pollutants and their effects on individual species and populations (Debus and Hund, 1997; Wahle and Kördel, 1997). It implies that within a given timeframe, only a fraction of the total amount of a chemical substance present in an environmental sample, e.g., soil, is available for uptake by living organisms, and subsequently may induce adverse effects (Peijnenburg et al., 2004). Bioavailability of PHCs is markedly affected by soil properties, e.g., SOM content and the clay content (Chung and

Alexander, 2002; Nam et al., 1998). However, laboratory-based methods are not suitable for biological assessments of polluted soil as they do not evaluate the toxicity, synergistic or antagonistic effects of given pollutants (Juvonen et al., 2000) on soil organisms. Consequently, a battery of bioindicator-based and toxicity-based assays (Bispo et al., 1999; Greene et al., 1988) on soil biota is used to determine the possible hazards of pollutants on soil ecology (Tang et al., 2011).

2.3. Bioindicator-based assays

Enzymes are directly involved in the degradation of hydrocarbons to simple molecules such as water and carbon dioxide and hence, may be used as potential bioindicators. Enzymes that have been used for this purpose include soil lipases (Margesin et al., 2000), dehydrogenases (Casida, 1977), catalases and ureases (Margesin and Schinner, 1997). Biolog plates have been widely used to characterize stressed soils (Bossio and Scow, 1995; Bundy et al., 2002) that measure the specific dehydrogenase activity of environmental samples upon exposure to water-soluble carbon substrates. The main advantage of enzymatic tests is that they offer an easy method of measurement, but a disadvantage is their indirect approach. Not all enzymes are synthesized by the bacterial cells at the same amount and both enzyme production and enzyme activity are highly regulated biomechanisms (Brock and Madigan, 1991). These methods have limited *in-situ* applicability as they also require pollutant extraction from the soils (Preston-Mafham et al., 2002).

Antibodies have an ability to bind selectively to the specific physical structure of the target analyte present in a sample matrix, such as soil. The availability of antibodies against small molecules, such as PAHs, accompanied by detection systems such as amperometric, capacitative, conductometric, potentiometric, fluorimetric, radioactive and UV detection

(González-Martínez et al., 1999) allowed the use of immunochemical procedures for environmental monitoring (Van Emon and Gerlach, 1998). The most commonly used immunoassay for this purpose is the enzyme linked immunosorbent assay (ELISA) (Fillmann et al., 2007). Immunoassays are sensitive, selective, reliable, easy to use and can be operated at low cost, but the development of bioassays especially for small molecules, such as PAHs, is time consuming, costly and intensive (Fähnrich et al., 2002).

2.4. Toxicity assays

Toxicity tests measure lethal and/or sublethal effects that are known as measurement or response endpoints i.e., they are easily measurable ecological attributes that may be adversely affected by exposure to organic contaminants and are related to an assessment endpoint. Hence, a measured endpoint can be used to approximate the assessment endpoint if the assessment endpoint itself cannot be measured directly (U.S. EPA, 1992).

2.4.1. Acute toxicity tests

Acute toxicity tests are short-term survival assays that measure the effects of exposure to relatively high concentrations of contaminants. Percent mortality of organisms exposed to a polluted sample is compared to percent mortality of organisms exposed to an unpolluted sample. Alternatively, dilution of the pollutant at which 50 percent of the organisms died, called the LC50, is the median lethal concentration (U.S. EPA, 1989). Aquatic bioassays are used for the assessment of the ecotoxic potential of soils towards aquatic and soil-dwelling organisms. The two most frequently used bioassays in aquatic ecotoxicity testing are the luminescence-based *Vibrio fischeri* test and the immobilization-based *Daphnia magna* test (Bispo et al., 1999). In a luminescence-based assay, bacterial luminescence is linked to electron transport (Ismailov et al.,

1981) and is negatively correlated with an increase in the toxicity of a pollutant after bacterial luminescence. *Vibrio fischeri* are used as test organisms in commercial luminescence inhibition assays (Microtox Assay; ISO, 1998a) as well as in the Solid-Phase Flash-Assay (Lappalainen et al., 2000). In the immobilization-based assay, the water flea *Daphnia magna* is immobilized during an exposure period of 48 hours. Immobilisation and abnormal behaviour is recorded at 24 and 48 hours and compared with control values (Daphtoxkit^{TM} Assay; OECD, 2012). These tests are performed on soil leachates that contain a pollutant (Tiensing et al., 2001)

2.4.2. Chronic toxicity tests

Chronic toxicity tests measure the sub-lethal effects of contaminants on test organisms during long term exposure. Sublethal effects include growth reduction, reproductive impairment, nerve function damage, lack of motility, behavioural changes, and the development of structural abnormalities (U.S. EPA, 1994b). Seed germination and earthworm survival assays are widely used for measuring chronic soil toxicity (Greene et al., 1988). These tests are sensitive to changes in soil toxicity during remediation of PHC contaminated soil (Dorn et al., 1998). Other standard methods include eco-toxicology tests for plants (ISO, 1995) and earthworms (ISO, 1993, 1998b) to measure acute and chronic soil toxicity. Also, reproduction of *Ceriodaphnia magna* over 7 days (AFNOR, 2004) is used as a chronic toxicity indicator. Chronic toxicity tests are less frequently performed than acute tests (Francois-Férard and Ferrari, 2005). Toxicity tests are useful to identify the bioavailability of pollutants and they have a simple methodology and moderate sensitivity (Maila and Cloete, 2002); nevertheless, they involve considerable time, cost and expertise and have limited *in-situ* applicability as they also involve an organic solvent and a vacuum/ speed extractor (Grossmann and Udluft, 1991) for pollutant extraction from soil solid. Moreover, tests with soil-dependent organisms are more important with respect to soil

ecotoxicity testing (Keddy et al., 1995), but few ecotoxicity studies have used soil organisms (Sverdrup et al., 2002). Hence, the focus has shifted to finding a way to overcome the limitations of soil toxicity assessments.

2.5. Soil toxicity and soil microbial ecology

The notion of soil "quality" refers to soil functionality (Doran and Zeiss, 2000) whereas soil "health" is defined by the ability of the soil to promote plant, animal and human health and sustainability (Gil-Sotres et al., 2005). However, due to the environmental impact of contaminants and treatments (Cébron et al., 2011), the quality and health of polluted and remediated soils may not be the same as native soils. In soils, carbon (C) and nutrient cycles (e.g., C mineralization, nitrification etc.) are driven by microorganisms (Nannipieri et al., 1990) and play an important role in both soil quality and soil heath. Hence, it becomes crucial to assess the microbial ecology of contaminated and treated soils.

Recent research has shown that PHCs have a significant impact on microbial community abundance (Chen et al., 2014), composition and diversity in soil depending upon the degree of hydrocarbon contamination (Hawrot-Paw, 2012; Sutton et al., 2013). Bacteria and fungi present in soil are susceptible to the toxic effects of such contaminants (Dawson et al., 2007) and once affected, they disturb the proper functioning of the soil (Cébron et al., 2011). Thus, the soil microbial activity in a hydrocarbon-contaminated soil can be used as an indicator of the level of hydrocarbon contamination present in the soil.

2.6. Soil respiration

One of the simplest methods to measure soil microbial activity is based on soil respiration. Assessment of soil respiration by measuring the soil CO_2 production or O_2

consumption allows the metabolic activity of soil micro-organisms to be quantified (Nannipieri et al., 1990). The basal respiration (BR) is defined as respiration without adding any organic substrate to the soil. The SIR involves the measurement of microbial respiration of soil after adding an excess of a readily available nutrient source, usually glucose, to trigger microbial activity (Anderson and Domsch, 1978).

Many studies have reported soil respiration measurements as a means of determining soil toxicity (Martí et al., 2007; Montserrat et al., 2006). Anderson and Domsch (1978) suggested that the respiration rate induced by glucose is proportional to the size of the original soil microbial biomass and hence, can be used as an indicator to determine the microbial biomass in the sample. The quotient of the actual (basal) and potential (substrate-induced) respiration rates was correlated with PAH concentration at a contaminated site (Hund and Schenk, 1994). Margesin et al. (2000) also used SIR as one of the monitoring parameters during the decontamination of a mineral-oil-contaminated soil. Currently, CO_2 evolved by microbial respiration from soil samples is determined by a simple colorimetric reaction in gas absorbent alkali (Campbell et al., 2003), but this is cost ineffective when a large number of samples are involved as the method involves replacing the CO_2 probe with each sample (Brinton and Haney, 2013).

2.7. Substrate-induced respiration

The reason that SIR could be adapted to a rapid sensor-based detection method is due to the short time-frame of microbial response. Upon the addition of the substrate, respiration rapidly increases to a maximum and remains at a constant rate for more than 4 h (Drobník, 1960). The strong differences, in terms of time of incubation, between the reported studies arise from the different objectives of the various studies. Anderson and Domsch (1978) made CO_2

emission measurements after 1 h incubation with glucose and correlated them with absolute soil microbial biomass. Lin and Brookes (1999) chose 0.5-2.5 h after glucose addition as the best estimator of the SIR rate. Ananyeva et al (2011) recorded CO_2 emission within 2-5 h after the application of glucose to evaluate respiration differences between unamended and amended soil samples with added solid and aqueous glucose. Dilly (2001) measured CO_2 emission within 4-24 h after the addition of glucose and targeted the calculation of microbial respiration quotients. The recent studies support a time span of 8 - 24 hours (Brinton and Haney, 2013; Haney et al., 2008) to estimate soil C, N and P mineralization and to correlate soil biological activity with potentially mineralizable N.

The SIR rate is strongly affected by soil water content (SWC; Wardle and Parkinson, 1990). For reliable SIR measurements, the substrate should be distributed evenly throughout the soil sample. Adding a substrate solution instead of a powder gives the best distribution of substrate in soil and is analytically convenient (Lin and Brookes, 1999). However, using a substrate solution might cause an underestimation of CO_2 due to its retention in solution (West and Sparling, 1986). The quantity of substrate needed to achieve a saturated respiration response also needs to be determined for each soil and depends upon the physical and chemical properties of the soil (Anderson and Domsch, 1978).

2.8. CO_2 sensors

To improve the efficiency of soil microbial respiration based methods, a number of simplified CO_2 sensors are available now (Yasuda et al., 2012). A sensor is a device or a system which has the function of converting a physical variable input into a signal variable output. Voltage (electrical circuits), displacement or forces (mechanical systems) are commonly used as

signal variables (Holman, 2011). A number of simplified sensors have been developed for monitoring CO_2 concentrations.

Based on the sensing mechanism, these CO_2 sensors can be broadly classified as electrochemical (or solid state) and optical sensors (Neethirajan et al., 2009). Electrochemical CO_2 sensors are based on a variety of principles (ampere-, conducto-, and potentiometry) and materials (metal oxides, polymers, ceramics, or sol-gel) (Capone et al., 2004; Moseley, 1997). They can be further divided into metal oxide (Barsan and Weimar, 2001), NASICON (Zhu et al., 2005) and polymer-based CO_2 sensors (Tongol et al., 2003). They make use of micro-electro-mechanical systems (MEMS) and nanotechnologies and are highly sensitive, but have problems with limited measurement accuracy and short-time stability (Neethirajan et al., 2009). The most common commercially available CO_2 sensors are non-dispersive infrared detectors (NDIR) because of their low cost, compact size, easy process control, mass production, and continuous measurement (Lee and Lee, 2001).

The basic principle of NDIR CO_2 sensors is the energy absorption characteristics of CO_2 in the infrared region (Adachi et al., 1992). CO_2 is known to absorb infrared radiation at wavelengths of 2.7, 4.3 and 15 μm (Skoog et al., 2006). Generally, the wavelength of the near 4.3 μm (Oberly et al., 1968) region is preferred because of maximum absorption and insignificant interference at this band. The radiation emitted at this wavelength is associated with CO_2 by the Lambert-Beer law (Kwon et al., 2009; Wagner et al., 1991) given by:

$$\frac{I_d}{I_0} = e^{-\alpha.c.l} \tag{1}$$

where, I_d is the intensity of the radiation detected at a wavelength of 4.28 µm, I_0 is the intensity if the incident radiation, α is the absorption coefficient of the CO_2, c is the CO_2 concentration and l is the optical path length from the source to the detector.

NDIR CO_2 sensors (Figure 2.1) consist of a pulse-driven IR lamp (i.e., light source), a perforated sampling tube (or chamber), two optical filters, and two IR detectors (thermopiles) (Pandey and Kim, 2007). A reflection mirror is attached behind the IR lamp, and an inner wall of the pipe is plated to make emitted IR reach the thermopiles. One thermopile monitors the intensity of light through an optical bandpass filter with 4.0 µm center wavelength, and the other measures the IR absorption due to CO_2 concentration through an optical bandpass filter with a 4.26 µm center wavelength. The difference of these two raw signals provides the signal output (Wang et al., 2005).

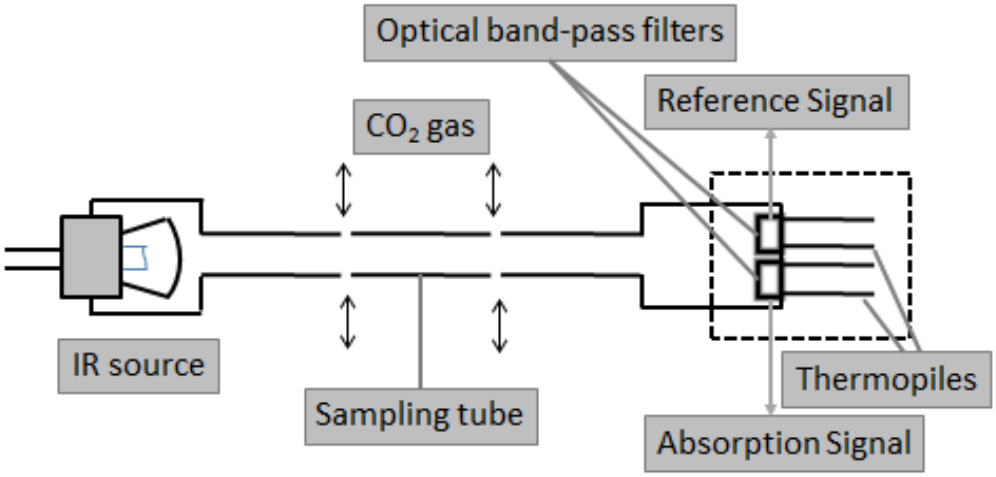

Fig. 2.1. Internal structure of an NDIR CO_2 sensor (adapted from Wang et al., 2005)

2.9. *In-situ* measurement of soil respiration

Different chamber techniques have been developed to measure soil CO_2 efflux *in-situ*. These chambers can be divided into three categories: closed static, closed and open dynamic chambers (Livingston and Hutchinson, 1995). In closed chambers (e.g., static and dynamic),

there is no exchange with the surrounding air and CO_2 efflux is calculated from the slope of the CO_2 concentration increase within the chamber over time (Hutchinson and Mosier, 1981; Jensen et al., 1996). Currently, portable infrared gas analyzers (IRGAs; Koepf, 1954) have been integrated in both open (Fang and Moncrieff, 1996) and closed (Rochette et al., 1992) chambers to develop commercially open (e.g., PP-Systems CFX-1) and closed chambers (e.g., Li-Cor Li-8100). Eddy covariance systems (Norman et al., 1992) measure CO_2 efflux at a height of 1-2 m above the ground and are mounted on towers above the vegetation (Law et al., 1999) to measure their net ecosystem exchange. The cost of these systems is a major hindrance.

Hence, the availability of these low cost NDIR CO_2 sensors can be harnessed to measure soil respiration by integrating these sensors into small systems developed to measure soil CO_2 efflux suitable for *in-situ* deployment and can easily be modified to determine SIR responses and hence, assess the "potential" microbial community present in the soil.

3. MATERIAL AND METHODS

3.1. Sensor system development

The CO_2 Engine® K30 CO_2 Sensor (SenseAir, Delsbo, Gävleborg, Sweden)[1] (Fig. 3.1.) was used for this study. It is inexpensive (under $100 USD) and has a measurement range between 0 - 5000 ppm with an accuracy of ± 30 ppm and ± 3% of measured value. Its small size (51 × 57 × 14 mm) enabled its integration into a small, closed CO_2 system to be used for the study. Its sampling chamber is a gold-plated labyrinth.

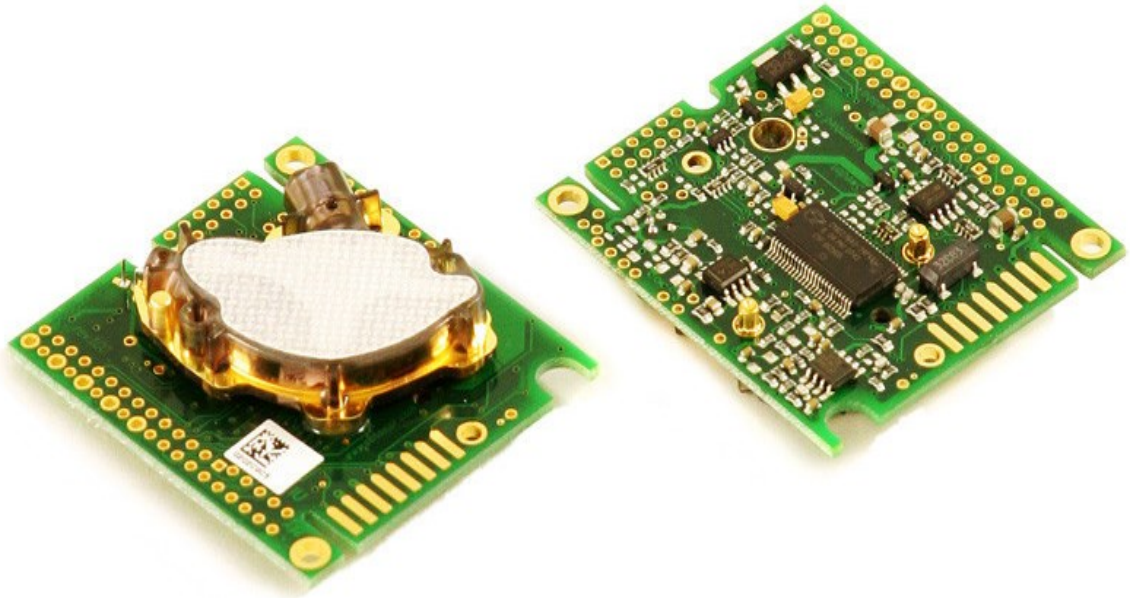

Fig. 3.1. Front and back view of CO_2 Engine® K30

No calibration is required during testing because of the built-in self-correcting ABC (Automatic Baseline Correction) algorithm. Infrared CO_2 sensors are prone to drift of the zero baseline of the calibration curve, which is set by default at the fresh air value of 400 ppm CO_2. The ABC algorithm is a "low pass filter" that takes advantage of the fact that the CO_2 level

[1] Mention of a trade name, proprietary product, or company name is for presentation clarity and does not imply endorsement by the authors, the McGill University, nor does it imply exclusion of other products that may also be suitable.

nearly falls to outside fresh air in buildings when unoccupied. It constantly keeps track of the sensor's lowest reading over a 7.5 day interval (by default) and slowly rescales the sensor probe for any long-term drift detected as compared to the expected 400 ppm CO_2, hence, updating the sensor calibration regularly.

A small closed static system (non-steady-state non-thorough-flow system) for CO_2 gas collection and quantification from soil samples was constructed (Fig. 3.2.). The K30 CO_2 sensor was placed on top of the system. To spray glucose solution over the soil sample uniformly, two nozzles attached to two clear PVC (polyvinyl chloride) tubes were integrated into the system. These pipes were attached to a syringe that aided the introduction of glucose solution into the pipes that fed glucose to the nozzles.

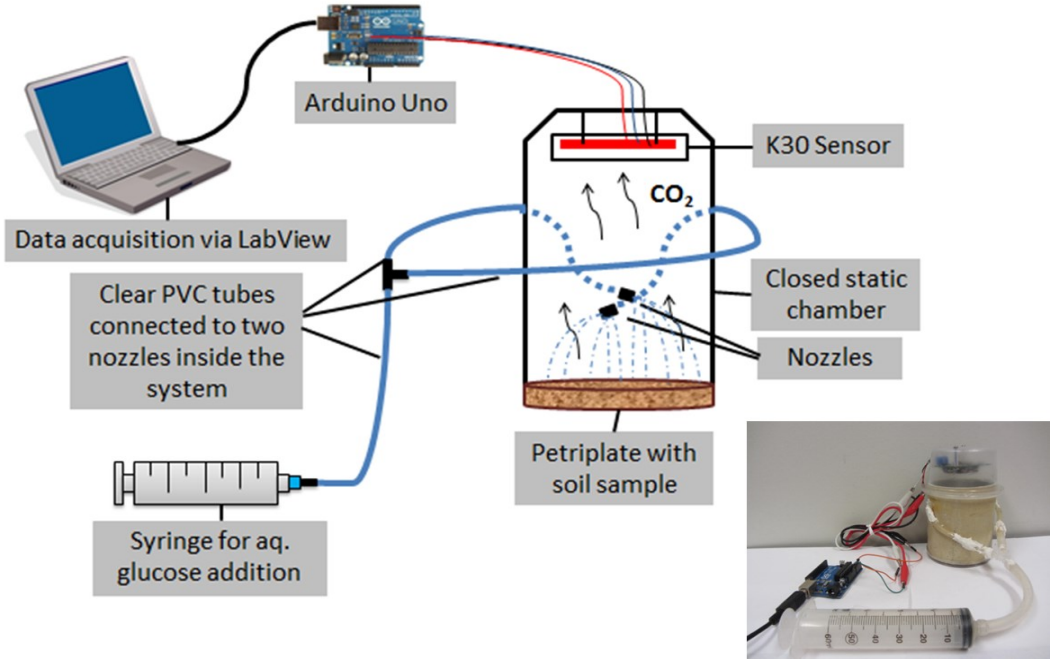

Fig. 3.2. Glucose-induced CO_2 emission sensing system for soils integrated with NDIR-based K30 CO_2 sensor

Data acquisition software for the K30 sensor was developed using LabView (National Instruments Corporation, Austin, TX) software. An Arduino UNO (Smart Projects, Strambino, Turin, Italy) was used as a microcontroller to power the sensor and to receive analog output; it

was connected to a laptop computer via a USB serial port. Analog voltage data output received from the sensor at 1 Hz was logged in a tab delimited text file. The collected data were analyzed using the Statistical Analysis System (SAS) 9.4 (SAS Institute Inc., NC, USA) software suite.

An illustration of the sensor response for sterile and control samples of organic soil over an extended period of 15 minutes is shown in Fig. 3.3. For data acquisition, the system was placed over the petriplate containing 20 g of the sample, the glucose solution was sprayed 6 minutes after the start of the experiment and the CO_2 emission data were collected for another 9 minutes. The data collected between 8 and 11 minutes (3 minutes in total), after glucose addition, were used for the calculation of CO_2 emission min^{-1}. For regular experiments, glucose was added 1 minute after the start of the experiment. The experiment spanned 6 minutes. The difference in CO_2 concentration between sterile and control soil samples at t = 0 is due to different experiment execution time and CO_2 concentration varies indoors throughout the day.

3.2. Sensor system evaluation

3.2.1. Soil Sampling

Three soil samples (1: organic, classified as histosol, 2: sandy loam, classified as gleysol and 3: sandy clay loam, classified as gleysol (WRB, 2006)) were collected from Field 26 of the Macdonald Campus Farm, McGill University, Quebec, Canada (45° 30'N, 73° 35'W); they were selected on the basis of the variability of their key physical and chemical properties (Table 3.1) and total count derived from gamma-ray spectrometry using SoilOptics™ (Practical Precision Inc., Tavistock, Ontario, Canada) (Fig. 3.4.). The soil samples were air dried for 7 days prior to analysis to reduce the contribution of roots to the total SIR response (West and Sparling, 1986), but the moisture content was maintained between 10-12% (gravimetric moisture content)

to sustain the activity of the soil microbial community. No grinding or sieving of the soils was done, so that the soil aggregate structure was maintained to simulate *in-situ* conditions.

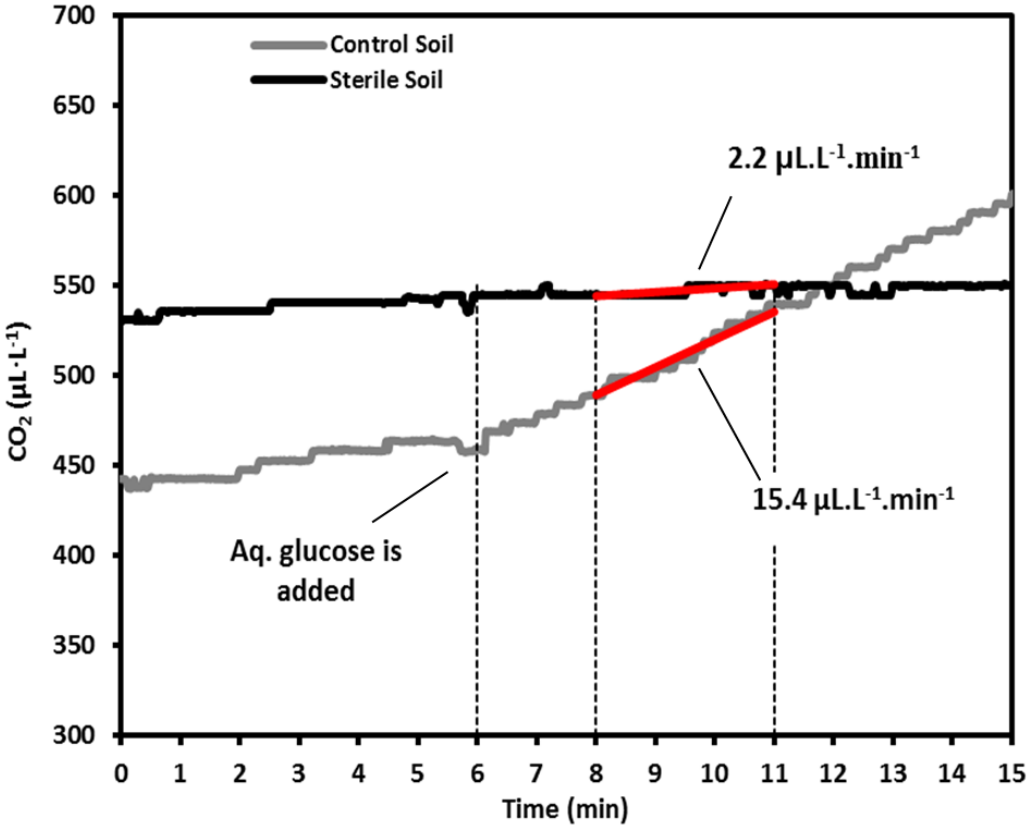

Fig. 3.3. Illustration of the response of NDIR-based CO_2 sensor system for sterile and control samples of organic soil. The glucose solution was added 6 minutes after placing the system over a petriplate containing the soil sample and the CO_2 emission data collected between 8 and 11 minutes were used for the calculation of CO_2 emission min^{-1}.

3.2.2. Substrate optimization

Glucose was chosen as a substrate for the study because it can be utilized as a carbon source by most soil microorganisms (Stotzky and Norman, 1961). It was purchased from Sigma-Aldrich Canada Co. (Oakville, Ontario, Canada). For each soil, five replicates of 20 g air-dry (a. d.) soil, were amended with a series of glucose concentrations (0, 5, 10, 15, 20 and 25 mg g^{-1} of soil) in solution (to adjust soil moisture to 80% of water holding capacity (WHC) of each soil) and CO_2 concentration was recorded for 5 minutes with the sensor described in Fig. 3.2., to determine the optimal glucose concentration.

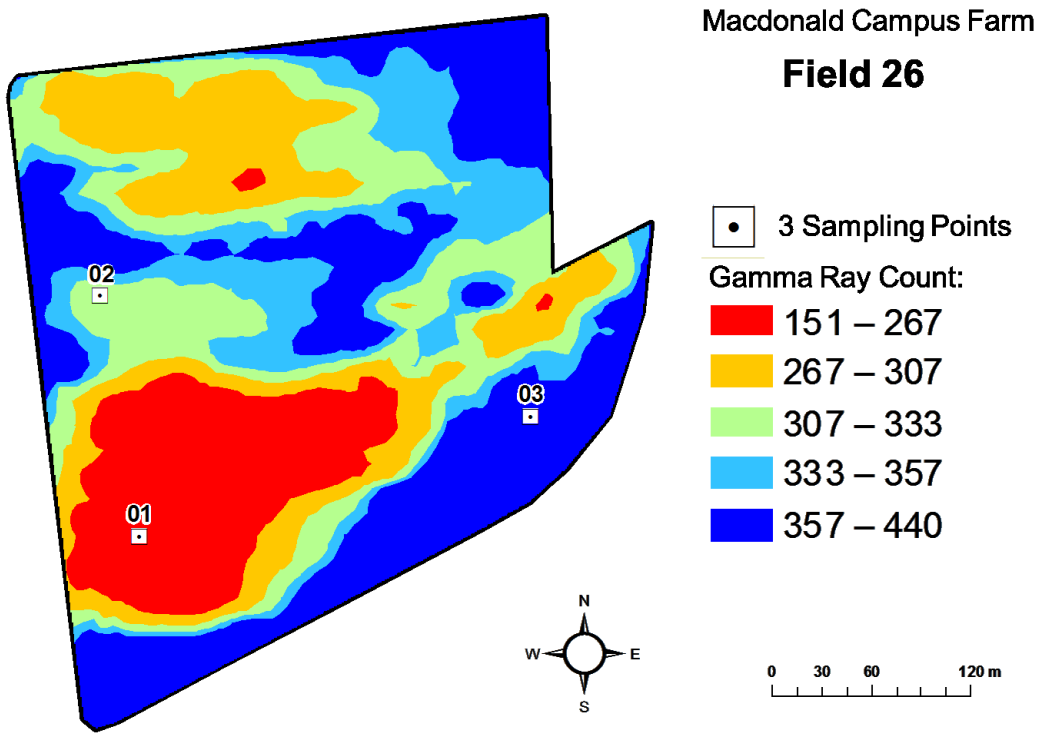

Fig. 3.4. Three sampling locations on the gamma ray count map of Field 26 of Macdonald Campus Farm, McGill University.

Table 3.1. Physical and chemical properties of collected soil samples

Sample No.	Sand, %	Silt, %	Clay, %	OM, %	WHC, %	pH	P	K	Ca	Mg	Al
							mg kg^{-1}				
1	36.5	40.2	23.3	63.3	50	6.9	78	108	10700	1700	206
2	62.4	24.6	13.0	7.8	16	5.9	104	56	1460	190	1082
3	46.5	27.9	25.6	7.5	20	7.4	100	124	3600	622	461

3.2.3. Preliminary evaluation

Next, an initial experiment was performed to check the applicability of the designed system for soils with, and without, microbial activity. All three soils were divided into four sub-samples, consisting of untreated soil for optimal glucose solution addition (control-glucose, CG), untreated soil for deionized water addition (control- deionized water, CD), autoclaved soil for glucose solution addition (sterile-glucose, SG) and autoclaved soil for deionized water addition

(sterile-deionized water, SD). Sterilization of soil samples was done by autoclaving the samples 3 times at 121°C and 15 psi for 1 h on alternate days (Greene et al., 2000).

3.2.4. PAH treated soils experiment

Phenanthrene (C_4H_{10}, Fig. 3.5), a neutral, 3-ring organic compound, was selected as a representative PAH. Its environmental properties, such as aqueous solubility, octanol–water partition coefficient (K_{ow}), and vapor pressure are similar to other PAHs, such as acenaphthene, fluoranthene, and fluorine (Schwarzenbach, 2002). It is predominant in coal-derived oils and automobile exhaust (Shabad, 1980) along with pyrene. Similarity of its parent molecular structure to the structure of higher molecular weight and more carcinogenic PAHs, such as benzo(a)pyrene, makes it an adequate representative compound that can give a general indication of PAH behaviour (Khodadoust et al., 2004). It has an aqueous solubility of 1.1 mg/L at 25°C (Schwarzenbach, 2002).

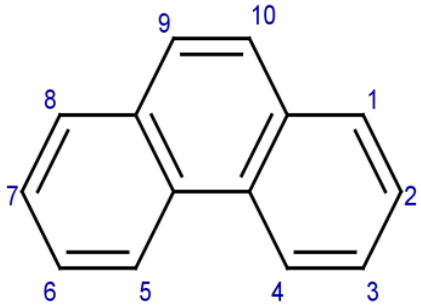

Fig. 3.5. Molecular structure of phenanthrene

To treat the soil samples with different concentrations of phenanthrene, stock solution of phenanthrene (1 g/L) was prepared in acetone. Each of the three soils were moistened to 10-12% (gravimetric moisture content) and homogenized with a stainless steel spatula. 0 µL,10 µL, 50 µL, 100 µL, 0.5 mL, 1 mL, 2.5 mL and 6.4 mL of the stock solution was taken and the final volume of 8 mL was made adding acetone to obtain a final concentration of 0, 0.1, 0.5, 1, 5, 10, 25 and 64 mg of phenanthrene kg^{-1} of soil, respectively. Approximately 10 g of the wet soil was

spiked with the above solutions at first. The soil was placed in a 1 L glass jar and mixed manually with a stainless steel spatula. The rest of the soil was gradually added to the glass jars in 5 g aliquots and extensively mixed with the spiked soil. This procedure was repeated until the entire amount of soil (100 g) was added and mixed (Brinch et al., 2002). The jars were closed hermetically for 5 minutes and left overnight under a flow hood. 5% (v/w) extra distilled water was added to cover water evaporation losses overnight (Scelza et al., 2007) and the samples were incubated for 20 days at 22°C. The moisture content was maintained at 10-12% over the period of incubation by periodically adding appropriate amounts of deionized water to the samples.

After incubation, triplicate subsamples (20 g of moist soil) were taken from each control and phenanthrene-contaminated soil and transferred onto petriplates, for a total of 72 petriplates (3 replicate subsamples × 8 phenanthrene treatments × 3 soil types). For each soil type, an optimal concentration of glucose (described above) was added into the volume of water required to reach 80% of WHC of each soil and was sprayed uniformly over the soil sample in the petriplate. The CO_2 concentration was determined for five minutes with the sensor system shown in Fig. 3.2., to determine toxicity response.

3.2.5. Diesel treated soils experiment

Each of the three soils were moistened to a moisture content of 10-12% (gravimetric moisture content), and homogenized with a stainless steel spatula. Five diesel treatments (0, 5, 20, 60 and 150 mg g^{-1} of soil) were applied to each soil. For diesel incorporation, soil samples (500 g each) were spread on aluminum trays to a depth of 1 cm. Diesel fuel (density 836 g L^{-1} at 15 °C) was applied over the surface of 1 cm deep soil by spraying uniformly from a spray bottle, so that it completely covered the surface of the soil with a thin layer of diesel. It was then allowed to penetrate the soil for 5 to 10 minutes, after that it was mixed thoroughly by hand

several times (Siddiqui and Adams, 2002). Then the control and diesel-contaminated soils were transferred to 1 kg plastic containers. The pots were covered with loosely fitted perforated lids and were incubated at 22 °C for 7 days. After incubation, triplicate subsamples (20 g of moist soil) were taken from each control and diesel-contaminated soil and transferred onto petriplates, for a total of 45 petriplates (3 replicate subsamples × 5 phenanthrene treatments × 3 soil types). An optimal concentration of glucose (described above) was added to the volume of water required to reach 80% of WHC of each soil; this was sprayed uniformly over the soil sample in the petriplate and the CO_2 concentration was determined.

4. RESULTS AND DISCUSSION

4.1. Glucose optimization

The data (Fig. 4.1) represented for optimization of glucose concentration for SIR is a result of five replicates. Analysis of variance (ANOVA) was conducted to determine the effect of various glucose concentrations using PROC GLM (the General Linear Model procedure). There was a significant effect of the addition of glucose on CO_2 evolution min^{-1} at the $p < 0.05$. Post hoc comparisons using the Tukey's HSD (Honest Significant Difference) test indicated that the mean score for the 10 mg g^{-1} glucose addition was significantly greater than the no sugar control, but no other glucose addition rates were different from the control among soil types. Hence, CO_2 emission following the addition of glucose solution increased rapidly to a maximum rate at 10 mg glucose g^{-1} soil for all three soil types. Variability of CO_2 emission among soil replicates can be explained by micro-habitats and micro-environments found in soil subsamples that leads to micro-scale heterogeneity in composition of the soil microbial community (De Bellis et al., 2007; Carson et al., 2009; Haack et al., 1995) The CO_2 emission rates increased by 2.5-fold, 4.6-fold and 2.8-fold for soil 1, 2 and 3, respectively when the glucose concentration in the solution was increased from 0 mg g^{-1} soil to 10 mg g^{-1} soil and decreased with higher glucose concentrations. Hence, a glucose concentration of 10 mg g^{-1} soil was used to induce SIR for all soils in the subsequent tests.

Anderson and Domsch (1978) found a range of 5 to 50 µM glucose (g soil $solution^{-1}$) soil to be optimal for 12 soils and Ananyeva et al. (2011) reported the range of 2-15 mg g^{-1} to result in a similar optimal respiration response. West and Sparling (1986) found optimal respiration rates at 10 mg glucose g^{-1} soil (60 mg mL^{-1} soil water) for three soils. They also reported a

significant decrease in CO_2 emission after the glucose addition exceeded 10 mg mL^{-1} soil water due to increased water osmotic potential to levels which inhibit respiration in this soil. These findings support the selection of glucose concentration of 10 mg glucose g^{-1} soil for the SIR test.

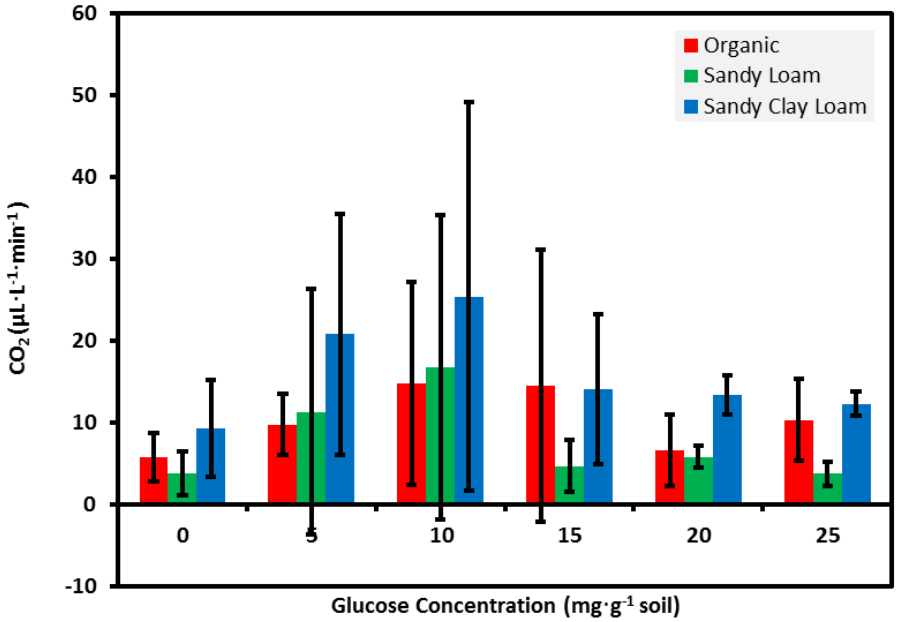

Fig. 4.1. Emission of CO_2 measured with the NDIR-CO_2 sensor from three soils amended with aq. glucose at concentrations from 0 to 25 mg g^{-1} soil. Error bars represent standard deviation of five replicates.

4.2. Preliminary evaluation: untreated and sterile soils

ANOVA was carried out using PROC GLM to determine the effect of four treatments for testing the applicability of the sensor to differentiate between control and sterile soils. The treatment means were compared by Tukey's HSD test at $\alpha = 0.05$. The results showed that there was a significant effect of different treated soils ($p \leq 0.0001$) and soil type ($p \leq 0.01$) on CO_2 emission min^{-1} (Fig. 4.2; Table 4.1). The interaction term between soil and treatment type was significant at $p \leq 0.05$, but non-significant at $p \leq 0.01$. Post hoc comparisons of treatment means showed that CO_2 emission from control soil samples with added aq. glucose (CG) was significantly greater than the sterile soil samples with added aq. glucose (SG) for all three soil types ($p \leq 0.0001$). CO_2 emission from sterile soil samples was found to be negligible in the

closed chamber over a period of 5 minutes, which led to the estimated slope of CO_2 concentration over time being close to 0 µL L^{-1} min^{-1}(±5 µL L^{-1} min^{-1}). The results supported the assumptions that the sensor is able to differentiate between CO_2 emission from soils with variable amounts of microbial activity and the glucose solution incorporation significantly increases the microbial respiration in soils under toxicity stress. The latter proves that the respiration rate of microorganisms increases within a few minutes after adding glucose.

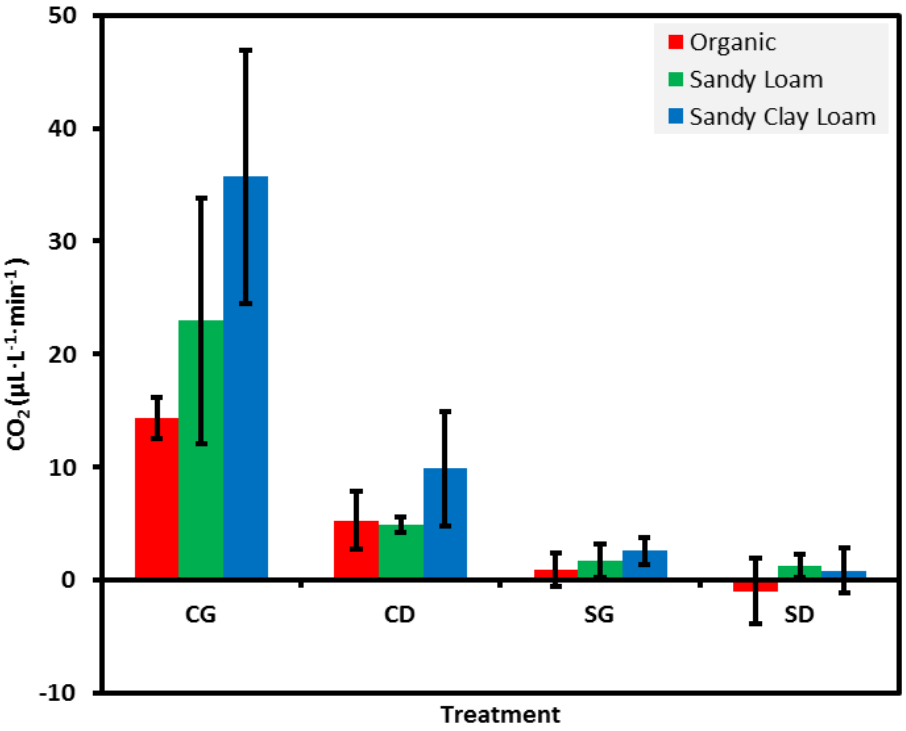

Fig. 4.2. Emission of CO_2 measured with the NDIR-CO_2 sensor from three soils for the treatments: CG: control soil sample added with aq. glucose, CD: control soil sample added with deionized water, SG: sterile soil sample added with aq. glucose and SD: sterile soil sample added with deionized water. Error bars represent standard deviation of three replicates.

0-6 hours following the addition of substrate is usually considered as representative of the initial microbial response before biomass growth (Degens and Harris, 1997). Our purpose is to investigate the *in-situ* applicability of an NDIR CO_2 sensor-based system for evaluation of soil toxicity in terms of diesel amendment. We assumed that the microbes in the toxic soil do not need a long time to metabolize the glucose and their respiration will be triggered quickly. Hence,

the CO_2 emission data between 2-5 min after aq. glucose addition were used for the measurement of CO_2 emission min^{-1} from the soil samples.

Table 4.1. Analysis of variance of the CO_2 concentration from sterile and control soil samples

Source	Degree of Freedom	Sum of Squares	Mean Squares	F value	Pr > F
CO_2 min^{-1} (between group)	11	1.13	0.11	15.16	< 0.0001
Error (within group)	24	0.16	0.01		
Total	35	1.29			

4.3. Soil toxicity evaluation: phenanthrene treated soils experiment

The phenanthrene treatment at different rates showed a significant ($p \leq 0.0001$) impact on soil CO_2 emission (Fig. 4.3.). The post hoc comparison of treatment means using Tukey's HSD test at $\alpha = 0.05$ showed that a significant difference existed only between control and phenanthrene-contaminated soils ($p = 0.05$) irrespective of the level of contamination or soil type. Significantly decreased CO_2 emission from control soil samples treated with acetone was observed.

Acetone is routinely used as an organic solvent to spike soil with PAHs in the laboratory (Brinch et al., 2002), but has been reported to kill soil microorganisms (Klimkowicz-Pawlas and Maliszewska-Kordybach, 2008) and to decrease soil CO_2 emission more than 10 times (Núñez et al., 2009). This explains the decreased CO_2 emission from control soil samples treated with acetone in the present study. Acetone was also found to suppress CO_2 emission from phenanthrene-contaminated soil samples. Thus, avoiding the use of organic solvents for spiking the soils with PHCs was considered crucial for the experiment and was achieved by choosing diesel as a PHC contaminant for the three soil samples.

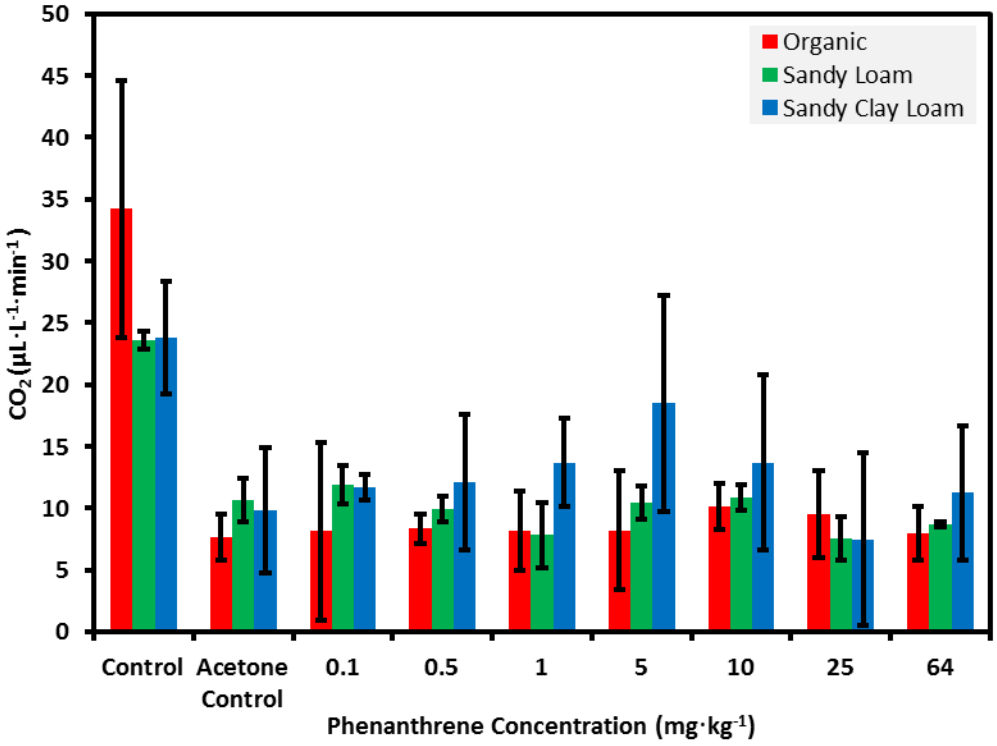

Fig. 4.3. Emission of CO_2 measured with the NDIR-CO_2 sensor from three control or phenanthrene-contaminated soils incubated for 20 days, prior to the glucose addition. Error bars represent standard deviation of three replicates.

4.4. Soil toxicity evaluation: diesel treated soils experiment

The diesel treatment at different rates indicated different CO_2 emission patterns in terms of the level of SOM. There was a significant effect of both degree of diesel contamination ($p \leq 0.0001$) and soil type ($p \leq 0.0001$) on CO_2 emission min^{-1} (Fig. 4.4.; Table 4.2.) after an incubation period of 7 days. The interaction term between soil type and rate of diesel contamination was significant at $p = 0.005$. Post hoc comparison of treatment means, using Tukey's HSD test, showed that it was possible to differentiate between the control and contaminated soil ($p \leq 0.0001$) and that all three soils were distinguishable from one another at $p \leq 0.05$.

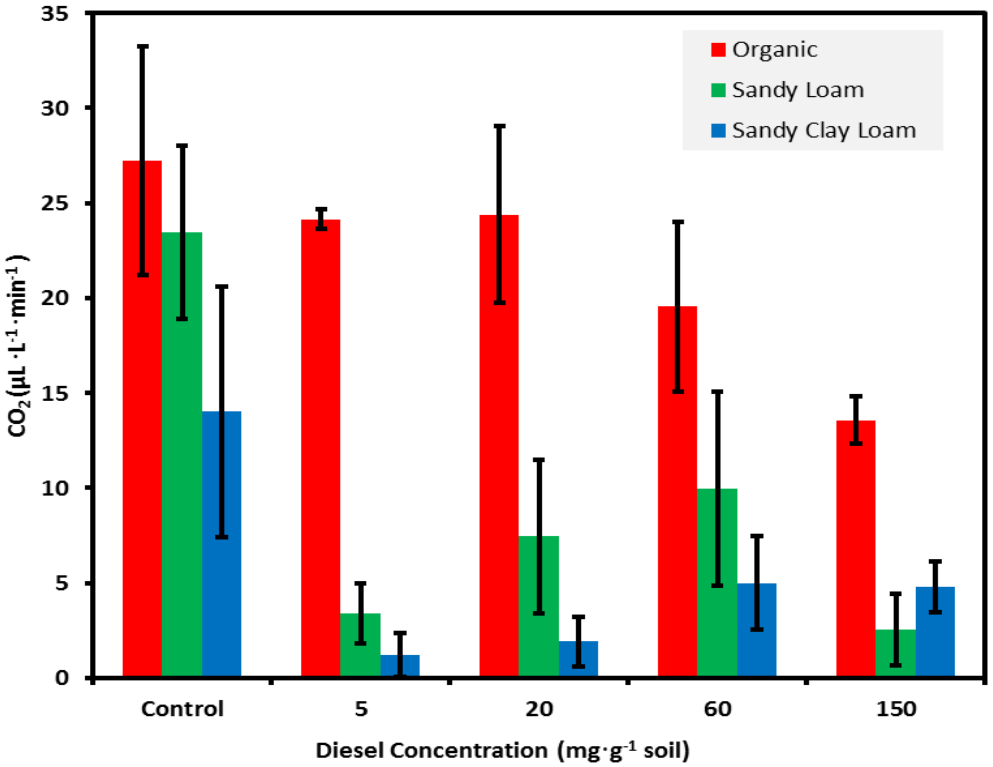

Fig. 4.4. Emission of CO_2 measured with the NDIR-CO_2 sensor from three control or diesel-contaminated soils incubated for 7 days, prior to the glucose addition. Error bars represent standard deviation of three replicates.

Table 4.2. Analysis of variance of the CO_2 concentration from diesel-treated and control soils

Source	Degree of Freedom	Sum of Squares	Mean Squares	F value	Pr > F
CO_2 min^{-1} (between group)	14	1.03	0.07	19.13	< 0.0001
Error (within group)	30	0.11	0.01		
Total	44	1.13			

The presence of high rates of SOM may decrease the accessibility of pollutant to micro-organisms. In such a case, the hydrophobic compounds get partitioned into the organic fraction of soil and their bioavailability is reduced due to their entrapment in the solid phase of the organic matter (Nam et al., 1998) resulting in a decrease in their toxicity towards microbial populations (Liu et al., 2012).

Soil 1, organic soil containing 63.3% SOM, showed the microbial SIR rate similar to that of the control when diesel contamination was used, showing a slight decrease in respiration with increasing diesel concentrations. It confirms the lesser bioavailability of diesel hydrocarbons to soil microorganisms as a consequence of higher SOM. Similar results have also been observed by several other authors (Bauer et al., 1991; Labud et al., 2007). The soils 2 and 3 (mineral soils) contained 7.8 and 7.5% of organic matter, which implied the soil has a major amount of mineral matter, acts as an adsorbent (Bosma et al., 1997; Chiou, 2002). Thus, most of the diesel hydrocarbon was adsorbed on the soil solid; it was bioavailable to micro-organisms and proved detrimental to their survival and activity. Significantly low SIR rates in diesel-contaminated samples of soils 2 and 3 as compared to the control samples indicated the inability of the microorganisms to metabolize added PHC as a substrate during the first week after the addition. An initial lag phase, during which microbial respiration was inhibited, has also been reported in polluted soil samples with low SOM in previous studies (Labud et al., 2007; Siddiqui and Adams, 2002). This lag phase suggests that microorganisms need an initial period of time to adapt to the presence of hydrocarbons in the medium before using them as substrates (Löser et al., 1999). Hence, microbial respiration hit a lag phase immediately after the diesel addition, before its expected increase (Hawrot-Paw, 2012).

The results reported above and the previous literature favour the usage of the SIR and the proposed NDIR CO_2 sensor-based system for toxicity evaluation of hydrocarbon contaminated soils. Bauer et al. (1991) found SIR to be inappropriate for the determination of microbial activity of contaminated and non-contaminated soils, but cited low sensitivity of the method used as one of the probable reasons. Many remediation studies have been reported that correlated SIR to the level of organic toxicity present in soil, in addition to other biological indicators. Shi et al.

(2005) found that there were characteristic differences of glucose-induced microbial respirations in the response of contaminated and non-contaminated soils. Pietravalle and Aspray (2013) found distinct catabolic diversity between hydrocarbon contaminated soils using multiple SIR assays. Degens and Harris (1997) also utilized differences between the SIR responses of microbial communities to simple organic compounds to quantify catabolic diversity of soil microbial communities. However, the integration of the SIR approach with a NDIR CO_2 sensor-based system for soil respiration measurements has not been reported yet.

To put this in perspective, a longitudinal study on a contaminated site can be performed using the sensor system and the correlation of CO_2 emission data collected with physicochemical parameters can be evaluated over time.

5. SUMMARY AND CONCLUSION

Increased use and production of petroleum-based products has led to an increase in the PHC concentrations in soils. The application of eco-toxicological indicators to evaluate soil quality of contaminated sites is preferable to arbitrary chemical criteria for contaminated soil remediation initiatives. But the high cost, sophistication and *in-situ* inapplicability of such biological indicators are major hindrances. This research presented a sensor based approach that indirectly measures potential (induced) biological activity in the soil and hence, could be used to support physiochemical criteria for site assessment and remediation. The performance of a simple CO_2 sensing system integrated with an inexpensive NDIR CO_2 sensor was successfully evaluated for its ability to differentiate between three different diesel-contaminated and non-contaminated soils. The current design can be modified easily for *in-situ* applicability. The sensor's ability to differentiate between glucose-induced CO_2 emission from sterile and control soil samples ($p \leq 0.0001$) and diesel-contaminated and non-contaminated soil samples ($p \leq 0.0001$) supported its applicability to determine soil toxicity, specifically, and soil quality, in general.

As the design and development of sensor integrated CO_2 sensing systems to evaluate both active (BR) and potential (SIR) soil biological activity and hence, soil quality, has not yet been explored, the outlook for such research is promising. It eliminates the barriers for *in-situ* applications of the evaluation of biological activity of environmental sites. Such methods may prove to be promising tools for the initial assessment of the level of contamination and for the determination of highly contaminated areas to become the focus of the final physiochemical evaluation. Moreover, they can be used as an indicator of soil quality of agriculture soils as they determine the potential microbial community present in the soil.

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APPENDICES

A. Block diagram of data acquisition system

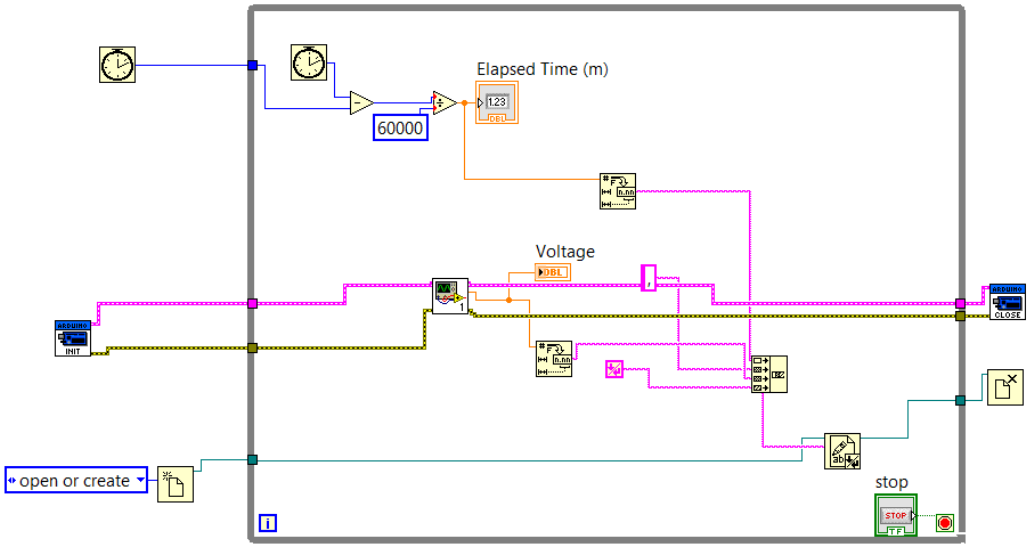

Fig. A-1. Main VI to receive CO_2 sensor analog output from Arduino UNO and log into a text file

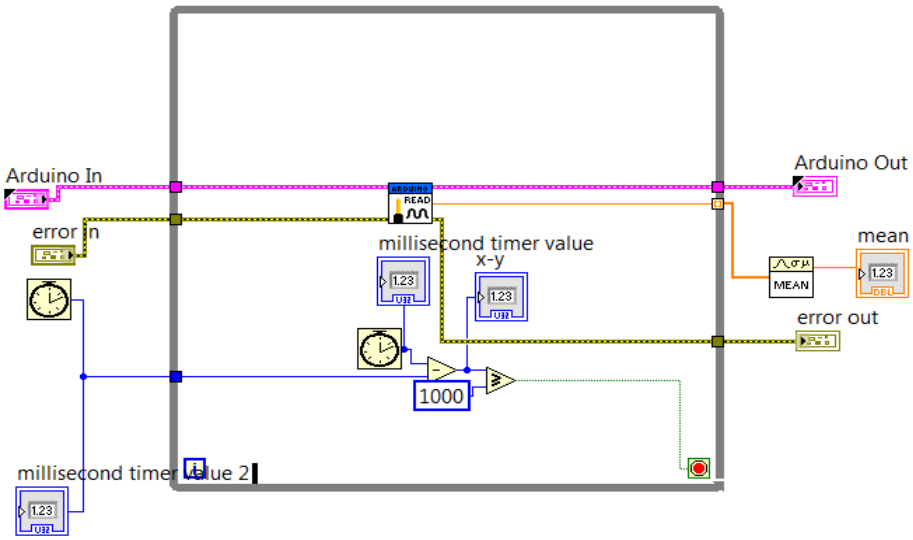

Fig. A-2. Sub-VI to read and average CO_2 sensor analog output collected via Arduino UNO

B. SAS models and codes for different experiments

B-1. Glucose optimization experiment

B-1a. SAS model

$$Y_{ijk} = \mu + a_i + B_j + (aB)_{ij} + \varepsilon_{ijk}$$

Assumptions: 1. μ is the unknown overall mean response.

2. a_i is a fixed treatment effect corresponding to the i^{th} level of factor a with $\sum_{i=1}^{6} a_i = 0$. Treatments are six glucose concentrations (0, 5, 10, 15, 20 and 25 mg of glucose g^{-1}soil).

3. B_j is a random column effect due to j^{th} level of factor B. The Bjs have independent normal distributions, with mean 0 and variance σ_B^2. Column effects are three soil types selected from the soil classification.

4. $(aB)_{ij}$ is a random effect due to the interaction of the i^{th} level of factor a with the j^{th} level of factor B. The $(aB)_{ij}$s have independent normal distributions with mean 0 and variance σ_{aB}^2.

5. The a_is, Bjs and $(aB)_{ij}$s are mutually independent.

B-1b. SAS code

```
DATA CO2;
INPUT SOIL TRT SLOPE;
DATALINES;
1 1 0.16
***
;
PROC GLM;
```

```
CLASS TRT SOIL;
MODEL SLOPE= TRT SOIL;
MEANS TRT/TUKEY CLDIFF;
MEANS SOIL/ TUKEY CLDIFF;
RUN;
```

B-2. 2 by 2 factorial design for preliminary sensor evaluation

B-2a. SAS model

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijk}$$

Assumptions: 1. μ is the unknown overall mean response.

2. A_i is a random effect due to j^{th} level of factor A. The Bjs have independent normal distributions, with mean 0 and variance σ_A^2. Factor A is microbial activity and has two levels: control and sterile.

3. B_j is a random effect due to j^{th} level of factor B. The Bjs have independent normal distributions, with mean 0 and variance σ_B^2. Factor B is glucose addition and has two levels: 10 mg of glucose g^{-1}soil and none.

4. $(AB)_{ij}$ is a random effect due to the interaction of the i^{th} level of factor a with the j^{th} level of factor B. The $(AB)_{ij}$s have independent normal distributions with mean 0 and variance σ_{aB}^2.

5. ε_{ijk} is a random effect due to all the other factors with ε_{ijk}s independently, normally distributed with mean 0 and variance σ_ε^2

5. The A_is, Bjs, $(AB)_{ij}$s are mutually independent.

B-2b. SAS code

```
DATA CO2;
INPUT SOIL TRT SLOPE;
DATALINES;
1 1 -0.04
***
;
PROC GLM;
CLASS TRT SOIL;
MODEL SLOPE= TRT SOIL TRT*SOIL;
MEANS TRT/TUKEY CLDIFF;
MEANS SOIL/ TUKEY CLDIFF;
RUN;
```

B-3. PAH treated soils experiment

B-3a. SAS model

$$Y_{ijk} = \mu + a_i + B_j + (aB)_{ij} + \varepsilon_{ijk}$$

Assumptions: 1. μ is the unknown overall mean response.

2. a_i is a fixed treatment effect corresponding to the i^{th} level of factor a with $\sum_{i=1}^{8} a_i = 0$. Treatments are eight phenanthrene concentrations (0, 0.1, 0.5, 1, 5, 10, 25 and 64 mg of phenanthrene per kg of soil).

3. B_j is a random column effect due to j^{th} level of factor B. The Bjs have independent normal distributions, with mean 0 and varianceσ_B^2. Column effects are three soil types selected from the soil classification.

4. $(aB)_{ij}$ is a random effect due to the interaction of the i^{th} level of factor a with the j^{th} level of factor B. The $(aB)_{ij}$s have independent normal distributions with mean 0 and variance σ^2_{aB}.

5. The a_is, B_js and $(aB)_{ij}$s are mutually independent.

B-3b. SAS code

```
DATA CO2;
INPUT SOIL TRT SLOPE;
DATALINES;
1 1 0.53
***
;
PROC GLM;
CLASS TRT SOIL;
MODEL SLOPE= TRT SOIL;
MEANS TRT/TUKEY CLDIFF;
MEANS SOIL/ TUKEY CLDIFF;
RUN;
```

B-4. Diesel treated soils experiment

B-4a. SAS model

$$Y_{ijk} = \mu + a_i + B_j + (aB)_{ij} + \varepsilon_{ijk}$$

Assumptions: 1. μ is the unknown overall mean response.

2. a_i is a fixed treatment effect corresponding to the i^{th} level of factor a with $\sum_{i=1}^{8} a_i = 0$. Treatments are five diesel concentrations (0, 5, 20, 60 and 150 mg of diesel g^{-1} of soil)

3. B_j is a random column effect due to j^{th} level of factor B. The Bjs have independent normal distributions, with mean 0 and varianceσ_B^2. Column effects are three soil types selected from the soil classification.

4. $(aB)_{ij}$ is a random effect due to the interaction of the i^{th} level of factor a with the j^{th} level of factor B. The $(aB)_{ij}$s have independent normal distributions with mean 0 and variance σ_{aB}^2.

5. The a_is, Bjs and $(aB)_{ij}$s are mutually independent.

B-4b. SAS code

```
DATA CO2;
INPUT SOIL TRT SLOPE;
DATALINES;
1 1 0.36
***
;
PROC GLM;
CLASS TRT SOIL;
MODEL SLOPE= TRT SOIL TRT*SOIL;
MEANS TRT/TUKEY CLDIFF;
MEANS SOIL/ TUKEY CLDIFF;
RUN;
```