TAMPERE UNIVERSITY
OF APPLIED SCIENCES

CARBON DIOXIDE TITRATION METHOD FOR SOIL RESPIRATION MEASUREMENTS

Luis Martín Rubio

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Energy and Environmental Engineering

ABSTRACT

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Carbon Dioxide Titration Method for Soil Respiration Measurements

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This thesis was commissioned by Tampere University of Applied Sciences, which was interested in studying and developing a titration measurement method for soil respiration and biodegradability. Some experiments were carried out measuring soil respiration for testing the method and others adding some biodegradable material like polylactic acid compressed material and 100% biodegradable plastic bags to test its biodegradability and the possibility to measure it via titration.

The thesis was done between January and May 2015 and consists of two parts. In the literature research part, a measuring method for soil respiration via titration was found, as well as an interesting theory of soil respiration that can be useful for the development and conditions of the experiments. The other part of the thesis was the experimental work in the laboratory applying the method found in literature for studying soil respiration and biodegradability. This method consists in creating the samples with Oxitop® oxygen experiment and the titration of them.

The results of the experimental part showed that it is possible to measure soil respiration via titration and the experimental method is established according to the standard SFS EN-ISO 16072. It was found that is possible to detect and measure biodegradability of PLA, soil and commercial biodegradable product applying soil respiration titration method. In addition, it seems that CO_2 titration is a reliable method when compared to Oxitop® soil respiration by pressure measurement.

Key words: Soil respiration, titration, carbon dioxide, biodegradability.

CONTENTS

ABBREVIATIONS AND TERMS

TAMK	Tampere university of applied sciences
SOM	Soil organic matter
ATP	Adenosine triphosphate
NADH	Nicotinamide adenine dinucleotide, oxidized form
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
TCA	Tricarboxylic acid cycle
R_p	Plant respiration
R_m	Microbial respiration
R_a	Aboveground plant respiration
R_b	Belowground plant respiration
R_s	Respiration in soil surface
R_e	Ecosystem respiration
GPP	Gross primary production
NEP	Net ecosystem production
PLA	Polylactic acid

1 INTRODUCTION

The principal aims of this project are to study the carbon dioxide (CO_2) formation in soil respiration and biodegradability of some materials and measure it by titration, which leads to test the measuring method described in SFS EN-ISO 16072 "Laboratory methods for determination of microbial soil respiration". Therefore, a reference measurement system will be developed for Tampere University of Applied Sciences (TAMK) Degree Programme in Energy and Environmental Engineering laboratory, studies in the field of soil respiration and biodegradability.

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The literature search consists in the topic of soil respiration, emphasising in the CO_2 processes in it, like production of the gas, transportation, factors which control and affect soil respiration and the relationship between soil respiration and other topics as carbon balance or climate change. All this can be found in chapter 2.

The experimental part was developed in TAMK laboratory and consists in the preparing of samples using Oxitop® device and the study of the samples via titration. The explanation and principle of the experiments is located in chapter 3, as well as their procedure and a commentary of all the test rounds done in the laboratory.

In chapter 4 the results of all the measurements and experiments are presented, while the discussion and conclusions made from them are in chapter 5.

2 SOIL RESPIRATION

2.1 INTRODUCTION

Soil respiration can be defined easily as processes carried by microorganism for decomposition of organic matter in water, carbon dioxide and energy. This decomposition is outlined in figure 2.1.1, with the example of glucose, showing the two possible ways of breakdown. The first way consists in the transformation of organic matter in water and carbon dioxide, conserving energy in adenosine triphosphate (ATP), corresponding to dissimilatoric metabolism. In the second way, this energy is consumed again for transforming the organic compounds in acetoacetamide ($C_4H_7O_2N$) (catabolic metabolism). These two processes are connected by nicotinamide adenine dinucleotide (NADP), which provides the needed electrons (Platen and Wirtz, 1999a, 3)

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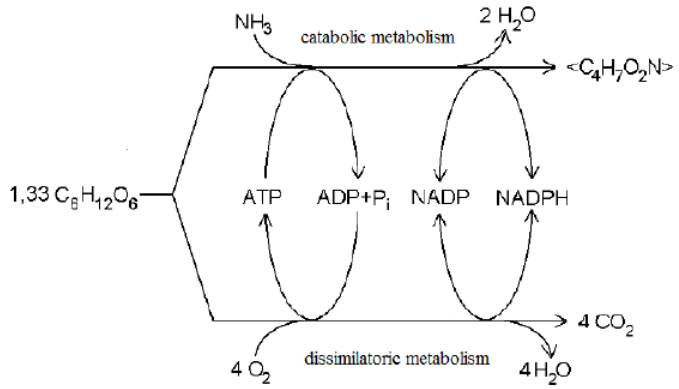

FIGURE 2.1.1 Aerobic decomposition of an organic substance (glucose) (Platen and Wirtz, 1999a, p. 3)

Soil respiration can be also defined as the production of carbon dioxide by organisms and the plant parts in soil. Soil can be considered as a mixture of organic matter, air, water, rock or even alive organisms can be included, so it makes sense to say that soil can breathe. Therefore, soil respiration means that the living biomass of soil respires CO_2, while soil organism gain energy from catabolizing organic matter to support life (Lou & Zhou 2006, 5).

2.2 PRODUCTION OF CO_2 IN SOIL

CO_2 is produced through several processes, root respiration being one of the most important. Other sources of carbon dioxide are the rhizosphere respiration and the decomposition of dead plant material, called litter. Adding the oxidation of organic matter to these three sources, soil respiration is completed (Lou & Zhou 2006, 36).

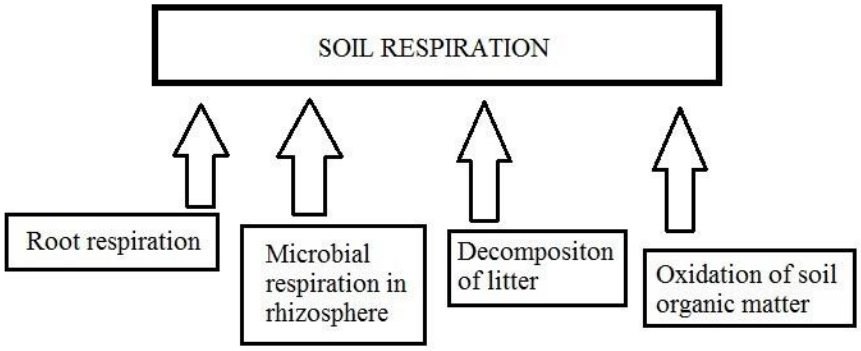

FIGURE 2.2.1 Sources of soil respiration (based on Luo & Zhuo, 2006)

2.2.1 Biochemistry of CO_2 production processes

At the biochemical level, the CO_2 production takes place by some different processes. The most common process is the tricarboxylic acid cycle (TCA), also known as Krebs cycle, but there are other ways as the fermentation of glucose or methanogenesis. Krebs cycle and methanogenesis occur in aerobic condition, while the fermentation happens without oxygen presence (Lou & Zhou 2006, 35-36).

Aerobic process

The general chemical reaction for the oxidation of glucose or other carbohydrates (or aerobic respiration) is:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$ *Equation 1*

This process is composed by the glycolysis, the pentose phosphate pathway, TCA cycle and the electron transport for oxidative phosphorylation. The pentose phosphate pathway produces nicotinamide adenine dinucleotide phosphate (NADPH) and other intermediates, while glycolysis converts glucose into malate in the case of plants and pyruvate in the case of animals, which enters in the TCA cycle to produce CO_2 and ATP.

In the glycolysis process, each molecule of glucose produces two molecules of pyruvate or malate. If pyruvate is the product, two molecules of ATP are produced but ATP is not produced if malate is formed (besides, CO_2 is needed for the formation of malate). These molecules of pyruvate or malate are oxidized in the TCA cycle, where three molecules of CO_2, four molecules of nicotinamide adenine dinucleotide (NADH), one molecule of flavin adenine dinucleotide ($FADH_2$), and one molecule of ATP are obtained per molecule of pyruvate; and an additional molecule of CO_2 and NADH in case of malate. Therefore, the oxidation of a molecule of glucose produces the same amount of CO_2 in both cases, because of the needs of CO_2 in the formation of malate (Lou & Zhou 2006, 38).

Anaerobic process

When the concentration of O_2 is not enough for the oxidation, anaerobic respiration takes place. This respiration occurs during fermentation, which can have many different ways, so in some of them CO_2 is not produced. The ways that produce CO_2 are shown in table 2.2.1. However, most of the studies do not consider anaerobic respiration, since its importance is low comparing with aerobic respiration (Lou & Zhou 2006, 39-40).

TABLE 2.2.1. Biochemical processes in roots and microorganisms that result in CO_2 production (Based on Luo & Zhuo, 2006. Modified)

Reductant	Oxidant	Products	Microorganisms
Sugars	O_2	CO_2, H_2O	Roots, protozoa, fungi, many bacteria
Sugars and related compunds	Organic compounds	Lactic acid, ethyl alcohol, CO_2	Lactic acid and bacteria
Sugars	Organic compounds	Ethyl alcohol, CO_2	Yeasts
Sugars	Organic compounds	Acetic, succinic, lactic acids and formic acids, ethyl alcohol, CO_2	Escherichia
Sugars	Organic compounds	Butanediol, lactic and formic acids, ethyl alcohol, CO_2	Enterobacter
Sugars, organic acids	Organic compounds	Propionic, succinic and acetic acids, CO_2	Propionibacterium, veillonella
Sugars, starch, pectin	Organic compounds	Butyric and acetic acids, CO_2, H_2	Clostridium
Aminoacids	Organic compounds	Acetic acids, NH_3, CO_2	Clostridium

2.2.2 Root respiration

When the respiration process takes place in the roots, it is called root respiration and it corresponds about half of the total soil respiration, consuming between 10 and 50% of the total carbon assimilated in photosynthesis. Therefore, soil respiration (and so, produced flux of CO_2) is linked to the root density, so that the more root density, the more CO_2 is released (Lou & Zhou 2006, 42).

This production depends on the plant species, locations, ecosystems and seasons. It also varies with the age of the plant, the amount of nutrients, the availability of water and levels of light (Lou & Zhou 2006, 43).

2.2.3 Rhizosphere respiration

The rhizosphere is the narrow region next to the root surface that is directly influenced by root secretions and associated with soil microorganisms. Its compounds vary from simple oligosaccharides to complex polymers and it is a very favourable habitat for mi-

croorganisms. The space between the roots and soil is covered of a substance called mucigel, which is responsible for allowing plants to continue the uptake water and nutrients (Lou & Zhou 2006, 46).

There are different groups of delivered compounds: exudates (sugars, amino acids, vitamin) which are released without metabolic energy; secretions (carbohydrates and enzymes) that are products of metabolic activity; and lysates, released when cells autolyse. The majority released compounds are water-soluble, like carbohydrates, amino acids and organic acids, and they are quickly decomposed by bacteria, which are able to grow really fast in rhizosphere zones, due to the small size but big surface of this layer. The amount of carbon lost as exudates and secretions varies a lot with the specie and site, but between 10 and 70% of the total assimilated carbon is transferred to the roots (Lou & Zhou 2006, 47).

2.2.4 Litter decomposition and soil organisms

Litter corresponds to all the plant biomass that is delivered to the soil as dead organic matter. Its decomposition carries a big amount of CO_2 production in the soil (Wikipedia 2017).

Litter decomposition is measured as the mass remaining of initial litter after a period of incubation. According to Lou and Zhou 2006, the decomposition is fast during some time from the beginning of the incubation, but after that, the remaining mass does not decrease so fast (figure 2.2.3).

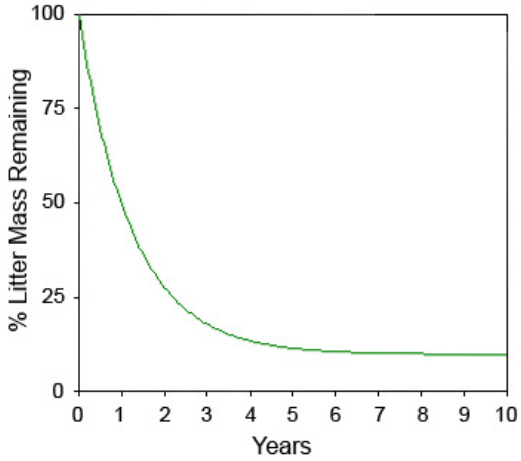

FIGURE 2.2.2. Theoretical litter decomposition (based on Lou & Zhou 2006)

This behaviour is because decomposition can be organized in three phases: leaching, fragmentation and chemical alteration of dead matter. In leaching, rapidly growing bacteria can take really fast soluble materials as amino acids, organic acids and sugars for metabolic activities. During fragmentation, soil animals transform big pieces of litter in smaller ones. Chemical alteration consists in the degradation by bacteria and fungi of organic compounds to obtain energy, as some exoenzymes are released. These exoenzymes convert macromolecules in soluble products, that are processed by microbes and CO_2 is released as a product (Lou & Zhou 2006, 51).

Litter decomposition depends on factors as climatic conditions (temperature and precipitation), litter quality (N content, lignin content) and vegetation type.

The exchange of litter mass can be described as:

$$X = X_0 e^{-kt} \qquad \textit{Equation 2}$$

Where X is the mass of litter, X_0 is the initial mass and k is the specific decomposition rate (litter mass lost per unit of time and per unit of litter mass). Therefore, the equation

shows the same as the graphics, that the mass remaining decreases exponentially with time. (Lou & Zhou 2006, 54).

2.2.5 Oxidation of soil organic matter

Soil organic matter is the organic fraction of the soil and has functions like supplying nutrients to the plant, maintaining the fertility of the soil and improving its structure.

Organic matter oxidation is controlled by many factors as the type of soil, texture, water availability, ion exchange capacity, temperature or amount of oxygen available. An increase of the degradation is induced by an increase of the temperature, an increase of the aeration, an appropriate nitrogen supply or some disturbances as agriculture activity. Other factors as deforestation or biomass burning also reduces the carbon amount in soil (Lou & Zhou 2006, 55).

Wadman and Haan found with their experiments that the decomposition of organic matter decreases with time and every type of soil studied follow the same pattern:

$$Y(t) = b + cr^t \qquad \textit{Equation 3}$$

Where Y is the organic matter content, b is the non-decomposable mass content, c is the decomposable mass content and r is the relative decomposition rate (Lou & Zhou 2006, 59).

2.3 CO_2 TRANSPORTATION FROM SOIL TO THE ATMOSPHERE

Once the carbon dioxide is produced in soil, it is transported to the atmosphere. First of all, CO_2 is transported through soil layers before arriving to the soil surface and there, is released to the air by diffusion and air turbulence. Then, is mixed in the plant canopy, where a part is absorbed by photosynthesis and the majority is released to the atmosphere though the boundary layer (Lou & Zhou 2006, 61).

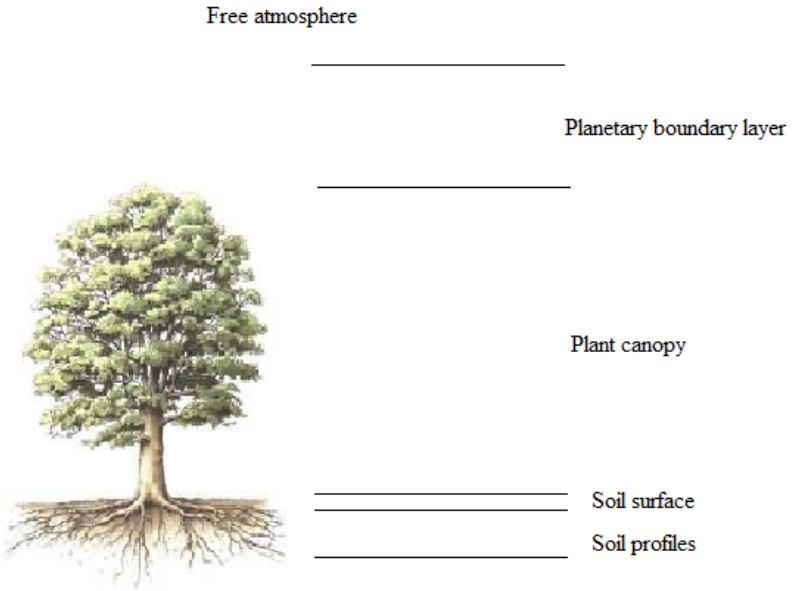

FIGURE 2.3.1. Diagram of the four segments of CO_2 transportation in soil (based on Luo & Zhuo, 2006, p. 62)

These processes of transport are important because they are the mechanisms for the measurement of soil respiration.

2.3.1 CO_2 transport within the soil

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Carbon dioxide has different concentrations in the different layers of soil. In the deep soil, concentration is much higher than in soil surface, up to 100 times. Therefore, there´s an important concentration gradient, so the way of transportation to the soil surface is the diffusion (Lou & Zhou 2006, 62).

Nevertheless, CO_2 is produced by roots and organisms more in the surface than in the deep layers, so the concentration gradient should be the opposite. This happens because of the slow movement of CO_2 from sources of production, and leads into a build-up of

CO_2 gradients; and because of the molecular weight of the carbon dioxide, which is higher than the air´s, so CO_2 tends to go to deeper layers (Lou & Zhou 2006, 62-63).

The CO_2 concentration profile and gradients depend on various factors like soil porosity, in the way that the smaller porosity, the higher CO_2 gradient; or precipitation and water infiltration, whose presence result in a degassing, where CO_2 is forced out of the soil, so its concentration decreases. Other factor is the CO_2 production, if it is high, a higher concentration gradient is needed to diffuse (Lou & Zhou 2006, 62-63).

As in all diffusion process, the movement occurs from the zone with more concentration to the low concentration area, so in this case CO_2 diffusion goes from the deeper layers of soil to the surface.

2.3.2 CO_2 release to the surface

While the movement of CO_2 in the soil layers was controlled by diffusion, CO_2 releases at the soil surface are controlled by wind speed and turbulence (Lou & Zhou 2006, 67).

The gas exchange in soil is influenced by barometric pressure fluctuations and pressure fluctuations caused by wind or air turbulence. Kimball estimated that barometric pressure fluctuations can cause up to a 60% variation in the diffusion rate of gases in soil (1983) and pressure fluctuations caused by wind can increase the gas exchange by at least 25% (Lou & Zhou 2006, 67).

Changes in soil surface of temperature and wind velocity can regulate the CO_2 efflux. At night, cooler temperatures decrease the production of CO_2 and reduce turbulence; and during daytime, due to the surface heating, a raise of respiratory activity is caused and turbulence and CO_2 production increase (Lou & Zhou 2006, 68).

As well, litter layers are a factor for the CO_2 efflux, being its existence a resistance for the CO_2 diffusion from soil to atmosphere (Lou & Zhou 2006, 70).

2.3.3 CO_2 transfer in plant canopy

CO_2 released from the soil surface is mixed within canopy and here, most of it is mixed with respiratory CO_2 to be transported upwards, but a part can be absorbed by photosynthesis during daytime. The transfer of CO_2 in the canopy depends on wind speed and CO_2 concentrations (Lou & Zhou 2006, 70).

At night, wind speed is low and no photosynthesis occurs, so the concentration is the highest in the soil surface and decreases along the profile in the ideal case (one-dimensional gradient-diffusion model). This model is not applicable in daytime, due to the high probability of strong turbulence, which controls the process and may cause counter gradient fluxes for heat, water vapor and CO_2, so the profile is not that simple (Lou & Zhou 2006, 70).

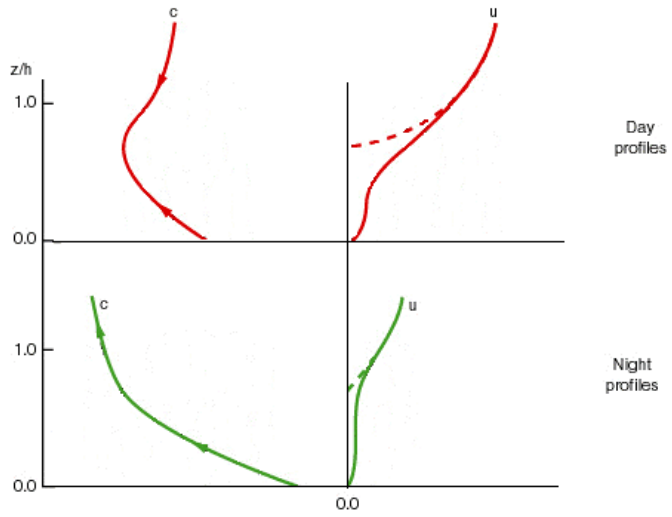

FIGURE 2.3.2 Idealized profiles of CO_2 concentration (c) and wind speed (u) in the height h plotted as a function of z/h (Based on Luo & Zhuo, 2006)

2.3.4 CO_2 transfer in the planetary boundary layer

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The planet boundary layer is that one above soil or vegetation whose size could be from 100 to 3000 meters and where turbulence has a dominant role in transfer of CO_2. This layer varies with time, location and weather conditions and the turbulence depends on factors as vegetation roughness, solar heating or evapotranspiration (Lou & Zhou 2006, 74).

CO_2 transport is fast because turbulence is much more effective at transporting gases than diffusion. This transportation is influenced by photosynthesis and thermal convection, so that in the growing session, the photosynthetic uptake of CO_2 is related with strong thermal convection, so the rapid transport and the plant uptake result in uniform concentrations of CO_2 in the boundary layer. Nevertheless, in winter, thermal convection is weak, so ecosystem respiration is the main component of CO_2 fluxes and the transport is slower with big gradient of carbon dioxide (Lou & Zhou 2006, 74).

2.4 CONTROLLING FACTORS

This chapter has the objective of explaining those factors which soil respiration is influenced in. They are factors as substrate supply, temperature, moisture, oxygen content, nitrogen content, and others like the texture or pH of the soil.

These factors do not affect soil respiration separately, but they have interactions and soil respiration usually responds to the most limiting factor. For example, it is not sensitive to moisture at low temperatures (below 5ºC), but it is at high temperatures (10 to 20ºC); and vice versa, soil respiration is not sensitive to temperature at low water content (below 7,5%) but it responds with moistures from 10 to 25%. When the concentrations are not extreme, both factors take place (Lou & Zhou 2006, 104; Guntinas, Gil-Sotres, Leiros, and Trasar-Cepeda 2013).

In this chapter are explained the single effects for each controlling factor on soil respiration.

2.4.1 Substrate supply

Respiratory activity results in a CO_2 release from the breakdown of carbon-based substrates, so the amount of carbon dioxide produced is directly proportional to the substrate availability. Nevertheless, there are multiple types of substrates, and it is not the same situation for all of them. For example, sugars can be processed in very short residence times, but others like cellulose, lignins and phenols need more time, or humic acids can need hundreds of years (Lou & Zhou 2006, 79-80).

Therefore, the more substrate content, the more CO_2 is produced, but the rate at which they are converted to CO_2 vary with the type of substrate, so the heterogeneity and the multiple sources of supply make very difficult to obtain simple relationships between substrate supply and CO_2 production (Lou & Zhou 2006, 80).

2.4.2 Temperature

Temperature is a very important controlling factor in soil respiration. It is well known that respiration increases exponentially with temperature until its maximum around 45ºC and then declines again with above temperatures. This is because respiration involves numerous enzymes, whose activity is limited in low temperatures but they degrade if more than the limit temperature is reached (figure 2.4.1).

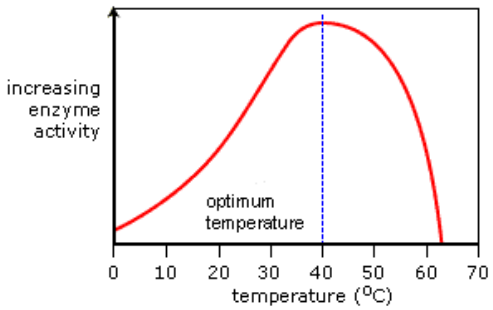

FIGURE 2.4.1. Relationship between enzyme activity and temperature (http://www.bbc.co.uk/schools/gcsebitesize/science/add_aqa/proteins/proteinsrev3.shtml)

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The relationship between temperature and respiration processes is described by the Arrhenius equation:

$$R = Ae^{\frac{-E}{RT}}$$ *Equation 4*

Where R is the respiration activity, A is the Arrhenius constant, E is the activation energy of the reaction and T is temperature. This equation shows the exponential increasing with temperature increasing (Lou & Zhou 2006, 86).

Root respiration has a similar behaviour of increasing exponentially with the temperature, but at temperatures above 35ºC the transport of substrates by diffusion start to decrease. Temperature also has influence in root respiration via its effects on root growth, since there is an optimum temperature for root growth. Besides, temperature response is not the same for young roots than for old ones, being the young roots more sensitive to the temperature than old roots (Lou & Zhou 2006, 86-87).

In the case of soil microorganisms, they also have an exponential response to temperature, reaching a maximum of microbial activity around 20-23ºC and then decreasing because of the microbial death (figure 2.4.2) (Lou & Zhou 2006, 88).

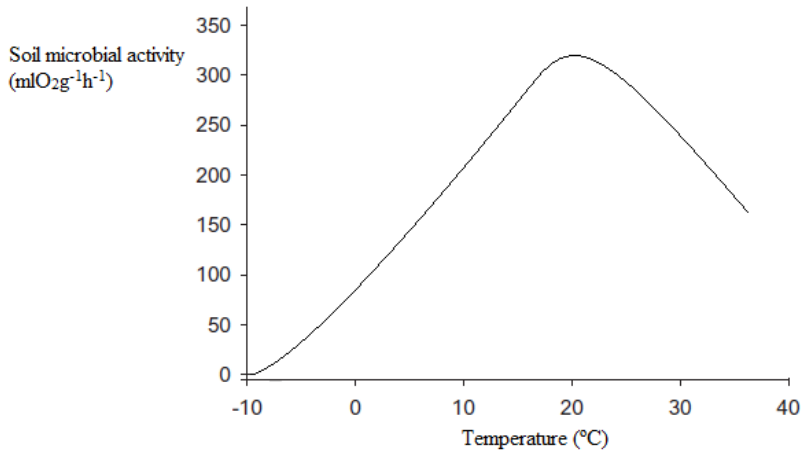


FIGURE 2.4.2 Relationship between microbial activity and temperature (Based on Flanagan and Veum 1974).

In the ecosystem scale, temperature influences the seasonality (periodic and predictable variation in a period of time of a calendar year or less) of substrate supply to the below-ground system, and partially determines soil respiration, due to its effects in shoot and root growth. Changes of few degrees lead to big changes in photosynthesis and soil respiration. Other factors that have strong seasonality are root biomass, rhizosphere activity and litter carbon input (Lou & Zhou 2006, 89).

2.4.3 Soil moisture

Soil moisture is also an important factor in soil respiration. In dry conditions, soil respiration (CO_2 efflux) is low, then reaches the maximum activity in intermediate water content and decreases again with high moisture levels, due to the lack of oxygen. This relationship is shown in figure 2.4.3 (Lou & Zhou 2006, 92-93).

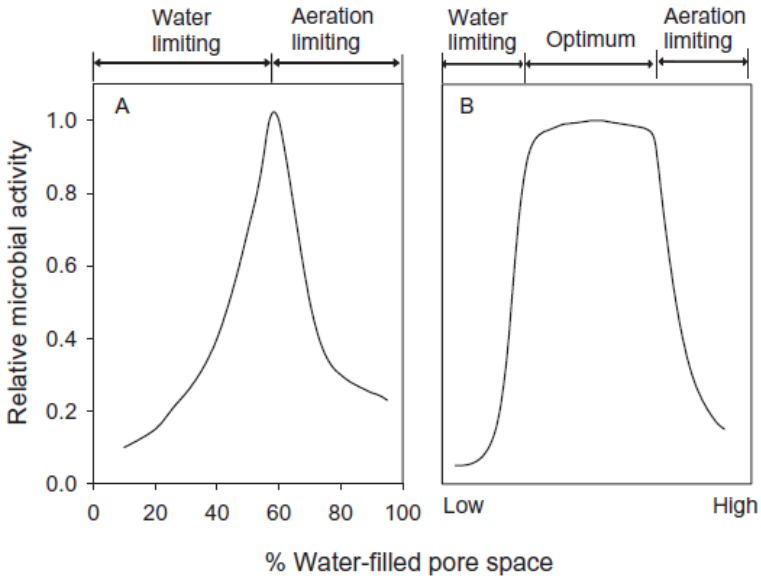


FIGURE 2.4.3 Idealized relationships between relative microbial activity and water-filled pore space (https://microbewiki.kenyon.edu/index.php/The_Effects_of_Global_Climate_Change_on_Soil_Respiration)

Figure 2.4.3 A shows the results in laboratory studies, with say that the maximum activity corresponds to a single value of soil moisture. Nevertheless, field observations show that soil respiration is only limited in very low or very high levels of water content, taking place many points of maximum activity (figure 2.4.3 B).

The optimum rate occurs when the macro pore spaces are air-filled, which makes the O_2 diffusion easier; and de micro pore spaces are water-filled, which facilities diffusion of soluble substrates (Lou & Zhou 2006, 92-93).

Soil moisture influences respiration by its relationship with physiological processes of roots and microorganisms and with diffusion of substrates and O_2. Extreme dry conditions induce microorganisms to a dormancy state, so a basic metabolism remains, reducing substantially the respiration rate. In less extreme dry conditions, soil moisture controls respiration trough substrates and O_2 diffusion. The physical distribution of water in soil can difficult the movement of microorganisms and the diffusion of nutrients and exudates of respiratory activity and in addition, with high water content, CO_2 and O_2 diffusion descends a lot, because the diffusion coefficient of gases in water is much lower than in air. Therefore, low water content is as few desirable as very high soil moistures (Lou & Zhou 2006, 93).

In nature, soil moisture changes a lot because of rainfall, so CO_2 fluxes also vary with time. Before rainfall, soil can be very dry and the CO_2 release is very little. Just after precipitation, soil moisture increases, which activates microbe activities and soil respiration is also higher, but its level depends on the precipitation rate, so if rainfall too strong, CO_2 release is no as high as with less precipitation rate. A period after rainfall, soil loses water via evaporation and the fluxes of CO_2 decline again (Lou & Zhou 2006, 94).

Besides, when rainfall is taking place, water fills soil pores that are full of air with high concentration of CO_2 and degassing occur. Degassing is not properly soil respiration, buy helps to release CO_2 from past activity (Lou & Zhou 2006, 96).

2.4.4 Soil oxygen

As explained before, when soil water content is higher than the optimum, soil respiration is paralyzed because of the lack of oxygen (the more water content, the less oxygen concentration). Therefore, oxygen becomes a limiting factor of soil respiration in wetlands, flooding areas and rain forests.

The concentration of O_2 affects root and microbial respiration, so that, as oxygen concentration decreases, root and microbial respiration decrease too and less CO_2 is released (Lou & Zhou 2006, 98).

2.4.5 Soil nitrogen

Respiration is affected by nitrogen by several ways. For example, respiration generates energy for root nitrogen uptake and assimilation, CO_2 is needed for the uptake of NO_3^- in roots and also for reducing it to NH_3 (before being assimilated into amino acids). Also, the fixation from N_2 to NH_3 needs CO_2 supply. If nitrogen content is high, protein content will also be high, and respiration will be needed for protein repair and maintenance. As well, high nitrogen content is related with high growth rate, and that will result in a high root respiration rate. Nitrogen can affect to litter decomposition, in the way that if nitrogen content is high, litter decomposition is improved, so more CO_2 from microbial respiration will be released. Degradation of cellulose is also controlled by nitrogen content and usually increases with it (Lou & Zhou 2006, 100).

Nevertheless, condensation of nitrogen compounds affects negatively the soil organic matter and the microbial respiration decreases. As well, NH_4^+ salts can inhibit microbial activity (Lou & Zhou 2006, 100).

2.4.6 Soil texture

Soil has different textures depending on the amount of sand, silt and clay that it contains. Soil texture is related with porosity, so it has influence in water movement, gas diffusion

and fertility. For example, in warm and dry periods soil respiration in sandy sites is suppressed, while in clayish soils the moisture is released slowly and its effects on soil respiration are reduced (Lou & Zhou 2006, 101).

Soil texture also influences root growth and, therefore, root respiration. If sandy content in soil is elevated, roots grow slower than in less sandy sites, so root respiration and microbial respiration in rhizosphere will be lower. As well, litter decomposition is influenced by soil texture, being faster in soils with clay than in sandy soils (Lou & Zhou 2006, 101-102).

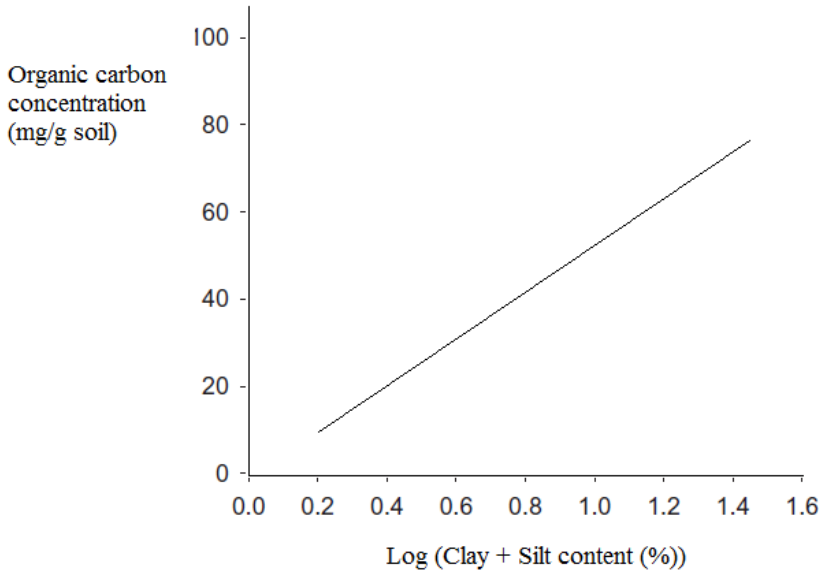


FIGURE 2.4.4 Relationship between organic carbon concentration and clay and silt content (based on Lou & Zhou 2006)

Water infiltration and gas diffusion and therefore CO_2 production vary a lot with soil texture. CO_2 production is 20 to 40% less in clayish soils than in silty ones, but around 50% greater than sandy soils. However, the proportion of total carbon respired is higher if the concentration in clay and silt increase (Lou & Zhou 2006, 102).

2.4.7 Soil pH

Many chemical reactions are regulated by pH, and many enzymes in soil depend on it. Bacteria grow with pH interval between 4 and 9, and fungi from 4 to 6, so pH is important for the growth and proliferation of soil microbes, and therefore soil respiration. Soils with pH 3 produce much less CO_2 than soils with pH 4 due to the adverse effect of low pH on soil microbial activity. Production of CO_2 usually decreases with an increase of pH if pH is above 7; and CO_2 production increases with pH if it is lower than 7 (Lou & Zhou 2006, 102-104).

2.5 IMPORTANCE AND ROLES OF SOIL RESPIRATION

The aim of this chapter is to explain the importance and roles soil respiration has in topics which is related with, as the ecosystem carbon balance, nutrient processes, carbon cycling, carbon storage or a topic that is always present talking about nature, climate change.

2.5.1 Soil respiration and ecosystem carbon balance

The carbon cycle in an ecosystem starts with photosynthesis, where plants take CO_2 from the air and convert it to organic compounds. Some of these compounds are used to grow leaves, stems and roots; and some are broken down to supply energy to the plant (Lou & Zhou 2006, 17-18).

During this process, CO_2 is released again to the atmosphere by plant respiration. Dead plant materials are decomposed by microorganisms to provide energy and, at the same time, CO_2 is released back to the atmosphere by microbial respiration. This microbial respiration also takes place during the decomposition of soil organic matter (SOM), which is a mixture of live microbial biomass and organic residuals of dead plants and dead microbes (Lou & Zhou 2006, 17-18).

Therefore, through the ecosystem cycle, CO_2 is produced by plant respiration (R_p), also called autotrophic respiration and microbial respiration (R_m), also called heterotrophic

respiration. Autotrophic respiration can be separated in aboveground plant respiration (R_a) and belowground (root) plant respiration (R_b).

$$R_p = R_a + R_b \qquad \textit{Equation 5}$$

In the steady state, the CO_2 flux in the soil surface (R_s) is the sum of root respiration and microbial respiration:

$$R_s = R_b + R_m \qquad \textit{Equation 6}$$

And the total CO_2 emission in the ecosystem (Re) is:

$$R_e = R_p + R_m = R_a + R_b + R_m = R_a + R_s \qquad \textit{Equation 7}$$

Approximately, R_s accounts for the 70% of the total respiration and the aboveground respiration represents the rest of the total (Lou & Zhou 2006, 18-19).

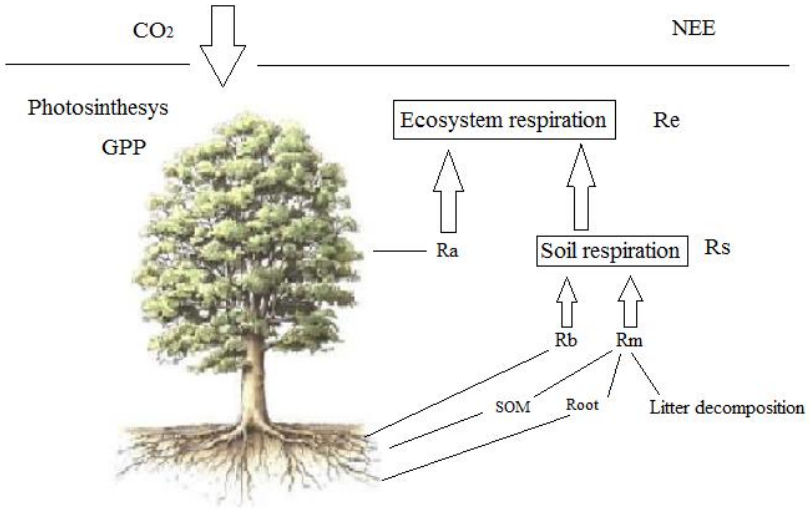


FIGURE 2.5.1 Diagram of ecosystem carbon processes. (Based on Luo & Zhuo, 2006, p. 18)

Soil respiration is also related to ecosystem production, which is the synthesis of organic compounds from atmospheric or aqueous carbon dioxide. Gross primary production (GPP) is the annual carbon assimilation by photosynthesis ignoring photorespiration. Net ecosystem production (NEP) is GPP minus ecosystem respiration R_e (Lovett, Cole, & Pace 2006):

$$NEP = GPP - R_e = GPP - R_a - R_s \qquad \textit{Equation 8}$$

This relationship between production and respiration can be also done through net primary production (NPP) as:

$$NEP = NPP - R_m = NPP + R_b - R_s \qquad \textit{Equation 9}$$

Net primary production is the rate at which all the plants in an ecosystem produce net useful chemical energy; it is equal to the difference between the rate at which the plants in an ecosystem produce useful chemical energy (GPP) and the rate at which they use some of that energy during respiration (Wikipedia 2017).

Respiration can be related as well to aboveground litterfall. Litterfall is the dead plant material that has fallen to the ground. Raich and Naderhoffer (1989) generalized the relationship as:

$$R_s = aL_a + b \qquad \textit{Equation 10}$$

Where a and b are parameters and L_a is the aboveground litterfall. L_a and R_s are in $gCm^{-2}yr^{-1}$. a is usually about 3, which indicates that the carbon released is nearly three times the carbon input from aboveground litter (Lou & Zhou 2006, 19-20).

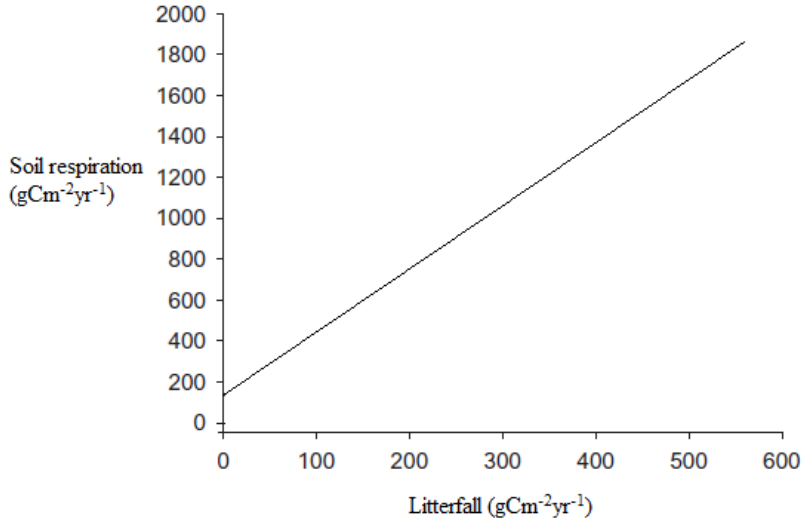


FIGURE 2.5.2 Relationship between soil respiration and the amount of aboveground litterfall (Based on Luo & Zhuo, 2006, p. 20)

2.5.2 Soil respiration and nutrient cycling

An important part of soil respiration is the decomposition of litter and soil organic matter, in which CO_2 is released meanwhile nutrients are immobilized or mineralized. At the beginning of the decomposition, the mineralized nitrogen from litter is simultaneously immobilized by microbes for their own growth, leading to an increased nitrogen concentration in the mixture of litter substrate and microbes. This carbon release and nitrogen immobilization causes an increase of carbon-nitrogen ratio (C:N) until mineralized nitrogen is greater than needed for microbial growth. When this happens, litter decomposition leads to a net release of nitrogen (Lou & Zhou 2006, 21-22).

Because of the carbon and nitrogen release, the rate of mineralized nitrogen (*Nmin)* is usually correlated with respiration (*Rm).* For example, Zak et al. (1993) found this relationship: $Rm = 15.9\ Nmin + 27.4$ with $r = 0.853$ for litter and $Rm = 7.1\ Nmin + 159.9$ with $r = 0.616$ for soil organic matter from laboratory incubation (Lou & Zhou 2006, 22).

2.5.3 Soil respiration and carbon cycling

The carbon cycling consists in the exchanges of CO_2 between the atmosphere, biosphere, oceans and the earth´s crust. Soil respiration is an important factor of this cycle (figure 2.5.3) (Lou & Zhou 2006, 22-23).

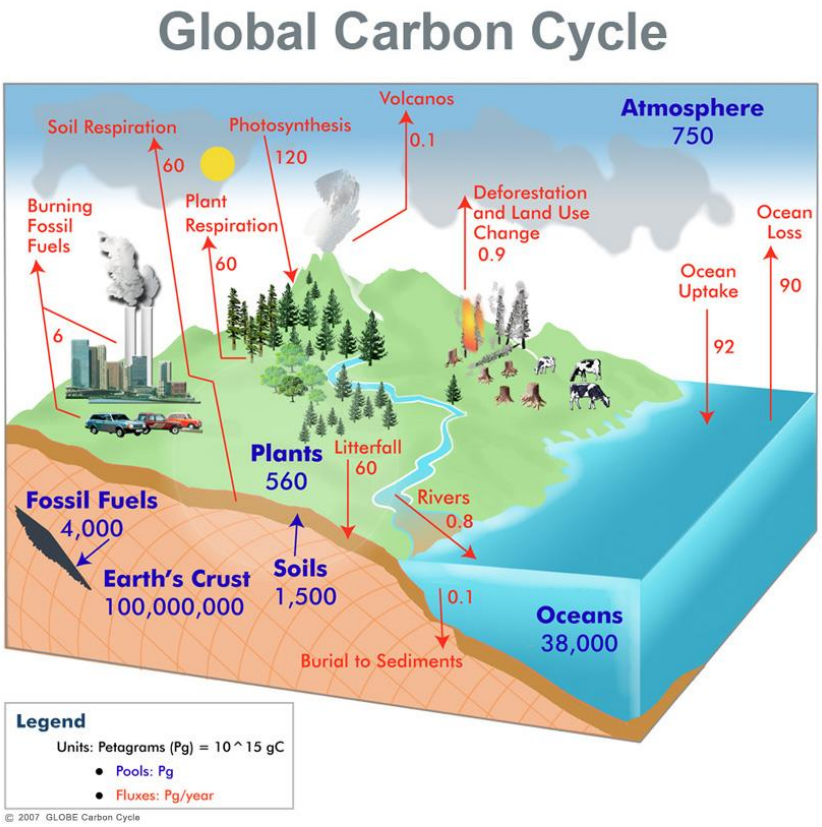


FIGURE 2.5.3 Global carbon cycle. Pools in Pg (= 10^{15} g) C and fluxes in Pg C yr^{-1} as indicated by arrows (Wikipedia)

Photosynthesis takes about 120 PgC per year from the atmosphere and a similar amount is released back due to respiration. Oceans and atmosphere exchange like 90 PgC per year. Through the human activity, approximately 7.2 PgC per year are released to the atmosphere. This amount seems to be very small compared with the natural fluxes, but only a small change it is necessary to perturb the balance (Lou & Zhou 2006, 123-24).

The soil pool from which soil respiration releases carbon (3150 PgC) is much bigger than the atmospheric pool (750 PgC), so a little change in soil respiration can seriously alter the CO_2 concentration balance. Therefore, soil respiration has to be well studied for predicting changes in the carbon cycle (Lou & Zhou 2006, 23-24).

Soil respiration is very sensitive to environmental changes, and the human activity causes an increase of the CO_2 concentration in the atmosphere, which induces at the same time to an increase of the global temperature. As seen in chapter 2.4.2, temperature affect a lot to soil respiration, so the global balance will be disturbed (Lou & Zhou 2006, 24).

2.5.4 Soil respiration and climate change

Soil respiration is very associated with climate change because the CO_2 released from respiration is one of the greenhouse gases. A greenhouse gas is the one which can absorb and emit radiation in the infrared range, so they permit incoming solar radiation but restrict the outgoing one. Therefore, they trap heat within the atmosphere, resulting in a climate warming near the earth´s surface (Lou & Zhou 2006, 26; Wikipedia 2017).

The increase of the concentration of greenhouse gases induces to a higher altitude from which the earth´s radiation is emitted, so less energy is released and the temperature increases (Lou & Zhou 2006, 26).

Therefore, an increase of the CO_2 concentration causes global warming, which at the same time stimulates respiration, and more CO_2 will be released. Thus, the climate system and the global carbon cycle form a positive feedback loop to reinforce each other (Friedlingstein *et al.* 2003).

This loop was studied by Cox *et al.* (2000) with three simulations, one of them raising the CO_2 concentration, the second one with only global warming and the third one with both phenomena.

In this simulation was seen that increasing CO_2 concentration as predefined by the IS92a scenario, would induce to an increase of the temperature of 5.5ºC, while with both global warming and increase of CO_2 the change of temperature was 8ºC. This temperature increasing largely due to stimulated respiration and oxidation of organic matter in warmer soils, and therefore, is shown that soil respiration is a critical process referred to climate change (Lou & Zhou 2006, 26-28).

3 METHODS

3.1 MEASUREMENT PRINCIPLES

3.1.1 Oxitop® principle

The global chemical reaction for the oxidation of an organic substance is:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O \qquad \textit{Equation 1}$$

This example is the oxidation of glucose but it can be written for all organic substances. In this equation, it is shown that oxidation leads to a consumption of oxygen and a release of carbon dioxide.

The oxygen consumption measurement has been always the way of studying respiration and specifically, manometric measurement. As the processes takes place, oxygen is consumed and CO_2 is released but, if CO_2 is absorbed by an absorbing agent, the change in pressure is only because to the oxygen consumption. Some conditions are needed, for example, the sample needs to be in a vessel impermeable to gas. If not, oxygen and carbon dioxide of the air can affect the results. As well, there must be enough space in the vessel for the oxygen supply. Another condition is that the absorbing agent must be inside the vessel and the temperature must be constant to avoid pressure fluctuations. The vessel must be situated in a dark place too, because the light can increase the pressure in the vessel (Platen & Wirtz 1999a; Guntinas, Gil-Sotres, Leiros, and Trasar-Cepeda 2013).

The absorbing agent is an alkaline compound, usually NaOH. When the absorbing process takes place, CO_2 reacts with NaOH in the next way:

$$CO_2 + 2\ NaOH \rightarrow Na_2CO_3 + H_2O \qquad \textit{Equation 11}$$

This reaction allows to calculate the amount of needed absorbing agent.

The pressure change and oxygen consumption is related with the ideal gas equation:

$$\Delta p = \frac{\Delta n R T}{V} \qquad \textit{Equation 11}$$

Where Δp is the pressure variation, Δn is the amount of substance variation (mol), R is the ideal gas constant, T is the temperature and V is the volume of the vessel. With constant volume and temperature, a decrease of the pressure is expected because of the oxygen consumption (Platen & Wirtz 1999b; Hartikainen 2015).

The Oxitop® device consists of a measuring vessel and a lid for locking it, an Oxitop® – C measuring sensor (measuring head) an Oxitop® OC110 controller. The moisture (soil) is placed in the bottom of the vessel, and a plastic cup with the absorption agent (NaOH) is placed in the holder plate. As the process follows, the measuring head reads the pressure every certain period of time, and the data can be collected with the controller. After finishing the experiment, it is possible to transfer all the measurements to the computer via ACHAT OC PC software, and then to Excel. After finishing the experiment and collecting all data, soil respiration can be calculated as:

$$BA = \frac{M_{O_2} V_f \Delta p}{R T m_s} \qquad \textit{Equation 12}$$

Where BA is the soil respiration in mgO_2/kg dry substance, M_{O2} is the molar mass of oxygen (32000 mg/mol), V_f is the free gas volume in L (see "Previous measurements"), Δp is the measured pressure change in mbar, R is the gas constant (83,14 L mbar mol^{-1} K^{-1}), T is the temperature in K and m_s is the mass of dry soil used in the experiment in kg (equal to the total mass of the sample multiplied for the dry mass fraction) (Platen & Wirtz 1999b; Hartikainen 2015).

3.1.2 Titration principle

As mentioned in chapter 3.1.1, when respiration process takes place, CO_2 is released and absorbed by NaOH, so some NaOH will be neutralized. It is possible to know the amount of released CO_2 by titrating the NaOH that has not been consumed. Before titration, it is necessary to precipitate the absorbed CO_2, and this is done by adding barium chloride solution needed (SFS EN-ISO 16072):

$$BaCl_2 + Na_2CO_3 \rightarrow BaCO_3 + 2\ NaCl \qquad \textit{Equation 14}$$

Titration is a common laboratory method of quantity analysis that is used to find out the concentration of a substance (analyte) in a solution. Another solution called titrator or titrant is prepared and a known amount and concentration of this solution reacts with the analyte to determine concentration. (Khopkar 1998).

An acid-base titration consists in the neutralization between an acid and a basic solution. An appropriate pH indicator has to be added. An indicator is a substance that changes its colour in a range of pH, so it indicates the end of the titration with the colour change. For example, phenolphthalein is colourless in the acid side and changes its colour to pink in between pH 8 and 10. (Wikipedia 2017).

In the case of this project, HCl will be the titrant and phenolphthalein will be the indicator. After the titration, it is possible to calculate the released CO_2 with the next equation (Schinner, Öhlinger, Kandeler, Margesin 1995; SFS EN-ISO 16072 2002):

$$R_{CO_2} = \frac{2{,}2\ (V_b - V_p)}{m_s w_{sd}} \qquad \textit{Equation 15}$$

Where:

R_{CO2} is the rate of CO_2 evolution on a dry soil ($mgCO_2\ g^{-1}$).

V_b is the volume of HCl consumed in the control (ml).

V_p is the volume of HCl consumed in the test sample (ml).

m_s is the mass of the soil sample (g).

2,2 is a factor (1 ml of 0,1 molar HCl corresponds to 2,2 mg of CO_2) ($mg\ ml^{-1}$). In appendix 1 this factor is explained.

w_{sd} is the dry mass fraction of the soil.

This equation is only valid for the specified HCl concentration 0,1 molar. If the concentration is changed, equation has to be changed accordingly. (SFS EN-ISO 16072).

The concentrations of HCl and NaOH used are chosen so that less than 20% of the NaOH is neutralized by CO_2. SFS-EN ISO 16072 suggests HCl 0,1 molar and NaOH 0,05 M for 24 hours experiments. (SFS EN-ISO 16072).

3.2 PRELIMINARY MEASUREMENTS

Commented [SH7]: Preliminary

Before starting the oxygen experiment, some others must be done before in order to collect necessary data for using the equations afterwards. These experiments are the volume of free gas inside the Oxitop® vessel and determining the dry matter content of the fresh soil, that is, the percentages of water and soil in the initial mixture.

3.2.1 Free gas volume

The first thing to be known for the experiments is the free gas volume of the vessel. For obtaining this, the procedure is as follows:

First, weight the empty vessel with the lid and the plastic cup (X_2). After this, fill in the vessel with water and weight again (X_1). Resting these two data the volume of the vessel is obtained, but there is also soil and absorbing agent, whose volumes are necessary to consider (V_s and $V_{absorptionagent}$). Weighting the amount of soil used in the experiments and introducing it into a measuring glass, it is possible to obtain the volume occupied by the soil. The volume of alkaline solution is already known. Therefore, the volume free is calculated as (Platen &Wirtz 1999):

$$V_f = X_1 - X_2 - V_s - V_{absorptionagent} \qquad \textit{Equation 16}$$

This method assumes that the density of water is 1000 g/l.

3.2.2 Dry matter content

It is necessary to measure the dry matter content of the soil. To do that, the moisture measurer PRECISA XM 60 is used.

PICTURE 3.2.1 Precisa XM 60 (Photo: Luis Martín Rubio 2017)

The only thing to do is taking an aluminium plate and weight around 0,5 g of soil in it. The machine will increase the temperature and dry the soil, and it shows directly the dry matter content in percentage.

3.3 PROCEDURE OF THE EXPERIMENTS

3.3.1 Preparation of solutions

Before starting experimentation, some solutions must be prepared, NaOH, $BaCl_2$ and phenolphthalein.

Sodium hydroxide

For preparing this solution, the first step is knowing which concentration is going to be used in the following experiment. In the case of this project, three different concentrations were used, 0,05 M, 0,1 M and 1 M.

NaOH 0,1 M and 1 M were directly available from a commercial box. For preparing NaOH 0,05 M the only thing to do is take an amount of NaOH 0,1 M and dilute it in the same amount of ultrapure water. It is important to be ultrapure water because otherwise could have some absorbed CO_2 from the air, which would alter the results of the experiment. The volume of sodium hydroxide must be chosen knowing how many samples are going to be started so that it is necessary 20 ml per sample (SFS EN-ISO 16072).

Barium chloride 0,5 M

For preparing this solution, it is necessary to dissolve 10,4 grams of solid $BaCl_2$ in 100 ml of ultrapure water. It is common to find solid $BaCl_2 \times 2H_2O$. In this case, a previous drying must be done to eliminate the water. This drying consists in leaving the dehydrated barium chloride during 2 hours in an oven at 103ºC (SFS EN-ISO 16072).

Phenolphthalein

Finding commercial phenolphthalein is very common in laboratories. Nevertheless, for preparing the solution, it is necessary to dissolve 0,1 grams of phenolphthalein in 100 ml of aqueous ethanol, leading to a volume fraction of ethanol of 0,6 (SFS EN-ISO 16072).

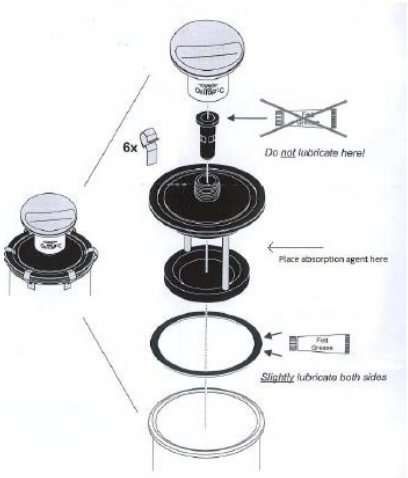
Hydrochloric acid

Like sodium hydroxide, the concentration of HCl must be decided. In this project, HCl 0,1 M has been used. If no commercial HCl 0,1 M is available, it is important to dilute it with ultrapure water for not alter the content of CO_2 in the samples. If other concentration is used, equation 15 must be changed accordingly (SFS EN-ISO 16072).

3.3.2 Oxitop® procedure

The first part of the experiment consists in the soil respiration process, using Oxitop® device. As said before, Oxitop® consists of a vessel, a lid with a plate, a measuring head and a controller. The procedure to do this part is as follows (Oxitop® user manual):

1. Check that measuring heads are free and with batteries ok. If not, empty them or change the batteries.
2. Weight the amount of soil wanted to study (in this case 25 or 50 g) and add it to the bottom of the vessel.
3. Fill in a plastic with of 20 ml of the absorbing agent, in this case NaOH 0,05 M, and put it in the plate of the lid.
4. Place the lid on the vessel and close it with 6 clips. These clips will help to avoid the CO_2 enter in the vessel and disturb the results.
5. Place a rubber stopper in the hole of the lid.
6. Adjust the measuring head.
7. Place the vessel in the isothermal chamber.
8. Start sample measuring. With Oxitop® controller, set the wanted measuring time and the I.D of the sample and start the measuring.



PICTURE 3.3.1 Assembly of the device (Oxitop® user manual)

After the measuring time, the measuring heads will have all the information of pressure evolution and it is possible to collect it with the controller pressing “Call up all data”. This also can be done during the experiment for controlling the temporal results. Once the data are in the controller, it is possible to bring them to a computer with the aid of ACHAT OC PC software, and then to Excel for their study.

PICTURE 3.3.2 Oxitop® device inside the isothermal chamber (Photo: Luis Martín Rubio 2017)

3.3.3 Titration procedure

Once the Oxitop® experiment has finished, titration experiment can start. The way to procedure is as follows (SFS EN-ISO 16072):

1. Take the cup with NaOH and absorbed CO_2 and transfer the content to a transparent glass.
2. Add 2 ml of $BaCl_2$ to precipitate the carbon as carbonates. Many solid particles can be seen in the glass.
3. Add one or two drops of phenolphthalein indicator. The solution should turn to pink colour.
4. Remove the air present in the pipes of the titrator. If there is air, the volume of expulsed air will be counted as HCl volume, so the results would be modified.

Commented [SH8]: Remove

5. Add a magnet and start the titration with the titration machine. With this machine HCl is added little by little to the stirred solution until the change of colour from pinkie to transparent.
6. The used HCl volume will appear in the screen.

Before or after the titration of the samples, three or four blanks need to be titrated. These blanks are 20 ml of fresh NaOH with the same concentration as the samples, also adding phenolphthalein and $BaCl_2$. The average result of the blanks will be calculated.
Having the results for the samples and blanks, it is possible to calculate the CO_2 absorbed with equation 15.

PICTURE 3.3.3 Sample before titration (Photo: Luis Martín Rubio 2017)

PICTURE 3.3.4 Sample after titration (Photo: Luis Martín Rubio 2017)

4 RESULTS

4.1 FREE GAS VOLUME

For measuring the free gas volume, three tries were done and the average result was calculated. This average result is the one used in next equations. The results are:

TABLE 4.1.1 Results in free gas volume experiment

	Try 1	Try 2	Try 3
x_2 (kg)	0,713	0,713	0,715
x_1 (kg)	1,643	1,653	1,654
m_{soil} (g)	24,751	25,259	24,776
V_{soil} (ml)	72	72	68
V_f (ml)	838	847,8	851,8

Commented [SH9]:

In this table, x_2 is the mass of the empty vessel with the lid and the plastic cup, x_1 is the mass of the vessel full of water with the lid and the plastic cup, m_{soil} is the mass of soil used, V_{soil} is the volume of that mass of soil seen in a measuring glass and V_f is the volume free.

Volume free is calculated as:

$$V_f = x_1 - x_2 - V_{soil} - V_{absorptionagent} \qquad \textit{Equation 16}$$

As an example, in the try 1:

$$V_f = 1643 - 713 - 72 - 20 = 838 \text{ ml.}$$

Average: V_f = 845,87 ml.

4.2 DRY MATTER CONTENT

In this measurement, four samples of fresh soil were dried and the average value was calculated. This average value is used in the equations. The results are presented in dry mass fraction and are shown in table 4.2.1.

Commented [SH10]:

TABLE 4.2.1 Results in dry matter content experiment

Dry matter content analysis (mass fraction)	
Sample 1	0,441
Sample 2	0,451
Sample 3	0,567
Sample 4	0,625
Average	**0,521**

Commented [SH11]:

4.3 TEST ROUNDS COMMENTS

As said in the previous chapter, there were six test rounds made in experimenting oxygen consumption and CO_2 release in soil respiration. The first round of smapless was built with the suggestions of SFS-EN ISO 16072. In this standard, it is indicated to use 20-25 g of fresh soil and 20 ml of NaOH 0,05 M in experiments with 24 hours of duration. After this time, add the indicator and $BaCl_2$ and titrate the solution with HCl 0,1 M.

Commented [SH12]:

In the case of this project, the measuring time was 11 days instead of 24 hours. This had an effect in the titration part that in the moment of adding the phenolphthalein, the solutions didn´t turn to a pink colour, and they stayed being white, so no titration was possible. In the first part of the experiment, NaOH is reacting with CO_2 in the form of equation 11:

$$CO_2 + 2\ NaOH \rightarrow Na_2CO_3 + H_2O \qquad \textit{Equation 11}$$

Therefore, sodium hydroxide is being consumed with the time. The lack of a pink colour after adding phenolphthalein indicates that too much NaOH has been consumed, and more quantity is needed. Therefore, 20 ml of NaOH 0,05 M is not enough for experiments with this kind of duration.

Following this conclusion, the second round of samples was built with the objective of figure out with concentration of NaOH is suitable for the titration. Therefore, 4 samples were built with NaOH 0,1 M, 4 with NaOH 0,5 M and 4 with NaOH 1 M. The titration of these samples showed that NaOH 0,1 M is enough, because all the expected results were obtained: pink colour when adding phenolphthalein and precipitation of carbonates

when adding $BaCl_2$, and change of colour after titration. Higher concentration can be also used, but more HCl is needed in the titration, so NaOH 0,1 M was selected as the correct one.

Commented [SH13]:

Once selected the correct concentration of chemicals, two more test rounds of 12 samples each one were built for having enough data and repeatability for a final conclusion. After these two rounds, it was decided to start another one adding a biodegradable material to see its influence on the results. The material selected is a PLA material and it was added to six of the samples, while the other six contained only soil. The results showed that this material need more time to start being degraded, so it was decided to build a last test round with a faster biodegradable material, Bioska® plastic bags, based in their web page (http://www.plastiroll.fi/en/products/biowaste-bags-sacks-films/biobags/bioska-bags-fruit-vegetables/). Thus, the sixth test round is composed by six samples containing soil and around 0,5 grams of bag, and other six blank samples containing only soil. The results show that it is possible to see biodegradability with the titration method.

Commented [SH14]: test rounds

PICTURE 4.3.1 Biodegradable PLA material (Photo: Luis Martín Rubio 2017)

PICTURE 4.3.2 Bioska® degradable plastic bags (Photo: Luis Martín Rubio 2017)

4.4 TEST RESULTS

In this part, the results of both oxygen and carbon dioxide experiments will be presented for each sample. These results include: pressure evolution with time, final pressure change, oxygen consumption, volume of HCl used in the titration and CO_2 release.

The way of name the samples is this type: RX-SY, where R comes from "Round" X is a number indicating the name of the test round, S comes from "Sample" and Y indicates the name of the sample in that round. For example, a sample called R2-S10 means that is in the second test round and sample number 10.

4.4.1 First test round

In this round, only the pressure development is presented because of the unexpected results that caused an impossible way to do a titration. Numeric results are not calculated because it is considered that the experiment is not finished so the final results can vary from other finished one. The evolution is presented in figure 4.4.1.

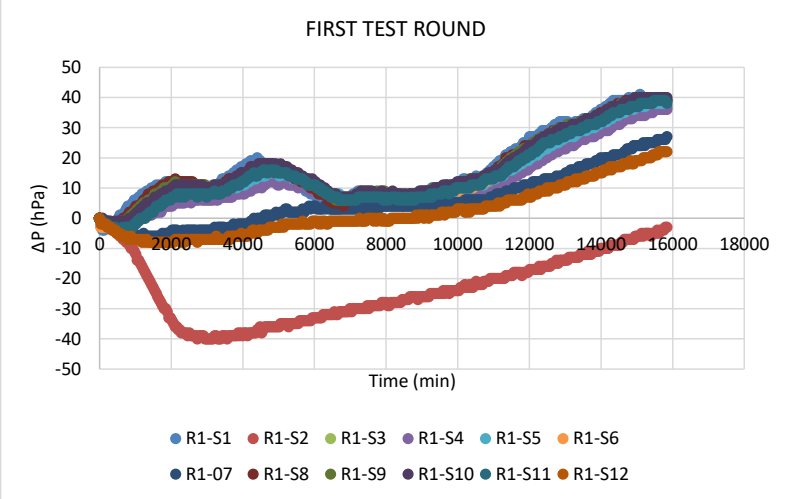


FIGURE 4.4.1 Graphical presentation of pressure change in the first test round

4.4.2 Second test round

As explained before, the second test round was built with the objective of finding a correct concentration of sodium hydroxide solution. The samples with NaOH 0,1 M were selected as valid and their pressure evolution is presented but it will not be considered for final results or conclusions. No calculations are presented for the same reason, they are not considered as a final round of samples.

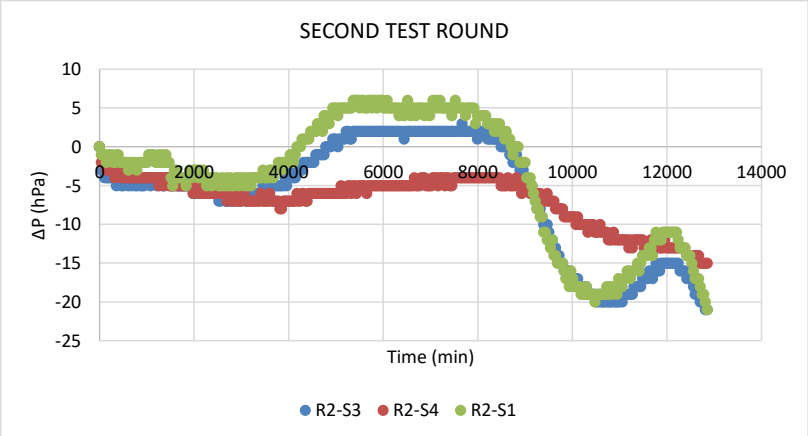


FIGURE 4.4.2 Graphical presentation of pressure change in the second test round

4.4.3 Third test round

Twelve replicates were built in this round. The pressure evolution of these samples is the next:

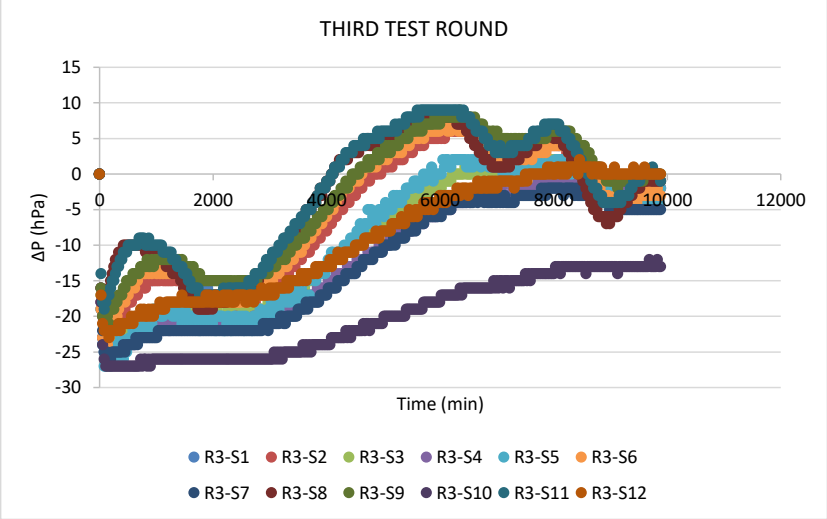


FIGURE 4.4.3 Graphical presentation of pressure change in the third test round

In table 4.4.1 the mass of soils of the samples, the maximum pressure change, O_2 consumption, volume of HCl needed in titration and CO_2 release in this trial are presented. The measuring time of this test round was seven days.

TABLE 4.4.1 Results in third test round

Sample	Soil mass (g)	Δp (hPa)	Vol HCl titr (ml)	BA (mg/kg)	CO_2 abs (mg/kg)	BA (mmol/kg)	CO_2 abs (mmol/kg)
R3-S1	25,54	25	12,224	2073,52	1275,85	64,80	29,00
R3-S2	24,96	24	12,194	2037,19	1310,80	63,66	29,79
R3-S3	25,92	25	12,858	2042,88	1153,72	63,84	26,22
R3-S4	24,24	26	11,922	2272,30	1397,00	71,01	31,75
R3-S5	25,59	27	11,328	2235,45	1421,48	69,86	32,31
R3-S6	25,07	25	11,398	2112,50	1438,97	66,02	32,70
R3-S7	24,69	26	10,762	2230,88	1569,94	69,72	35,68
R3-S8	25,09	20	11,352	1688,88	1445,76	52,78	32,86
R3-S9	25,37	21	10,336	1753,54	1598,75	54,80	36,34
R3-S10	25,24	27	11,288	2266,01	1447,60	70,81	32,90
R3-S11	24,96	19	9,78	1612,49	1718,96	50,39	39,07
R3-S12	25,25	23	11,696	1929,50	1378,77	60,30	31,34

Commented [SH15]:

The volume of HCl used for titrating blanks are in table 4.4.2:

TABLE 4.4.2 Volume of HCl in the titration of blanks in the third test round

	V blanks (ml)
1	19,886
2	20,048
3	19,888
Average	19,940

In the results table, Δp is the most negative value of pressure read by Oxitop® measuring head, indicating the point with maximum oxygen absorption.

Oxygen consumption (BA) has been calculated with equation X:

$$BA = \frac{M_{O_2} V_f \Delta p}{RT m_s} \qquad \textit{Equation 13}$$

As an example, in R3-S1:

$$BA = \frac{32000 mg/mol * 0{,}84587 l * 25 mbar}{83{,}14 \frac{mbar * l}{mol * K} * 295 K * 25{,}54 g * 0{,}521} = 2073{,}52\ mg/kg$$

CO_2 absorption is calculated with equation X:

$$R_{CO_2} = \frac{2{,}2\ (V_b - V_p)}{m_s w_{sd}} \qquad \textit{Equation 15}$$

Following with the same example of R3-S1:

$$R_{CO_2} = \frac{2{,}2 mg/ml\ (19{,}940 - 12{,}224) ml}{25{,}54/1000 kg * 0{,}521} = 1275{,}85\ mg/kg$$

These rates data are also shown in mmol/kg. For calculating this, the rate in mg/kg must be divided by the molar mass, 32 g/mol in case of oxygen and 44 g/mol in case of carbon dioxide.

4.4.4 Fourth test round

This round is also done with twelve replicates. Figure 4.4.4 shows the pressure development of these replicates.

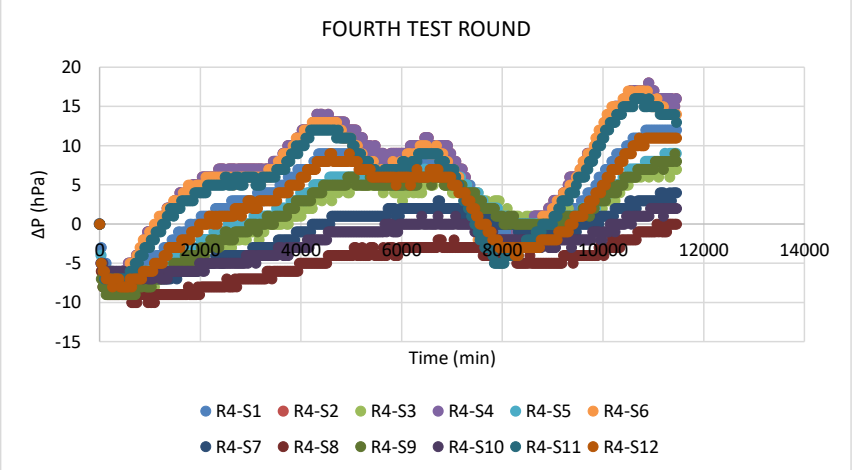


FIGURE 4.4.4 Graphical presentation of pressure change in the fourth test round

In table 4.4.3 the mass of soils of the samples, the maximum pressure change, O_2 consumption, volume of HCl needed in titration and CO_2 release in this trial are presented. The measuring time of this test round was seven days.

Table 4.4.3 Results in the fourth test round

Sample	Soil mass (g)	Δp (hPa)	Vol HCl titr (ml)	BA (mg/kg)	CO_2 abs (mg/kg)	BA (mmol/kg)	CO_2 abs (mmol/kg)
R4-S1	25,05	7	12,488	591,98	1425,42	18,50	32,40
R4-S2	24,41	7	9,13	607,41	2043,42	18,98	46,44
R4-S3	25,85	8	8,902	655,55	1966,96	20,49	44,70
R4-S4	25,61	7	10,304	579,09	1754,53	18,10	39,88
R4-S5	24,04	7	10,468	616,92	1840,34	19,28	41,83
R4-S6	26,91	8	6,264	629,81	2303,72	19,68	52,36
R4-S7	25,21	8	8,468	672,21	2089,63	21,01	47,49
R4-S8	26,52	10	9,312	798,77	1852,05	24,96	42,09
R4-S9	23,81	9	9,986	800,84	1943,60	25,03	44,17
R4-S10	25,40	7	7,626	583,77	2213,93	18,24	50,32
R4-S11	24,12	7	9,648	614,91	1977,94	19,22	44,95
R4-S12	25,17	8	9,162	673,36	1976,76	21,04	44,93

The volume of HCl used for titrating blanks are in table 4.4.4:

TABLE 4.4.4 Volume of HCl in the titration of blanks in the fourth test round

	V blanks (ml)
1	20,920
2	20,992
3	20,918
Average	20,943

4.4.5 Fifth test round

This round contains twelve samples, replicates from R5-S1 to R5-S6 contain 0,5 grams of biodegradable material and the rest of them are blanks with only soil. The pressure evolution of these samples is presented in figure 4.4.5.

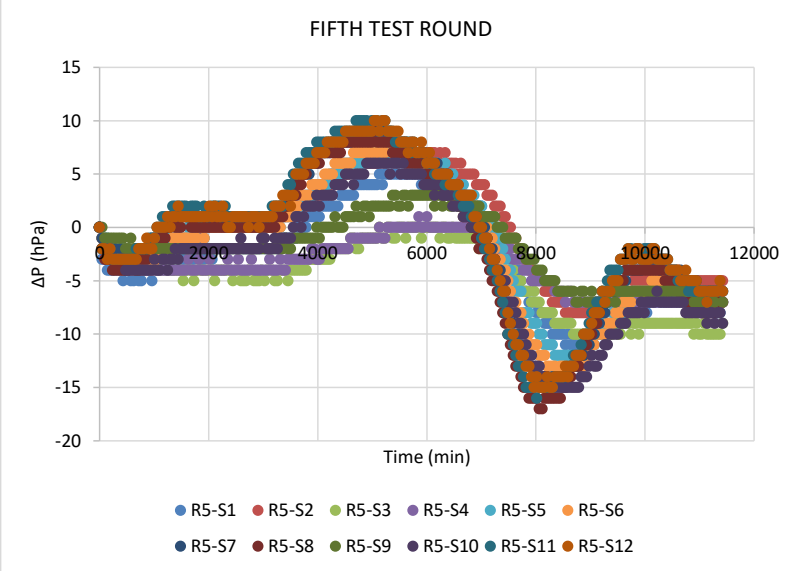


FIGURE 4.4.5 Graphical presentation of pressure change in the fifth test round

In table 4.4.5 the mass of soils of the samples, the maximum pressure change, O_2 consumption, volume of HCl needed in titration and CO_2 release in this trial are presented. The measuring time of this test round was eight days.

Table 4.4.5 Results in the fifth test round

Sample	Soil mass (g)	Material mass (g)	Δp (hPa)	Vol HCl titr (ml)	BA (mg/kg)	CO_2 abs (mg/kg)	BA (mmol/kg)	CO_2 abs (mmol/kg)
R5-S1	24,69	0,567	11	9,234	1069,90	2206,46	33,43	50,15
R5-S2	24,96	0,549	8	8,748	769,65	2275,67	24,05	51,72
R5-S3	25,05	0,575	10	9,046	958,60	2210,53	29,96	50,24
R5-S4	24,72	0,507	8	9,074	777,08	2234,52	24,28	50,78
R5-S5	24,86	0,558	12	9,05	1159,23	2226,88	36,23	50,61
R5-S6	24,82	0,505	14	8,852	1354,22	2268,01	42,32	51,55
R5-S7	25,28	-	15	9,014	1425,04	2196,82	44,53	49,93
R5-S8	25,06	-	17	9,102	1629,20	2199,27	50,91	49,98
R5-S9	25,38	-	7	8,87	662,25	2214,83	20,70	50,34
R5-S10	25,20	-	15	9,338	1429,35	2141,92	44,67	48,68
R5-S11	24,81	-	16	9,22	1548,72	2198,51	48,40	49,97
R5-S12	24,86	-	15	8,59	1449,14	2315,63	45,29	52,63

In this case, before the Oxitop® experiment, the soil was moisture looking for a more active soil. The results of the dry matter content experiments are shown in table 4.4.6.

TABLE 4.4.6 Dry matter soil in the fifth test round

Dry matter content	
Sample 1	0,424
Sample 2	0,441
Sample 3	0,514
Average	**0,460**

The volume of HCl used for titrating blanks are in table 4.4.7:

TABLE 4.4.7 Volume of HCl in the titration of blanks in the fifth test round

	V blanks (ml)
1	20,858
2	20,592
3	20,392
Average	20,614

4.4.6 Sixth test round

This round also contains twelve samples, replicates from R6-S1 to R6-S6 contain 0,5 grams of biodegradable plastic bags and the rest of them are blanks with only soil. The pressure evolution of these samples is presented in figure 4.4.6.

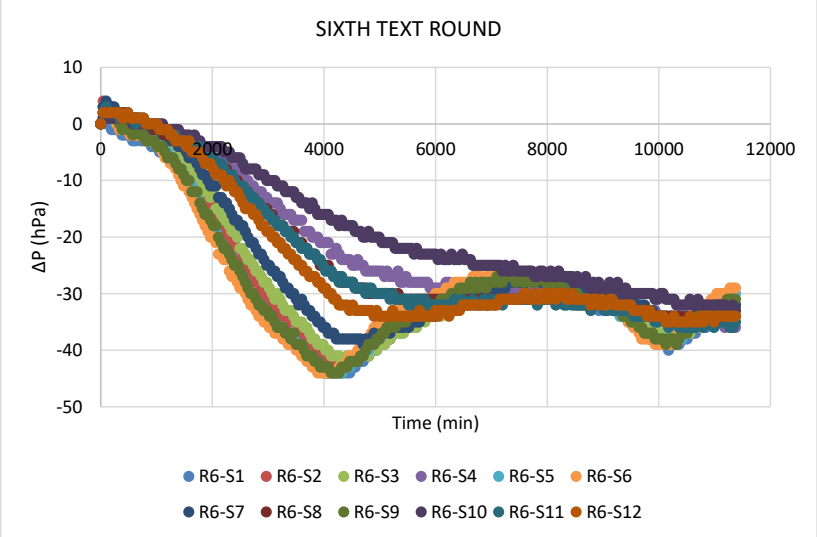


FIGURE 4.4.6 Graphical presentation of pressure change in the sixth test round

In table 4.4.8 the mass of soils of the samples, the maximum pressure change, O_2 consumption, volume of HCl needed in titration and CO_2 release in this trial are presented. The measuring time of this test round was eight days.

Table 4.4.8 Results in the sixth test round

Sample	Bag mass (g)	Soil mass (g)	Δp (hPa)	Vol HCl titr (ml)	CO_2 abs (mg/kg)	BA (mmol/kg)	CO_2 abs (mmol/kg)
R6-S1	0,4744	24,99	44	6,474	2546,87	130,91	57,88
R6-S2	0,5227	25,28	43	8,418	2152,45	126,44	48,92
R6-S3	0,5076	25,14	42	8,57	2136,00	124,20	48,55
R6-S4	0,4884	24,80	36	8,386	2200,70	107,93	50,02
R6-S5	0,5494	25,00	44	5,314	2765,83	130,85	62,86
R6-S6	0,5409	25,05	44	6,42	2550,42	130,57	57,96
R6-S7	-	25,13	39	8,77	2099,28	115,38	47,71
R6-S8	-	25,04	35	9,798	1911,70	103,90	43,45
R6-S9	-	25,09	44	8,068	2235,50	130,40	50,81
R6-S10	-	25,01	33	9,674	1937,43	98,08	44,03
R6-S11	-	25,05	36	9,168	2030,72	106,85	46,15
R6-S12	-	25,05	35	9,688	1931,73	103,86	43,90

In this case, before the Oxitop® experiment, the soil was moisture looking for a more active soil. The results of the dry matter content experiments are shown in table 4.4.9.

TABLE 4.4.9 Dry matter soil in the sixth test round

Dry matter content	
Sample 1	0,437
Sample 2	0,462
Sample 3	0,492
Sample 4	0,464
Average	**0,464**

The volume of HCl used for titrating blanks are in table 4.4.10:

TABLE 4.4.10 Volume of HCl in the titration of blanks in the sixth test round

	V blanks (ml)
1	20,1
2	19,58
3	20
Average	**19,893**

5 CONCLUSIONS AND DISCUSSION

In this chapter, conclusions of different aspects of the experimental work are commented.

5.1 Sodium hydroxide concentration

The first test round of samples of this project was done following the instructions of SFS-EN ISO 16072. In this standard, 20 ml of NaOH 0,05 M is used as the alkaline solution for absorbing the carbon dioxide released by the soil. SFS standard indicates a length of experiments of 24 hours. Nevertheless, in this project the length has been at least one week long.

After finishing the pressure measurement, the NaOH was taken and $BaCl_2$ was added for precipitating carbonates and some drops of phenolphthalein were also included. However, the pink colour did not appear, so no titration was possible. The lack of colour after adding an indicator solution shows that, in this case, the pH of the solution is not the correct one.

During the Oxitop® experiment, sodium hydroxide is reacting with CO_2 as follows:

Commented [SH16]: as follows

$$CO_2 + 2\ NaOH \rightarrow Na_2CO_3 + H_2O \qquad \textit{Equation 11}$$

Therefore, NaOH is being consumed, so it was concluded that too much sodium hydroxide had disappeared in these experiments and a bigger amount was needed and it can be obtained by increasing the concentration.

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In the next test round of samples, the same conditions were tested but changing the concentration from 0,05 M to 0,1 M. With the double concentration, when adding phenolphthalein, the solution turned to pink and the titration was possible.

Therefore, for soil respiration experiments longer than 24 hours, it is not possible to use 20 ml of NaOH 0,05 M, and at least a concentration of 0,1 M is recommended.

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5.2 Pressure evolution

After seeing the pressure evolution in the different test rounds, it is not easy to explain the behaviour of soil respiration.

A pressure decrease is common in the beginning of Oxitop® experiment. This high activity can be caused because of the moving and mixing of soil particles during the sample preparation, which leads to an aeration of the soil and therefore, to higher oxygen consumption.

After some hours, the activity decreases and a pressure increases is observed. According to Platen & Wirtz (1999), this can be because a big amount of the oxygen in the vessel is consumed and the pressure increase can indicate the formation of a gas.

While the experiment continues, other fluctuations take place. Soil contains a huge amount of types of microorganisms and it is difficult to understand their behaviour in the different environmental conditions. Soil respiration is not a single and easy chemical reaction, other transformations like inorganic reactions can take place and change pressure.

For further calculations, the most negative value of the pressure evolution was applied. This value indicates the point of the highest oxygen consumption, so it is the most interesting value for the aim of this project.

5.3 Oxygen consumption

Due to the differences in the pressure evolution with Oxitop® method between the test rounds, the rate of oxygen consumption is also different. Figure 5.3.1 resumes the results of the oxygen consumption rate, showing the average oxygen consumption in each test round and the standard deviation.

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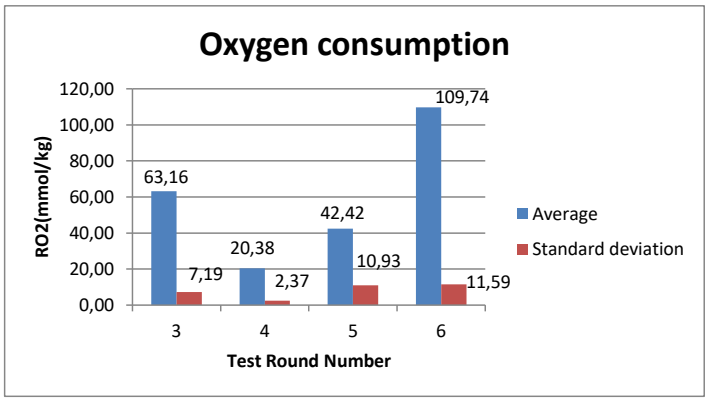

Figure 5.3.1 Oxygen consumption rate average and standard deviation values of the samples which contain only soil. Samples with biodegradable material are excluded in order to compare all the samples in the same conditions in every round. Therefore, in test rounds 5 and 6, the data in this graph is the average value between the six samples with only soil, excluding the other six.

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All the test rounds had the same conditions, so a similar behaviour between oxygen consumption results could be expected. However, the average value of oxygen consuption between the test rounds is different; for example, the average value in round three is three times higher than in round four. Moreover, calculating the average value and standard deviation within all oxygen data, an average oxygen consumption of 40,20 mmol/kg of soil is obtained. In addition, standard deviation is 19,26, which represents 47,91% over the average value of oxygen consumption measured in this report, and it shows a high deviation of the results.

These results differ from each other enough to conclude that the Oxitop® soil respiration measurements may have some factors affecting, therefore causing differences in measurement results.

5.4 Carbon dioxide absorption

As in the case of O_2 consumption, the average and standard deviations of the rate of CO_2 absorption are presented in the figure 5.4.1. It is possible to see less difference in the CO_2 release data than in O_2 consumption data between the test rounds. Soil respiration CO_2

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results analysed in this thesis by titration method could be considered more exact than the oxygen consumption results calculated based on pressure difference measurements.

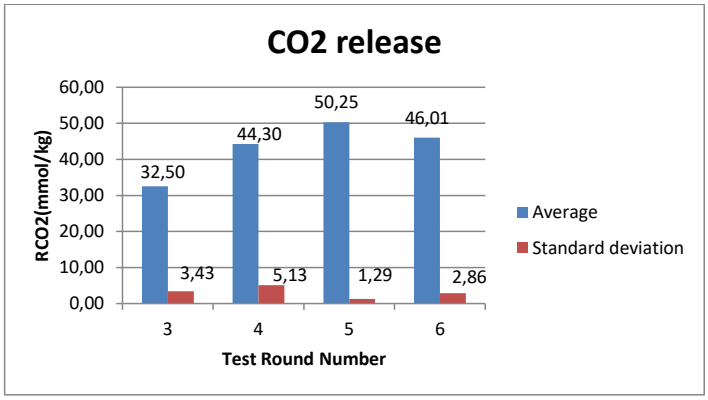

Figure 5.4.1 Carbon dioxide release average and standard deviation values of the samples containing only soil. Samples with biodegradable material are excluded excluded in order to compare all the samples in the same conditions in every round. Therefore, in test rounds 5 and 6, the data in this graph is the average value between the six samples with only soil, excluding the other six.

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Including all the data from samples with only soil, the total average is 42,73 mmol/kg with a standard deviation of 8,20, which represents a 19,19% over the average value, much less than the 42% of the oxygen consumption. That is the reason why this result seems to be more reliable than the obtained with the oxygen experiment, because of the smaller standard deviation of the data. As a conclusion, for the experiments of this project, the titration method for measuring soil respiration seems to be more reliable than the pressure measurement by Oxitop® method.

5.5 Biodegradable PLA material

Round five presents six samples with half a gram of PLA compressed degradable material (samples from R5-S1 to R5-S6) and other six samples with only soil (from R5-S7 to R5-S12).

Seeing the results of consumed oxygen on the test round five, it is possible to check that the samples containing degradable material have a less rate of consumed oxygen than the blanks, which is not expected because if the material is biodegradable, it means that the reactions would be increased and the rate of consumed oxygen would be accordingly higher. This result supports the conclusion of regarding the titration method more reliable than the pressure measurement method.

Referring to the titration results of CO_2 released in soil respiration, it seems that the samples with biodegradable material have a bit higher rate of released gas (figure 5.5.1), but it cannot be considered as a final result because the difference is so small that it could be caused by the standard deviation between similar samples. Another possibility is that the tested material does not start to degrade during the experiment time and a longer test period would be needed. For confirming, more experiments with this material could be done, but in this project, it was decided to test another round with a material known to be biodegradable.

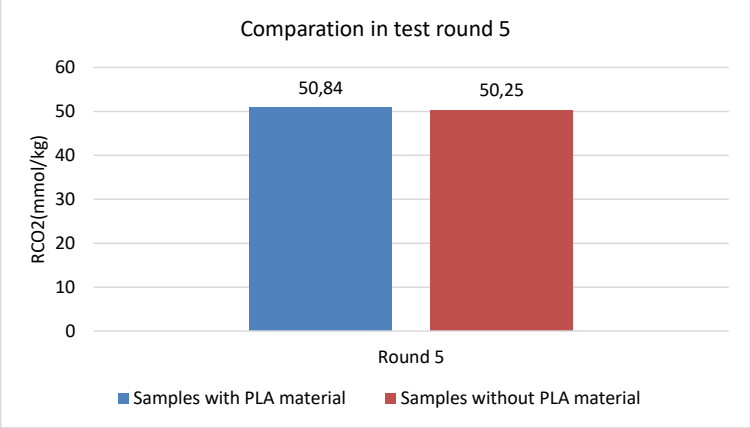

FIGURE 5.5.1 Comparison of the rate of released CO_2 in test round 5 between samples with and without PLA material.

5.6 Bioska® plastic bags

The sixth test round presents six samples with half a gram of biodegradable Bioska® plastic bag placed inside the soil (samples from R6-S1 to R6-S6) and other six samples with only soil (from R6-S7 to R6-S12).

In this case, it is possible to see a difference in the oxygen consumption between the samples with Bioska® bag and the samples without it, Bioska® bag consuming more oxygen than the samples with only soil. This result that was expected because the bag should contribute in the respiration process and increase the rate of consumed oxygen.

The same goes through the released CO_2 results analysed by titration method. Comparing the samples with plastic bags and samples without them, it can be seen that there is a difference between them, the samples with Bioska® material being the ones with higher rate of released CO_2. The average values are seen in figure 5.6.1.

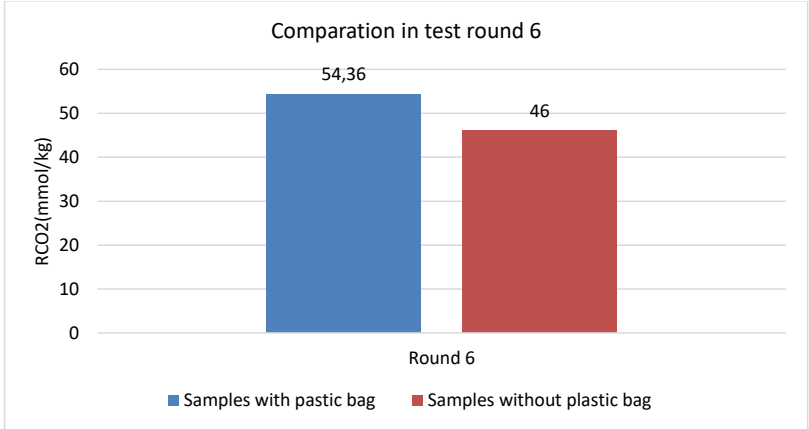

FIGURE 5.6.1 Comparison of the rate of released CO_2 in test round 6 between samples with and without Bioska ® biodegradable material

The difference between samples with Bioska® bags and samples without them is a 15,37%, which could be enough to conclude that with 8 days of experiment duration, the Bioska® bags started to degrade. This degradation process indicated by CO_2 release is possible to measure with the titration method applied from SFS EN-ISO 16072 and described in this thesis.

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5.7 Error evaluation

5.7.1 Free gas volume

There is always a possibility for errors in laboratory analyses, and error factors are several, but two main error factors can be considered in this experiment.

The first one is assuming that the density of water is 1000 g/l, since this value is valid for a pressure of 1 atm and a temperature of 4ºC. As the conditions in a laboratory are not exactly these, the density will vary a bit. Moreover, the water is taken from the tap in different moments, so the water temperature can vary between the test rounds, although it is tried to take the water at the same conditions. This could be fixed by using a densimeter in the moment of filling the vessel and calculating the exact volume of the water.

The second error of measuring the free gas volume is that the porosity of the soil is neglected. When measuring the volume of the soil in the measuring glass, there are some pores between the particles of soil, so the real soil volume is a bit lower the read in the measuring glass, but as the soil particles are very small, the space between them will be also small and it does not affect to the results in important terms, therefore no porosity is considered. If an exact data is wanted, it would be possible to measure the porosity by reading the volume occupied by soil in a measuring glass and then adding water and checking how much is it necessary until the volume in the glass starts to increase; in that moment, all the pores will be completely full of water.

5.7.2 Oxitop® and titration

The first error in this experiment affects the moisture of the soil. After measuring the initial water content, some time passes from the moment when the soil is placed inside the vessels and the measurement starts. This time is used for filling the cups with NaOH and introducing them in the vessels, as well as putting the lid and the closing clips. In this time, soil is in contact with the atmosphere, so it can lose moisture and the respiration in this period is not registered in the measuring heads.

In addition, soil is not 100% pure, it contains some stones, wooden pieces or other materials that do not respire, so some pieces can be weighted as soil when they are not. Therefore, less respiration will be measured and the result of respiration will be less than if all the weighted mass was pure soil.

Another error possibility is that despite the vessel of NaOH is closed, it could exchange and absorb some CO_2 from the atmosphere, so the concentration in titration could vary a bit. For minimizing this error, sodium hydroxide is exposed to air at the last possible moment but there is a time when the plastic cup is in contact with the air, so some absorption could occur.
During the titration, the change of colour is not immediate. From the addition of the last drop to the change of colour, a short time passes and in that period, it is possible to add extra volume of titration agent. For minimizing this error, the HCl should be added slowly so that it has time to react in a correct volume.

5.8 Resume conclusions

The principal aims of this project were to study the carbon dioxide formation in soil respiration and biodegradability of some materials and measure it by titration, testing the measuring method described in SFS EN-ISO 16072 for TAMK Environmental Engineering laboratory. These aims were achieved. The titration of CO_2 could be used as a reference measurement system for soil respiration measument by Oxitop® pressure mode.

Regarding to the experimental work, the most important conclusion of this thesis is that titration method seems to be more reliable for measuring soil respiration than the pressure experiment with Oxitop® for soil respiration and the tested materials. Besides, applying titration method needs careful attention when implemented.

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APPENDICES

Appendix 1. Explanation of CO_2 formula modified in this thesis

The rate of absorbed CO_2 is calculated by Equation 15:

$$R_{CO_2} = \frac{2{,}2\ (V_b - V_p)}{m_s w_{sd}} \qquad \textit{Equation 15}$$

This appendix has the objective to explain to explain the 2,2 factor in this formula.

In SFS-EN ISO 16072 is said that "1ml of 0,1 molar HCl corresponds to 2,2 mg of CO_2 per day". The first thing to say is that the correspondence is not per day, but in this standard the experiments have a duration of 24 hours, and so the CO_2 rate is presented in mg per gram of dry soil and day. In fact, SFS standard uses a factor of 24 h/day in the denominator to present the result per hour.

In this project, that factor of 24 h/day has been omitted and the 2,2 factor is only in mg of CO_2, because the experiments are longer than 24 hours and the total rate of CO_2 was wanted to be presented.

Taking 1 ml of 0,1 molar HCl and passing it to mol:

$$0{,}1\ \frac{mol}{l} * 10^{-3} l = 0{,}0001\ mol\ HCl$$

As HCl reacts mol to mol with NaOH in the neutralization reaction (reaction happening in the titration):

$$HCl + NaOH \rightarrow NaCl + H_2O$$

Thus, 0,0001 mol of HCl corresponds to 0,0001 mol of NaOH.

The reaction between NaOH and CO_2 is the next:

2(2)

$$2\ NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$

This reaction indicates that 0,0001 mol of NaOH corresponds to 0,00005 mol of CO_2, that is, 2,2 mg.

Therefore, 1ml of 0,1 molar HCl corresponds to 2,2 mg of CO_2 per day. So, multiplying this factor (mg CO_2/ml HCl) by the volume of HCl used (ml) resting the blanks and dividing by the dry mass of soil, it is obtained the rate of released CO_2 (mg CO2/g soil).