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RESPONSES OF SOIL CO_2 EFFLUX TO BIOTIC AND ABIOTIC DRIVERS IN AGRICULTURAL SOILS

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ABBREVIATIONS

AWCD: AVERAGE WELL COLOR DEVELOPMENT

BD: BULK DENSITY

$CaCO_3$%: CALCIUM CARBONATE

CFUS: COLONY-FORMING UNITS

CH_4: METHANE

CO_2: CARBON DIOXIDE

CO_2-EQ: CARBON DIOXIDE EQUIVALENT

CRDS: CAVITY RING DOWN SPECTROSCOPY

EC: EDDY-COVARIANCE

FID: FLAME IONIZATION DETECTOR

FTIR: FOURIER TRANSFORM INFRARED SPECTROSCOPY

GC: GAS CHROMATOGRAPHY

GHG: GREENHOUSE GASES

GPP: GROSS PRIMARY PRODUCTION

IPCC: INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE

LAI: LEAF AREA INDEX

$LLGHG_S$: LONG-LIVED GREENHOUSE GASES

LULUCF: LAND USE LAND USE CHANGE FOREST

MPN: MOST PROBABLE NUMBER

NDIR: NONDISPERSIVE INFRARED SENSOR

NEE: NET ECOSYSTEM EXCHANGE

NH^+_4: AMMONIUM

NOAA: NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION

NO_3^-: NITRATE

N_2O: NITROUS OXIDE

OCO-2: ORBITING CARBON OBSERVATORY-2

PAR: PHOTOSYNTHETIC ACTIVE RADIATION

PDH: PYRUVATE DEHYDROGENASE COMPLEX

PVC: POLYVINYL CHLORIDE

R_a: AUTOTROPHIC RESPIRATION

R_h: HETEROTROPHIC RESPIRATION

R_S: SOIL RESPIRATION

RTCA: TRI-CARBOXYLIC ACID CYCLE

RUBISCO: RIBULOSE-1, 5-BISPHOSPHATE CARBOXYLASE-OXYGENASE

SOC: SOIL ORGANIC CARBON

SOM: SOIL ORGANIC MATER

SWC: SOIL WATER CONTENT

T_a: AIR TEMPERATURE

TN: TOTAL NITROGEN

T_S: SOIL TEMPERATURE

UNFCCC: UNITED NATIONS FRAMEWORK CONVENTION ON CLIMATE CHANGE

VIGREEN: VIGREEN INDEX

WFPS: WATER FILLED PORE SPACE

WMO: WORD METEOROLOGICAL ORGANISATION

WRB: WORLD REFERENCE BASE FOR SOIL RESOURCES

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1. INTRODUCTION:

1.1. Foreword

Global warming has become a severe problem that cannot be overlooked (Pesce *et al.*, 2018). Climate change is accelerating and record greenhouse gas (GHG) concentrations are increasing global temperatures towards serious levels (WMO, 2016). Rising temperature has significant effects on ecosystem's functioning, especially on carbon cycling. In fact, soil respiration (Rs) is a dominant process of the global carbon cycle and it has a significant influence on global radiative forcing (Qin *et al.*, 2014). In terrestrial ecosystems, during photosynthesis atmospheric CO_2 is assimilated, and then released either via autotrophic respiration or through heterotrophic decomposition of carbon compounds differing in recalcitrance and sensitivity to temperature (Davidson, Janssens and Lou, 2006), the elevated atmospheric CO_2 concentration may not only cause climate warming but also cause a profound effect on the primary productivity of agriculture and natural ecosystems (Bazzaz, 1990).

Recent methodological advances in automated soil respiration measurement systems allowed high frequency measurements to be taken (Herran, Tachiiri and Matsumoto, 2019), providing insights into the variations of soil CO_2 efflux at different time scales (Yan, Li and Liu, 2014). The WMO Greenhouse Gas Bulletin showed that globally averaged concentrations of carbon dioxide (CO_2) reached 407.8 ppm in 2018, the growth rate of CO_2 averaged over three consecutive decades (1985–1995, 1995–2005 and 2005–2015) increased from 1.42 ppm yr^{-1} to 1.86 ppm yr^{-1} and to 2.06 ppm yr^{-1} with the highest annual growth rates observed during El Niño events (WMO, 2018).

Soil respiration and its components are under the control of a complex set of biotic and abiotic driving forces, and as croplands are one of the main sources of greenhouse gases into the atmosphere (Reichstein *et al.*, 2003) a study of the temporal dynamics of soil respiration has great significance. However, a wide range of studies proved that several factors, like vegetation (Balogh *et al.*, 2019), soil temperature (T_s) (Lloyd and Taylor, 1994; Risk, Kellman and Beltrami, 2002), soil moisture (SWC) (Davidson *et al.*, 2000; Manzoni, Joshua P. Schimel and Porporato, 2012), nutrient availability and N treatments (Al-Kaisi, Kruse and Sawyer, 2008; Janssens *et al.*, 2010), and management practices like tillage, harvesting, loosening and sowing (Li, Ou and Chen, 2014) can affect soil respiration rates (Davidson, Belk and Boone, 1998;

Shen, Li and Fu, 2015; Wang *et al.*, 2016). The large uncertainty in Rs estimations could be caused by the fact that Rs is regulated by these multiple biotic and environmental factors (Hanson *et al.*, 2000) and because of the error of measurements (Nagy *et al.*, 2011).

Soil respiration is the second-largest flux in the global C budget and returns as much as 50-90% of annual gross primary production (GPP) back into the atmosphere (Bahn *et al.*, 2008) depending on the cited drivers (Bao *et al.*, 2010; Carbone *et al.*, 2011). Combined experiments (field and lab studies) could provide new insights into these effects. Among these factors, soil temperature and moisture are generally acknowledged as the dominant drivers of Rs but soil temperature is generally considered the most dynamic on both diurnal and longer time scales, therefore it is used in the majority of Rs models (Lloyd and Taylor, 1994; Daly *et al.*, 2008) being a good predictor of the dynamics of the soil CO_2 flux rate. In addition, a strong positive correlation between CO_2 efflux and soil temperature was found in natural and agricultural ecosystems (Lopes De Gerenyu *et al.*, 2005).

Soil moisture influences the production of CO_2 both by directly affecting the activity of microorganism and plant roots and the diffusion of gases through the soil pores (Li, Ou and Chen, 2014), and indirectly affecting the change of the substrate supply and plant growth (Reichstein *et al.*, 2003; Davidson, Janssens and Lou, 2006; Wan *et al.*, 2007). The changes in soil water content can strongly modify the total soil respiration. Under dry conditions the soil CO_2 efflux is low because the activities of micro-organisms and roots are typically low. Increased soil water content normally increases the bio-activity in the soil but if the soil water content is very high the total soil CO_2 efflux is reduced and the diffusion of oxygen will be limited resulting in a subsequent suppression of CO_2 emission (Moyano, Manzoni and Chenu, 2013).

Rs is a highly complex process consisting of two main components including autotrophic (R_a) and heterotrophic respiration (R_h) (Bond-Lamberty, Wang and Gower, 2004; Savage, Davidson and Tang, 2013; Balogh *et al.*, 2016). Plants are the most important autotrophs contributing to CO_2 efflux from soil by root respiration, while heterotrophic respiration mainly comes from free-living soil microorganisms (bacteria, fungi and actinomycetes) that subsisted by decomposition of soil organic matter (SOM) and organic matter in litter layer (Ekblad and Högberg, 2001; Moyano *et al.*, 2009), and is primarily regulated by the root activity and plant photosynthate supply (Tang, Baldocchi and Xu, 2005; Vargas *et al.*, 2011). Although the direct contribution of nematodes and soil macro-fauna (macroscopic invertebrates and small mammals) to R_h is small, they can greatly increase microbial respiration not only by fragmentation and

comminution of plant residues (Bonkowski *et al.*, 2000) but also by predation of some groups of microorganisms (Bonkowski, 2004). Litter-derived respiration and soil organic matter (SOM) decomposition are considered to be the R_h component (Moyano *et al.*, 2009). Balogh *et al.* (2016) reported that R_a was repressed by drought more than R_h in at a grassland site in Central Europe highlighting the differences among the components in their response to the environmental variables.

Soil microbial dynamics are controlled through complex interactions with plants and are influenced by a range of organic compounds added to soils from plants as root exudates and as litter inputs (Bardgett *et al.*, 2005). Therefore, the coupling between aboveground gross primary productivity (GPP) and carbon allocation to roots and root-associated organisms varies depending on the season (Savage, Davidson and Tang, 2013), especially the amounts of carbon allocated to the mycorrhizal fungi partners and to roots being variable in the different seasons (Högberg *et al.*, 2010; Abramoff and Finzi, 2015). The droughts typically lead to reduced carbon assimilation in plants (Huang and Fu, 2000; Ingrisch *et al.*, 2018) and reduced carbon transfer to the roots and the rhizosphere (Hasibeder *et al.*, 2015; Karlowsky *et al.*, 2018), resulting in a lower soil CO_2 efflux (Dreesen *et al.*, 2014). Consequently, the reduced belowground carbon allocation weakens plant–microbial interactions (Brüggemann *et al.*, 2011), as soil microorganisms strongly depend on plant-derived carbon inputs (Bardgett *et al.*, 2005).

1.2. Objectives

The main goals of the current study were:

1. To investigate the temporal dynamics of CO_2 efflux from the soil surface in a temperate cropland site during a two year long study period.
2. To analyze the response of the soil respiration components to the main environmental factors of cropland systems such as soil temperature (T_s), soil water content (SWC), N fertilization and biotic drivers as plant growth. Both field and laboratory measurements were conducted within the framework of the study.

2. LITERATURE REVIEW

2.1. Climate change

The progressing global climate change caused by human-induced increases in greenhouse gases represents one of the biggest scientific and political challenges of the 21st century. One of the greatest scientific challenges is the need to better understand the biological mechanisms regulating carbon exchanges between the land, oceans and atmosphere and how these exchanges will respond to climate change through climate-ecosystem feedbacks, which could amplify or dampen regional and global climate change (Heimann and Reichstein, 2008). Terrestrial ecosystems play a major role in such climate-feedbacks because they release and absorb greenhouse gases, such as carbon dioxide, methane and nitrous oxides, while storing large quantities of carbon in living vegetation and soils, thereby acting as a significant global carbon sink (Schimel *et al.*, 1994). Many interacting factors affect the sink activity of terrestrial ecosystems, including natural and anthropogenic disturbances (Magnani *et al.*, 2007), agricultural land use (Smith *et al.*, 2008).

The influence of climate change on the soil carbon sink remains a major area of uncertainty, especially as there is scope for warming to increase the liberation of carbon dioxide from soil to atmosphere due to enhanced microbial breakdown of soil organic matter. Such acceleration in carbon loss from soil could significantly exacerbate the soil carbon cycle feedback if predicted climate change scenarios are correct (Friedlingstein *et al.*, 2006). Ultimately, the net effect of climate change on ecosystem carbon budgets depends on the balance between photosynthesis and respiration (that is, autotrophic root respiration and heterotrophic soil microbial respiration) (Nagy *et al.*, 2007).

2.2. Greenhouse Gases (GHG_S) emissions

Over the last few decades, climate change has been studied by researchers in different disciplines, who have predicted an increase in the atmosphere temperature and in the oceans mainly due to the emissions of greenhouse gases (IPCC, 2007). The GHGs are those gases that absorb infrared radiation in the atmosphere, trapping heat and warming the surface of the Earth. The three main greenhouse gases (GHGs) associated with agriculture are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Muñoz *et al.*, 2010). Other important GHGs include water vapor and many halocarbon compounds, but their emissions are not considered to be influenced by agriculture. Soils act as sources for carbon dioxide and negligible sinks for methane and nitrous oxide, globally. Since both storage and emission capacities may be large, precise quantifications are needed to obtain reliable global budgets that are necessary for land-

use management (agriculture, forestry), global change and for climate research (Oertel *et al.*, 2016).

Pursuant to the United Nations Framework Convention on Climate Change (UNFCCC), Hungary, as a Party of the Convention, has been preparing annual inventories of greenhouse gas emissions using the IPCC methodology since 1994, the following direct greenhouse gases are taken into account: carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and others. The most important greenhouse gas is the carbon dioxide accounting for 78-79% of total GHG emissions globally. The main source of CO_2 emissions is burning of fossil fuels for energy purposes, including transport. CO_2 emissions have decreased by 42% since the middle of the 80's (Table1).

Table 1. Trend of emissions of GHGs, excluding LULUCF (Gg CO_2-eq) in Hungary.

	BY	**1990**	**1995**	**2000**	**2005**	**2010**	**2015**	**2016**	**2017**	**2018**
CO_2	85.68	73.46	61.69	58.60	60.60	52.12	46.62	47.39	49.68	49.62
CH_4	12.75	11.72	8.06	8.56	8.01	7.71	7.35	7.30	7.37	7.27
N_2O	11.13	8.37	4.75	5.40	5.61	3.72	4.53	4.80	4.80	4.85

Base year (BY) = average of 1985-87

2.2.1 Summary of national emissions and removal related trends in Hungary

According to the last National Inventory Report for 1985-2018 in Hungary, the total emissions of greenhouse gases in 2018 were 63.2 million tons carbon dioxide equivalents (CO_2-eq) excluding land use, land-use change, and forestry (LULUCF) sector. Taking into account also the mostly carbon absorbing processes in the LULUCF sector, the net emissions of Hungary were 58.6 million tons CO_2-eq. Although total emissions had not changed significantly since last year, the growing trend of the previous four years stopped and a slight decrease of 0.9% could be detected in 2018. Being about 6 tons, the Hungarian per capita emissions are below the European average (Kis-Kovács, 2020).

2.2.2 The latest concentrations and GWP of the three GHGs (CO_2, CH_4 and N_2O)

Carbon dioxide: The WMO Greenhouse Gas Bulletin showed that globally averaged concentrations of carbon dioxide (CO_2) reached 407.8 ppm in 2018, up from 405.5 ppm in 2017. The increase in CO_2 from 2017 to 2018 was very close to that observed from 2016 to 2017 and just above the average over the last decade. Global levels of CO_2 crossed the symbolic and significant 400 ppm bench mark in 2015 (WMO, 2018). CO_2 remains in the atmosphere for centuries and in the oceans for even longer. The growth rate of CO_2 averaged over three

consecutive decades (1985-1995, 1995-2005 and 2005-2015) increased from 1.42 ppm/yr to 1.86 ppm/yr and to 2.06 ppm/yr with the highest annual growth rates observed during El Niño events (WMO, 2018). It is calculated that the temperature rise produced by high CO_2 concentrations, plus the water positive feedback, would increase by 3-5 °C the global mean surface temperature in 2100 (IPCC, 2014). The National Oceanic and Atmospheric Administration (NOAA) Annual Greenhouse Gas Index shows that from 1990 to 2018 radiative forcing by long-lived greenhouse gases (LLGHGs) increased by 43%, with CO_2 accounting for about 80% of this increase.

Methane and nitrous oxide: Methane (CH_4) is the second most important long-lived greenhouse gas, has a 28 times higher global-warming potential (per molecule) than CO_2 over a time horizon of 100 years (CDIAC, 2017) and contributes about 17% of radiative forcing. Approximately 40% of methane is emitted into the atmosphere by natural sources (e.g., wetlands and termites), and about 60% comes from human activities like cattle breeding, rice agriculture, fossil fuel exploitation. Atmospheric methane reached a new high of about 1869 ppb in 2018 and is now at 259% of the pre-industrial level. For CH_4, the increase from 2017 to 2018 was higher than both that observed from 2016 to 2017 and the average over the last decade.

Nitrous oxide (N_2O) is emitted into the atmosphere from both natural (about 60%) and anthropogenic sources (approximately 40%), including oceans, soil, biomass burning, fertilizer use, and various industrial processes. Nitrous oxide yields a 265 times higher global-warming potential (per molecule) than CO_2 over a time horizon of 100 years (CDIAC, 2017). Its atmospheric concentration in 2018 was 331.1 ppb. This is 123% of pre-industrial levels. The increase from 2017 to 2018 was also higher than that observed from 2016 to 2017 and the average growth rate over the past 10 years

2.3. Overview of sources and sinks of carbon dioxide efflux

2.3.1 Sources of carbon dioxide efflux

Soil carbon represents 80% of the global terrestrial ecosystem carbon stock, 2-3 times more than the terrestrial vegetation carbon pool (500–600 Gt), and twice of the atmospheric carbon pool (750 Gt; Hashimoto *et al.*, 2015). CO_2 is the main long-lived greenhouse gas in the atmosphere related to human activities (Cassia *et al.*, 2018), the latest estimates reveal that global CO_2 emissions are unstable and have grown significantly in the last century (Rehman, Ozturk and Zhang, 2019).

According to the Intergovernmental Panel on Climate Change (IPCC), CO_2 accounts for about 76.7% of anthropogenic greenhouse gas emissions; 56.6% is from the fossil fuels which is

the largest and most rapidly growing source of CO_2 emission into the atmosphere, about 17.3% is from deforestation (LULUCF) and 2.8% is from the other sources (IPCC, 2014). Some of CO_2 sources and sinks are shown in (Figure 1). In recent years, CO_2 emissions are considered the key source of the greenhouse effect and have garnered intense attention (Saidi and Hammami, 2015). The natural sources of CO_2 basically correspond to the respiration process of terrestrial and aquatic organisms (Rehman, Ozturk and Zhang, 2019). Other process like decomposition of plant residues and organic matter by the action of soil microbes and respiration of microbes and plant roots are the major sources of emission of CO_2 in soil (Oorts *et al.*, 2007). Although evapotranspiration is a key process in ecosystem functioning and has global significance, it was only recently found that it may play a direct and significant role in carbon cycling between the plants and the soil by decreasing root respiration rates (Bekku *et al.*, 2011; Grossiord, Mareschal and Epron, 2012).

In terrestrial ecosystems, during photosynthesis atmospheric CO_2 is assimilated, and then released either via autotrophic respiration or through heterotrophic decomposition of carbon compounds differing in recalcitrance and sensitivity to temperature (Davidson et al. 2006), while net ecosystem exchange (NEE) is the difference between photosynthesis and ecosystem respiration. A positive NEE indicates a CO_2 source, whereas a negative NEE reveals a CO_2 sink (Oertel et al. 2016). An estimated 60 Pg C yr^{-1} is emitted to the atmosphere by autotrophic respiration and a similar amount is emitted as a result of heterotrophic respiration (Reay and Grace, 2007), where the CO_2 emission resulting from soil respiration is 10 to 15 times greater than the CO_2 emission from fossil fuels (Raich and Schlesinger 1992). Rehman, Ozturk and Zhang (2019) mentioned that the emission of CO_2 due to volcanic activity is relatively minor on a global scale, accounting for 0.02 to 0.05 (Pg C yr^{-1}).

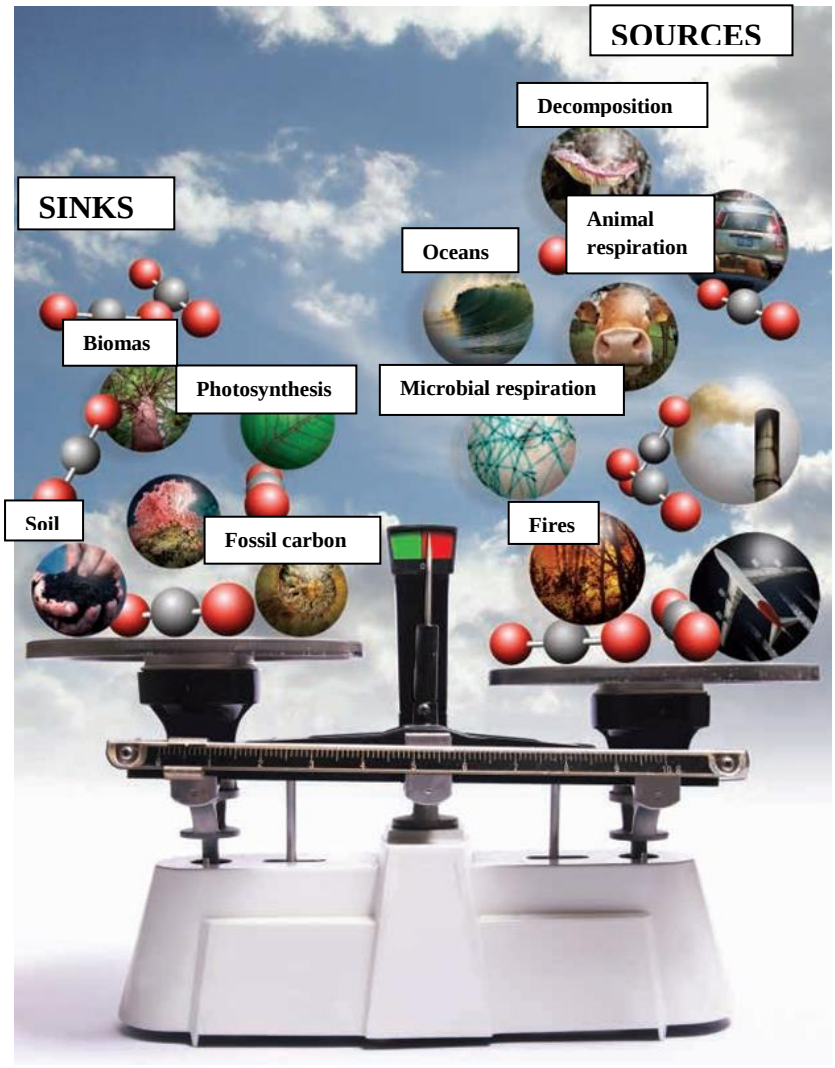

Figure 1. Natural and anthropogenic sources and sinks of carbon dioxide efflux

Image source: Orbiting Carbon observatory-2 (OCO-2).

Note that this diagram does not include all carbon sources and sinks.

Kuzyakov (2006) mentioned in his study that there are five main biogenic sources of CO_2 efflux from soils which have been distinguished and described according to the mean residence time of carbon and to their turnover rates. They are root respiration, rhizomicrobial respiration, decomposition of plant residues, the priming effect induced by root exudation or by addition of plant residues, and basal respiration by microbial decomposition of soil organic matter (SOM). These sources can be grouped in several combinations to summarize CO_2 efflux from the soil including: root-derived CO_2, plant-derived CO_2, SOM-derived CO_2, rhizosphere respiration, heterotrophic microbial respiration (respiration by heterotrophs), and respiration by autotrophs.

2.3.1.1 Soil respiration (Rs) and its components

Because the major part of the source activity is the result of soil respiration (Rs), the variability of this CO_2 flux has a significant relevance in the carbon balance (Claire L. Phillips *et al.*, 2017). Soil CO_2 efflux, commonly referred to as soil respiration (Rs; μmol CO_2 m^{-2} s^{-1}) is the primary path by which C fixed by land plants returns to the atmosphere (Barba *et al.*, 2018), Rs is the second largest carbon flux next to gross primary production between the terrestrial ecosystem and the atmosphere at the global scale (Xu and Shang, 2016). Thus, the magnitude of soil respiration can turn the carbon budget from a net sink to a net source in dry years (Nagy *et al.*, 2007).

Rs is an important part of the carbon cycle in terrestrial ecosystems, and its dynamics directly affect the carbon balance at the regional or global scale (Heimann and Reichstein, 2008; Zhang *et al.*, 2015). In fact, Rs is an enzyme-catalyzed biochemical process, and the enzyme activity is mainly mediated by temperature (Baldrian *et al.*, 2013). Variations in Rs have been identified both spatially and temporally by researchers and these variations are affected by both biotic and environmental factors (Wang, Zhao and Chen, 2015). Lellei-Kovács *et al.* (2016) mentioned that both soil moisture availability and temperature may alter with a changing climate, and this will affect the root activity and decomposition processes, potentially changing rates of CO_2 efflux from soils.

Autotrophic respiration (R_a) mainly comes from plant roots, mycorrhizae, and other microorganisms that are in obligate associations with living roots and the organic exudates provided by aboveground parts of the plant through photosynthates (Bond-Lamberty and Thomson, 2010)**.** Hiiesalu *et al.* (2014) mentioned that arbuscular mycorrhizal fungi (AMF) are obligatory symbiont soil fungi, forming intimate mutualistic associations in 70–90% of the plant species in grasslands. About 10–20% of the assimilated C may be attributed to AMF in exchange for acquiring essential nutrients for plant productivity and water (van der Heijden *et al.*, 2015). Therefore CO_2 efflux originated from SOM decomposition in planted soils is 'masked' by root-derived CO_2, also called rhizosphere respiration, comes from dead roots, root respiration *per se* and rhizomicrobial respiration of exudates. Root-derived CO_2 is thought to comprise 40–60% of total CO_2 flux (Raich and Schlesinger 1992), although these values strongly depend on growth stage of the vegetation especially in agriculture soils.

Subke, Inglima and Francesca Cotrufo (2006) found that the estimated ratios of the autotrophic respiration vary between 50 and 60% in forests, this value can range from 10% to 90% seasonally (Hanson *et al.*, 2000). In temperate grasslands the contribution of the autotrophic

component to the total soil respiration amounted to about 40% on annual scale (Heinemeyer *et al.*, 2012).

Many experiments suggest that R_a strongly depends on recent ^{13}C photosynthates as indicated by rapid and pronounced declines in soil respiration after clipping, shading or phloem girdling (Craine, Wedin and Chapin, 1999; Wan and Luo, 2003) while other studies have reported only minor effects (Zhou and Braun, 2007; Bahn *et al.*, 2009; Bond-Lamberty and Thomson, 2010). Dramatic increases in R_a have been found in strongly seasonal ecosystems at high latitudes in late as opposed to early summer (Högberg *et al.*, 2010) indicating that R_a is dependent on plant phenology and/or the season. Higher R_a is likely dominated by increased growth respiration, while maintenance respiration is assumed to undergo less seasonal change (Wieser and Bahn, 2004).

The decomposition depends on substrate availability and soil biota (Fang and Moncrieff, 1999; Drotz *et al.*, 2010; Feng *et al.*, 2017),their decomposition is attributed mainly to soil bacteria and fungi and has about 50-55% share in the total soil respiration in dry grasslands (Bao *et al.*, 2010; Gomez-Casanovas *et al.*, 2012).

Although the direct contribution of nematodes and soil macro-fauna (macroscopic invertebrates and small mammals) to R_h is small, they can greatly increase microbial respiration not only by fragmentation and comminution of plant residues (Bonkowski *et al.*, 2000) but also by predation of some groups of microorganisms (Bonkowski, 2004). The respiration rate of soil microbes is determined by the total amount of soil microbes and the ability of microorganisms in nutrient mineralization (Mgelwa *et al.*, 2019). Those studies indicated that the three major microorganisms in soils (bacteria, fungus and actinomycetes) work together to affect Rs. For example, Yang, Liu and Zhang (2019) found that the bacterial/fungus ratio is positively correlated with Rs.

There is evidence that fresh C input into soil can increase, decrease or have little or no effect on R_h (Domanski and Kuzyakov, 2000; Fontaine *et al.*, 2007). This variability of the R_h response to soil C availability may arise in part because soil organic matter (SOM) consists of several functional C pools with different levels of protection and recalcitrance (Six and Jastrow, 2002).

On average, R_a and R_h contribute equal amounts to total soil respiration, ranging from 10 to 90% in single studies (Hanson *et al.*, 2000), with the contribution of R_a increasing with annual soil CO_2 efflux (Subke, Inglima and Francesca Cotrufo, 2006; Bond-Lamberty and Thomson,

2010). Some studies indicated that heterotrophic and autotrophic components showed no differences in their temperature sensitivity (Sulzman *et al.*, 2005; Graham *et al.*, 2014), whereas other authors observed significant differences (Moyano, Kutsch and Schulze, 2007; Gomez-Casanovas *et al.*, 2012; Matteucci *et al.*, 2015). Heterotrophic respiration responds primarily to soil temperature and soil moisture, while mycorrhizal respiration responds more readily to photosynthetic active radiation in an indirect way in grasslands under sheep grazing (Heinemeyer *et al.*, 2012).

Both autotrophic and heterotrophic components were shown to be sensitive to water shortages (Carbone *et al.*, 2011; Moyano, Manzoni and Chenu, 2013). Balogh *et al.* (2016) and Papp *et al.* (2018) found that the autotrophic component of the soil respiration is more sensitive to drought than the heterotrophic ones in the dry grassland ecosystem studied. Thus, carbon source activities during drought periods identified by NEE measurements originated from carbon sources already stored, thereby decreasing the carbon content of the soil. In contradictory to some authors who found that the heterotrophic component of soil CO_2 efflux was more sensitive to the droughts stress than the autotrophic component (Scott-Denton, Rosenstiel and Monson, 2006; Suseela *et al.*, 2012), while other studies suggested that the drought stress period mostly decreased the rates of root-and mycorrhizal respiration compared to the heterotrophic component originating from microbial respiration (Lavigne, Foster and Goodine, 2004). These contradictory findings could be the functional differences of the study sites and vegetation types (Nagy *et al.*, 2011). However, both autotrophic and heterotrophic components receive assimilates from the shoots (Finzi *et al.*, 2015; Shahzad *et al.*, 2015), therefore the dynamics of belowground carbon allocation although the responses can vary with the type of vegetation (Casals *et al.*, 2011).

2.3.2 Sinks of Carbon dioxide efflux

2.3.2.1 Photosynthesis and phloem transport

Photosynthetic activity supplying carbohydrates from leaves to roots and rhizosphere is a key driver of soil CO_2 and it is acknowledged as CO_2 sink (Kuzyakov and Gavrichkova, 2010). Hoégberg *et al.* (2001) mentioned that trenching and girdling methods based on the idea of interrupting the phloem transport from the leaves to the roots were adopted in forests, while the clipping and shading methods were applied mainly in grasslands and shrub communities (Carbone *et al.*, 2011). It was newly found that photosynthesis has a time-lagged (a few hours) positive effect on the respiration of roots and root-associated soil microbes, Balogh *et al.* (2017) explained that by an increase in easily accessible non-structural hydrocarbon sources for the roots and root-associated organisms within the period of their study. The magnitude of this effect and the fast response (short time lags) should consider photosynthesis as one of the main drivers

of C fluxes. This calls for incorporating photosynthesis in soil C turnover models and carbon balance studies (Moyano, Kutsch and Schulze, 2007; Kuzyakov and Gavrichkova, 2010; Balogh *et al.*, 2011).

Plant-internal C allocation

Partitioning of the newly assimilated carbohydrates within the plant occurs via loading of sugars into the phloem, transport in the sieve tube system and unloading at the sites of demand (McQueen *et al.*, 2005). van bel (2003) reported that the pressure-driven mass flow system of the phloem allows C compounds to be transported over long distances in the plant from source to sink tissues. Consequently, the C partitioning is controlled by the supply of assimilates via photosynthesis, but also depends on the ability of different organs to use the available supply (Vardlaw, 1969). Generally, time lags determined as propagation of fluctuations in $\delta^{13}C$ at natural abundance raise with tree height, with transport rates between 0.07 and 0.5 m h^{-1} (Kuzyakov and Gavrichkova, 2010), although carbon translocation velocities in tall plants are often higher (Mencuccini and Hölttä, 2010), probably due to stronger root C sinks connected with a larger belowground biomass. In some studies, seasonal changes in belowground C allocation had no effect on the time lag between assimilation and use of assimilates in belowground respiration (Horwath, Pregitzer and Paul, 1994; Högberg *et al.*, 2010), proposing that phloem path length and structural differences were the main determinants of C transfer velocity.

Carbon transfer to soil biota

Carbon allocated to roots can stimulate exudation, which in turn increases microbial respiration in the rhizosphere (Bowling *et al.*, 2002; Tang, Baldocchi and Xu, 2005). Up to 40% of photosynthates are exudated by roots and are quickly respired or invested by rhizosphere microorganisms in biomass (Kuzyakov and Cheng, 2001). Among rhizosphere microorganisms, mycorrhizal fungi are of great relevance to plant-soil C interactions (Jones, Nguyen and Finlay, 2009). Several studies indicate that mycorrhizal fungi can use up to 35% of recent plant photosynthates (Hoégberg *et al.*, 2001; Heinemeyer *et al.*, 2007; Stuart Chapin III *et al.*, 2009).

Jones, Nguyen and Finlay (2009) mentioned that the large variability in C turnover times of soil microorganisms could be correlated with a switch between different functional groups of microbes, as e.g. mycorrhizal mycelium and rhizosphere bacteria can be used as C substrates by other soil microorganisms. It has been shown with ^{13}C-pulse labelling that also soil macrofauna (e.g. earthworms) may quickly incorporate plant exudates as a C source in addition to above- and

belowground plant litter inputs, probably by incorporating ^{13}C-labeled microorganisms (Ostle *et al.*, 2007).

The major factor hindering the quantification of the carbon allocation driven part of soil respiration is the temperature co-varying with gross primary productivity on seasonal and diel time scales (Gomez-Casanovas *et al.*, 2012; Savage, Davidson and Tang, 2013). Because the droughts typically lead to reduced carbon assimilation in plants (Harper *et al.*, 2005; Hasibeder *et al.*, 2015) and reduced carbon transfer to the roots and the rhizosphere (Hosen, Tsuruta and Minami, 2000; Högberg *et al.*, 2010), resulting in a lower soil CO_2 efflux (Dreesen *et al.*, 2014). Consequently, the reduced belowground carbon allocation weakens plant-microbial interactions (Brüggemann *et al.*, 2011), as soil microorganisms strongly depend on plant-derived carbon inputs (Bardgett *et al.*, 2005).

Moreover, soil moisture influences the quantity of water supplied by the xylem to the collection phloem, affecting the turgor pressure differences between two phloem ends. Potentially, all environmental factors which affect photosynthesis (vapor pressure deficit, radiation, CO_2 concentration, etc.) might have similar consequences (Brüggemann *et al.*, 2011).

Carbon isotopic signals

Isotopic signatures of soil respiration are a useful tool for estimating the contribution of its main components (Carbone *et al.*, 2011; Hopkins *et al.*, 2013) and for tracing the transfer of C in ecosystems (Johnson *et al.*, 2002; Carbone and Trumbore, 2007; Högberg *et al.*, 2008) and thus have the potential to provide insights into the coupling of photosynthetic assimilation and soil respiratory fluxes. Natural abundance techniques make use of the fact that different carbon pools in the environment can have different ratios of carbon isotopes; for example, the $\delta^{13}C$ of C3 plants (e.g. wheat) ranges from 25‰ to 35‰, whilst that of C4 plants (e.g. maize) ranges from 10‰ to 20‰ (Staddon, 2004). The difference in ^{13}C signatures of biological material occurs as a result of differing discrimination against ^{13}C in different biochemical pathways (Ehleringer, 1991; Lajtha, 1994). In C3 plants the enzyme RuBisCo contributes to most of the discrimination but this effect becomes less expressed as stomata close because of water stress (Farquhar, Ehleringer and Hubick, 1989).

Heterotrophs CO_2-fixation is estimated to be 4 to 7% net microbial respiration (Miltner *et al.*, 2005), is another pathway that drives to the different isotopic composition of amino acids and fatty acids (Feisthauer *et al.*, 2008) and could have a significant impact on the overall isotopic signal of microbial biomass and the CO_2 respired. Furthermore, autotrophic and

photoautotrophic CO_2-fixation must be considered in terms of C fractionation, autotrophic organisms may express a high level of isotopic fractionation, and fractionation has been reported to be interestingly high within the context of inorganic C fixation (Cowie *et al.*, 2009). Several reviews (Badeck *et al.*, 2005; Bowling, Pataki and Randerson, 2008; Cernusak *et al.*, 2009) have shown that heterotrophic organs (branches, stems and roots) are enriched in ^{13}C compared to autotrophic organs, which supply them with carbon.

Knohl *et al.* (2005) found that the seasonal changes are expected to reflect the changes in the contributions of source components rather than the changes in the isotopic signals of the component itself. Nevertheless, SOM $\delta^{13}C$ can also change during the year with fresh plant material being more depleted in ^{13}C than the older SOM components (Bowling *et al.*, 2002); therefore fresh litter may contribute to the decreasing $\delta^{13}C$ of the heterotrophic component. Moyes *et al.* (2010) mentioned that drying of the surface layers can also modify $\delta^{13}CO_2$ since heterotrophic respiration may be limited to the profound layers of the soil. Furthermore, Balogh *et al.* (2015) reported that drying of the soil can also change the amount of CO_2 produced in the top layer of soil by allowing greater atmospheric inroad and thereby enriching soil air in ^{13}C (Phillips *et al.*, 2010). Additionally, Pate and Arthur (1998) demonstrated that the ^{13}C abundance of recent photosynthates in the phloem varied dynamically depending on conditions of weather, with high ^{13}C of C compounds in the phloem occurring after sunny periods with low rainfall.

Carbon isotope fractionation in plants

Farquhar, O'Leary and Berry (1982) mentioned that carbon isotope fractionation in plants has been separated into (1) photosynthetic carbon isotope fractionation, including CO_2 diffusion, carboxylation, as well as dark and photorespiration, and (2) post-photosynthetic fractionation (von Caemmerer *et al.*, 1997). However, Gessler *et al.* (2008) reported that, if the distinction between the main fractionation step by RubisCO activity and all downstream fractionation steps should be made, the latter can be collectively addressed as post- carboxylation fractionation

Post-carboxylation fractionation is also responsible for differences in $\delta^{13}C$ between plant organs (Cernusak *et al.*, 2009). Next to photosynthetic also post-carboxylation carbon isotope fractionation might account for diel variations in the isotopic composition of carbon exported from the leaves to heterotrophic tissues (Tcherkez *et al.*, 2004) and of respired CO_2 (Werner *et al.*, 2011). One of the first post-carboxylation fractionation steps occurs in the Calvin cycle during aldolase condensation (i.e. synthesis of fructose 1,6-bisphosphate from triose-phosphates), enriching ^{13}C in the C3 and C4 atom positions of hexoses while leaving behind the light triose phosphates (Gleixner and Schmidt, 1997)

In the other hand, Decarboxylation of pyruvate by the pyruvate dehydrogenase complex (PDH), coupled to the glycolysis pathway, releases relatively ^{13}C-enriched CO_2, using the C-3 and C-4 atoms of glucose (Melzer and Schmidt, 1987). Consequently, acetyl-CoA is relatively depleted in ^{13}C, as are fatty acids or CO_2 released during the tri-carboxylic acid cycle (TCA).

2.4. Factors affecting soil CO_2 efflux

Soil-respired carbon dioxide (CO_2) integrates the release of CO_2 from soils via root (autotrophic) and microbial (heterotrophic) respiration through soil pores, and this release from the soil system can be measured at the soil surface (Rolston, 1986). The emitted CO_2 efflux between soil and atmosphere strongly affected by several environmental factors, with soil temperature and soil water content being the main abiotic drivers, also biotic factors such as vegetation type and functioning (Raich and Tufekcioglu, 2000; Balogh *et al.*, 2019) which can include canopy cover, leaf area, and litter deposits, soil properties (e.g. soil texture, pH and C/N ratio) and agricultural management practices (Flechard *et al.*, 2005); N input, tillage application (Li, Ou and Chen, 2014), irrigation and others which can all influence soil microbes and their activity.

Biotic and abiotic factors can directly influence each other and often interact. Soil moisture and soil temperature are considered the two most influential abiotic factors influencing soil respiration (Daly *et al.*, 2008).

2.4.1 Environmental factors

Soil temperature and soil moisture have an impact on emissions and uptakes of gases through their effects on microorganisms and root activity (Smith *et al.*, 2003). Rates of chemical and microbial processes generally increase exponentially with temperature, as long as other factors (substrate or moisture availability) are not limiting (Meixner, 2006). In field studies, the seasonal development of soil temperature and soil moisture usually is reflected in the seasonal course of soil gas emissions. Gasche and Papen (2002) and Kitzler *et al.* 2006) found that in temperate climates, soil emissions typically peak during summer when temperatures are highest.

2.4.1.1 The effect of soil temperature on CO_2 emissions

Temperature is often a predominant factor controlling biological metabolic processes and a broad spectrum of relationships between temperature and Rs has been tested (Subke and Bahn, 2010; Wu *et al.*, 2010). Soil temperature was found to be the principal factor influencing soil respiration on both diurnal and longer time scales (Yuste *et al.*, 2003; Balogh *et al.*, 2019), therefore it is used in the majority of Rs models (Lloyd and Taylor, 1994; Daly *et al.*, 2008) due to its general effect on soil microclimate conditions and the biological activity of below-ground

organisms (Yuste *et al.*, 2003; Dhital *et al.*, 2019) and being a good predictor of the dynamics of the soil CO_2 flux rate. In addition, a strong positive correlation between CO_2 efflux and soil temperature was found in natural and agricultural ecosystems (Ramirez, Craine and Fierer, 2010)

Oertel *et al.* (2016) also mentioned that soil temperature is important to explain the variations of trace gas emissions from soils. Rising temperatures stimulated soil respiration by accelerating rates of C cycling via autotrophic respiration and heterotrophic decomposition of organic matter (Bond-Lamberty and Thomson, 2010; Melillo *et al.*, 2011), therefore it is an important topic in climate change research as well. Methane and N_2O emissions are additionally forced by increasing soil respiration rates with increasing soil temperatures, leading to decreasing O_2 concentrations in the soil (Butterbach-Bahl *et al.*, 2013; Moyano, Manzoni and Chenu, 2013). The positive temperature effect may be overlain by soil water stress, since water is needed as a transport medium for nutrients required by microbes (Fowler *et al.*, 2009). Nitric oxide and CO_2 emissions increase exponentially with temperature (Fang and Moncrieff, 2001; Tang, Baldocchi and Xu, 2005).

Chengfang *et al.* (2020)Chengfang et al. (2020) suggested that the response of Rs to temperature is highly correlated with species diversity and hydrological changes and Luan *et al*, (2018) reported that tree species diversity can promote soil carbon stability by weakening Rs and its temperature sensitivity in temperate forests. Therefore, the results suggested that T_s was the primary factor limiting Rs, but when the temperature was suitable for Rs, it was also affected by other environmental factors (Wang *et al.*, 2018). The temperature dependency of gas emissions from soils can be described with the temperature sensitivity factor Q_{10}. It expresses the rate of change in a chemical or biological system with a temperature change of 10 °C (Berglund, Berglund and Klemedtsson, 2010) and usually increases with soil depth (Tang *et al.*, 2003). Q_{10} is 2.4 with a range of 1.3-3.3 for soil respiration; based on a data reviewed by Raich and Schlesinger (1992). The values have been confirmed by current studies of (Hu *et al.*, 2015) with a range of 1.7-2.5 and (Jiang *et al.*, 2015) with an average of 2.2.

Zou *et al.* (2018) found that the effects of soil temperature on CO_2 efflux could be described with a simple exponential regression model as mentioned above for both the warmed and control plots, consistent with a number of studies (Briones, Poskitt and Ostle, 2004; Zimmermann *et al.*, 2009; Laganière *et al.*, 2012; Shi *et al.*, 2012). Most commonly, the exponential function has been used to model the temperature-respiration relationship (Davidson et al. 2006; Beier et al. 2009; Vicca et al. 2014). In situ Rs studies covering a wide range of temperature and moisture conditions are rare and the limited availability of such data affects the

ability of modellers to fit Rs functions to empirical data (Vicca *et al.*, 2014). Consequently, to study Rs on a wide range of ecosystems and climatic conditions, the Arrhenius, Lloyd–Taylor, Gaussian, and Quadratic functions have been used (Lloyd and Taylor, 1994; Reichstein and Beer, 2008; Tuomi *et al.*, 2008; Lellei-Kovács *et al.*, 2011; González-Ubierna, de la Cruz and Casermeiro, 2014).

2.4.1.2 The effect of low soil temperature on CO_2 emissions (freezing)

Temperature is also important for the regulation of freeze-thaw events, forcing gas emissions from soils (Holst *et al.*, 2008). In contrast, winter CO_2 emissions are considered less important for the annual emission budget since root respiration is low in temperate or more polar environments (Groffman *et al.*, 2006). During wintertime, soil water content is close to saturation reducing the O_2 content (Groffman *et al.*, 2009). During freeze-thaw cycles, additional nutrients are released for microbial metabolism through the disaggregation of soil particles (Christensen and Christensen, 1991). After thawing, dead organic material (e.g., dead plant roots) forces increased microbial soil respiration (Mørkved *et al.*, 2006), thus, a gaseous exchange between the atmosphere and soil does not stop even in frozen soil, resulting in the accumulation of CO_2 during winter and its release into the atmosphere during spring thaw events (Drotz *et al.*, 2010). Also Drotz *et al.* (2010) argue that winter emissions are relevant for the temperate climate zone.

Microbial CO_2 production has been detected at temperatures down to –39 °C in frozen surface horizons of tundra (Panikov *et al.*, 2006), and anabolic activity has been determined in bacterial populations from permafrost down to –6 °C (Bergholz, Bakermans and Tiedje, 2009) and –20 °C (Rivkina *et al.*, 2000). It has also been suggested that there is no evidence of a minimum temperature for the metabolism of microbes in permafrost and ice (Price and Sowers, 2004). Both bacteria and fungi adjust their membrane composition in response to changes in temperature >0 °C in ways that maintain a lamellar liquid crystalline phase (Rilfors and Lindblom, 2002). This adjustment requires anabolic processes. Also, soil respiration from bacteria was observed down to soil temperatures of –7 °C (Brooks, McKnight and Elder, 2005).

Drotz *et al.* (2010) concluded that not only catabolic processes (CO_2 production) but also anabolic microbial processes (synthesis of biomass) proceed below 0 °C in frozen boreal forest soils. It has been suggested that no (or highly limited) microbial growth can take place at temperatures <0 °C because the severely limited fluidity of the cell membrane at low temperatures inhibits the utilization of substrates from the environment (Nedwell, 1999). It has also been suggested that the soil microbial community undergoes a shift from growth to survival-

related metabolism, with decreased carbon allocation to anabolic processes, when subjected to various stresses, including freezing (Schimel, Balser and Wallenstein, 2007).

2.4.1.3 The effect of soil water content on CO_2 emissions

Next to temperature and light availability, soil moisture is a main driver of net primary productivity and thus strongly affects the accumulation and cycling of soil carbon. Moisture in soils is essential for both plant growth (Huxman *et al.*, 2004) and soil microbial activity, thus affecting carbon inputs as well as the decomposition of litter and soil organic matter, and hence heterotrophic respiration and carbon outputs (Davidson, Janssens and Lou, 2006; Moyano, Manzoni and Chenu, 2013).

Soil moisture can be expressed using a number of different units: relative to soil mass (gravimetric moisture μ (kg water/kg dry soil)), relative to volume (volumetric moisture, θ (m^3 water/m^3 soil)), relative to soil pore space (water-filled pore space, WFPS (v%)), relative to water holding capacity WHC (inches), as water potential ψ (MPa) and other relative measures to be interpreted at the soil core scale (Hillel, 1998; Paul *et al.*, 2003; Moyano *et al.*, 2012). During dry periods soil water content decreases and water in soil pores becomes increasingly disconnected. As a result, the diffusion of solutes slows down, thus limiting substrate supply to microbial communities (Skopp, Jawson and Doran, 1990; Schjønning *et al.*, 2003). Additionally, as the matric potential in soil water decreases, cells must spend energy to attain osmotic equilibrium with the surrounding solution (Schimel, Balser and Wallenstein, 2007) and to produce extra- cellular substances that can buffer variations in soil moisture and improve diffusivity (Or *et al.*, 2007), thus reducing their growth and respiration rates. Because the diffusion rate of oxygen through air is much higher than through water (Cook and Knight, 2003), the metabolic activity of aerobic organisms also decreases as soil pore space fills with water and approaches saturation levels (Franzluebbers 1999). The relationship between heterotrophic respiration and soil moisture emerges from the interactions of physical (e.g., diffusion), biochemical (enzyme dynamics), and physiological (osmoregulation) processes.

Wallenstein and Hall (2012) mentioned that ecological factors may also play a role, with possible effects on microbial community composition and trophic interactions. Since all these factors vary through time, the resulting respiration response to water availability is also expected to be time-dependent. Despite a general understanding of the above processes, soil moisture effects on soil respiration are still the subject of active debate. For example, the tolerance to water stress varies significantly across soil microorganisms (Lennon et al. 2012; Manzoni et al. 2012).

Moyano, Manzoni and Chenu (2013) found a low tolerance corresponding to complete metabolic inactivity at ca. –1.5 MPa is found in strains of bacteria (spiral bacteria), while the highest tolerance of over –60 MPa has been observed in fungal species (yeasts, ascomycetes and xerophilic fungi). However, recent studies show that the response of microbial activity to water potential is very similar across soils of different properties and under different climates (Moyano, Manzoni and Chenu, 2013).

(Manzoni, Joshua P Schimel and Porporato (2012) suggested that the different tolerance of soil organisms to water stress is not apparent because activity in dry soils is primarily limited by the diffusion of substrates. This may not be the case for litter, where decomposition was found to stop at more negative water potentials than in mineral soils, indicating that decomposers are affected less by diffusion than by osmotic stress or enzyme deactivation. Therefore it seems that soil microbes might be adapted to wetter or drier conditions depending on the climatic situation of their site of origin (Sowerby *et al.*, 2005). Soil moisture may limit soil respiration in two ways, either by limiting aeration and therefore the diffusivity of CO_2 when the soil is wet, or by osmotic stress of soil microbial communities when it is too dry (Smith *et al.*, 2003). Therefore, the changes in soil water content can strongly modify the total soil respiration. Furthermore, Schaufler *et al.* (2010) concluded that intermediate soil moisture conditions (between 20% and 60% WFPS) produced the highest CO_2 emissions, which were related to site-specific rainfall and soil texture conditions. In addition, Lee *et al.* (2009) observed that CO_2 fluxes increased with greater SWC in soils planted with maize, but not sunflower or chickpea, implying an interaction between crops and SWC that influenced soil respiration. Han *et al.* (2018) reported that precipitation events may decrease Rs by increasing the soil moisture and anaerobic conditions of coastal wetlands

However, the frequency and intensity of drought are expected to increase in the future due to climate change, which may increase Rs temperature sensitivity and accelerate SOC loss (Chen *et al.*, 2018). In general, these studies on Rs involved almost all terrestrial ecosystems, such as forests (Borkhuu *et al.*, 2015; Gao *et al.*, 2018; Chen *et al.*, 2019), grasslands (Mukumbuta, Shimizu and Hatano, 2019), wet lands (Daniel *et al.*, 2015; Chen *et al.*, 2018; Han *et al.*, 2018) and other natural ecosystems at the local and global scales.

2.4.1.4 Relationship between soil moisture and Rs

The relationship between soil moisture and Rs has been modelled using many different functions that include linear (Leiros *et al.*, 1999), exponential (Rodrigo *et al.*, 1997), second- order exponential, that is, Gaussian (Howard and Howard, 1993; Mielnick and Dugas, 2000; Vicca *et*

al., 2014), lognormal (Balogh *et al.*, 2011) and reverse exponential (Zhou and Braun, 2007) relationships. Mechanistic studies of the relationship between soil moisture and Rs conducted by Davidson et al. (2006) revealed that not only CO_2 efflux is influenced by moisture-induced changes in soil physical properties, but also autotrophic root respiration and heterotrophic microbial decomposition are directly impacted by changes in soil moisture.

Evaluation of the impact of soil moisture is more difficult than that of temperature because the efficiency of water uptake is influenced by various soil physical properties and also by physiological processes of the organisms. At any given soil moisture content, water uptake may differ for numerous reasons such as soil texture (sand or clay), plant water use efficiency, stress tolerance and soil microbial composition (for example, fungal to bacterial ratio) (Moyano, Manzoni and Chenu, 2013)

2.4.2 Biotic factors

Kuzyakov (2006) mentioned that the most of soils in the world are covered with vegetation, and the vegetation may contribute strongly to the total CO_2 efflux by root and rhizo-microbial respiration.

2.4.2.1 Photosynthesis

Photosynthetic activity can influence below-ground respiration in many aspects. Soil respiration in forests decreased with stand age, caused by a lower fine root biomass (Oertel *et al.*, 2016). The decrease levelled out with stand age since lower root respiration rates in old forest were partly compensated for by higher microbial respiration due to higher organic inputs (Saiz *et al.*, 2006). Fornara and Tilman (2008) found that a high biodiversity with a balanced ratio of Leguminosae and C3 and C4 plants (carbon fixation) at grassland sites resulted in an increased C-sequestration potential. Furthermore, soil water content increases, since the opening time of stomata can be reduced, while soil temperatures decrease due to the higher leaf area and related shade (Dorodnikov *et al.*, 2009; Kim, 2013).

There has been a debate in the past about some studies saying the influence of vegetation on soil microclimate is sufficient enough to explain differences in Rs among vegetation types (Raich and Tufekcioglu, 2000). Others found that the correlation between climate characteristics, net primary productivity, and Rs has caused scientists to speculate which factors were driving the differences in Rs between different vegetation types (Raich and Schlesinger 1992). In a study by (Reichstein *et al.*, 2003), they corrected the ecosystem respiration data for soil water content and soil temperature influences resulting in site-specific, standardized respiration rates. These standardized rates were correlated with leaf area index (LAI) and leaf production indicating that

both climate and vegetation type played a major roles in explaining the spatial variability in Rs. On the contrary, another study found a correlation between net primary productivity (NPP) and Rs when comparing various ecosystem types. They concluded that the correlation was mainly caused by a background correlation of both factors with climate variables (J. W. Raich and Schlesinger, 1992). The negative correlation may be related to the fact that litter with higher lignin content is more difficult to break down by heterotrophic organisms, lowering Rs (F.S. Chapin III *et al.*, 2009) .

Balogh *et al.* (2017) mentioned that biotic drivers of soil respiration represented a significant supply-side (plant) control of the different process. Those biotic drivers that combine over longer time periods are useful in describing the physiological state of the vegetation and also phenological changes, but they are not suitable to explain the diel variability of soil respiration. Two plant physiological processes, acting in opposite directions, could be relevant at diel timescale; photosynthesis and transpiration. Firstly, it was recently found that photosynthesis has a time-lagged (a few hours) positive effect on the respiration of roots and root-associated microbes. Balogh *et al.* (2017) explained this by an increase in easily accessible non-structural hydrocarbon sources for the roots and root-associated organisms within this period. Secondly, it was found that the effect of transpiration could reduce root respiration due to CO_2 transport through the transpiration stream (Balogh *et al.*, 2017).

2.4.2.2 Fires

The greenhouse gas balance of soils can be affected by fires in ecosystems, depending on duration of the fire and temperature, with burned areas showing lower CO_2 fluxes than non-burned reference sites for around one month after burning (Kim, 2013). This is caused by reduced root respiration in the absence of plant cover and the related pH change. After burning, soil temperatures increase due to missing canopy, while soil moisture does not change since lowered plant transpiration compensates for missing or reduced plant canopy (Castaldi and Fierro, 2005).

2.4.3 Effect of agricultural management practices on soil respiration

Upendra *et al*, (2008) stated that the management practices can influence soil CO_2 emission and C content in cropland, which can effect global warming. Although soil C content was not altered, management practices influenced CO_2 flux within a short period due to changes in soil temperature, water, and aeration and nutrient contents. Lemke *et al.* (2007)(Lemke *et al.*, 2007) mentioned that agricultural activities are an important source of anthropogenic GHGs, contributing ~20% of the annual atmospheric increase.

2.4.3.1 N fertilization

Al-Kaisi, Kruse and Sawyer (2008) found that N application can have a significant effect on soil C pools, plant biomass production, and microbial biomass C processing. In the corn year, season long cumulative soil CO_2 emission was greatest with the zero N application. There was no effect of N applied in the prior year on CO_2 emission in the soybean year, except at one of three sites, where greater applied N decreased CO_2 emission. Soil CO_2 emission from aerobically incubated soil showed a more consistent declining trend with increase in N rate than found in the field. N fertilization of corn reduced the soil CO_2 emission rate and seasonal cumulative loss in two out of three sites, and increased microbial biomass carbon (MBC) at only one site with the highest N rate. N application resulted in a reduction of both emission rate and season-long cumulative of CO_2-C from soil. Another study has shown opposite results on impacts of N addition on soil respiration. For example, N applied to maize-cultivated soil in the northeast China enhanced soil respiration by 12% (Wang *et al.*, 2006).

Dick (1992) argued that soil microbial activity may increase due to N fertilization as a result of increased plant biomass production, which on incorporation, stimulates soil biological activity; or N fertilization reduces microbial biomass due to lowering soil pH (Smolander *et al.*, 1994).

Increasing soil N content generally leads to higher soil respiration and to higher net ecosystem exchange (NEE), if carbon is not limiting (Niu *et al.*, 2010; Peng *et al.*, 2011). With limited C availability, N fertilizer application has limited influence on soil respiration (Micks *et al.*, 2004). However, the increases in Rs are alleviated by nitrogen (N) fertilization and deposition in subtropical forests, suggesting that the increasing N deposition in the future will be beneficial for SOC sequestration (Liu and Wang, 2017; Gao *et al.*, 2018).

Yuan, Dai and Wang (2019) suggested that soil carbon decomposition might be retarded by fertilization when the litter quality and the growth rate of decomposers are both changed. Experimental additions of nitrogen to cropland soil usually result in increased N_2O emission (Wu *et al.*, 2017). Fertilization increased CO_2 fluxes by promoting the autotrophic respiration instead of heterotrophic (Yuan, Dai and Wang, 2019). A study by Xu and Wan (2008) also found that soil respiration was higher in the fertilized plots than in the unfertilized plots and this was attributable to stimulated root activity, plant growth and respiration. Nitrogen has been reported to play an important role in soil C storage, both by promoting crop dry matter production and by chemically stabilizing C in the soil (Paustian, Parton and Persson, 1992; Paustian, Robertson and

Elliott, 1995; Paustian, Collins and Paul, 1997). Many experiments have shown that fertilizing crops with N results in higher levels of soil C over time.

However, some other studies indicated that N addition alone exerts no obvious effect on Rs (Liu and Wang, 2017; He *et al.*, 2018). The inconsistency in N fertilization effects on soil CO_2 emission in these field and laboratory studies demonstrates the challenge in understanding the effect of N fertilization on soil C dynamics and loss as CO_2.

Wilts *et al.* (2004) reported that total soil organic C declined for all treatments, but at a slower rate in the fertilized treatments than in the unfertilized control. The difference resulted from increased accumulation of C in the soil with the isotopic signature of corn. Much of this corn C was considered to have come from roots and root exudates during growth.

Paustian, Parton and Persson (1992) reported data from a long-term experiment in Sweden that showed N stabilized C in soil. In this experiment, the addition of 80 kg ha^{-1} N as $Ca(NO_3)_2$ increased the growth of the cereal crop. The increased root growth provided additional C to the soil, but the net storage in the long-term was enhanced even more. Addition of N increased net C stored in response to additions of straw and sawdust as well. The authors speculated that nitrogenous compounds may react with lignin in the process of humus formation, as a mechanism of C stabilization. In addition, most SOM stabilizes with a C:N ratio of approximately 10:1, indicating again that if soil C storage is to increase, N is needed.

Khan *et al.* (2007) reported that the positive role of N fertilization in sequestering C may be offset by N_2O emissions, if care is not taken to properly manage the entire cropping and tillage system.

2.4.3.2 Tillage application

The production, consumption and transport of N_2O and CO_2 are strongly influenced by the changes in soil structural quality and in water content associated with tillage and compaction. One such soil quality influencing gas transport in soil is gas diffusivity. The influence of tillage and compaction on soil conditions (including gas diffusivity) and on consequent gaseous emissions may be important aspects of soil quality

Calderon *et al.* (2001) found that tillage caused short-term changes in nutrient dynamics and soil biology and could alter the microbial community structure of the soil within days of disturbance. Tillage can lead to C loss from agricultural soils because of the exposure and subsequent oxidation of previously protected organic matter (Reicosky *et al.*, 1995). Tillage may be followed by significant increases in water vapor flux, which result in the drying of soil

(Kessavalou *et al.*, 1998). Tillage is often followed by irrigation to provide sufficient moisture for seed germination. Rewetting of dry soil stimulates C and N mineralization from microbial and organic sources (Van Gestel, Merckx and Vlassak, 1993).

Ball, Scott and Parker (1999) found that tillage practices and weather affected the release of greenhouse gases. No-tillage may increase emissions of nitrous oxide N_2O and the fixation of carbon by decreasing carbon dioxide CO_2 emissions. Tillage may also decrease the oxidation rate of atmospheric methane CH_4 in aerobic soil. These effects are partly due to compaction as mentioned above and to the lack of both soil disturbance and residue incorporation. Also, Ball, Scott and Parker (1999) found that CO_2 emissions in the few weeks after sowing were not strongly influenced by tillage and diurnal variations were related to soil temperature. However, periods of low or zero CO_2 fluxes and very high N_2O fluxes under no- tillage were associated with reduced gas diffusivity and air-filled porosity, both caused by heavy rainfall.

Birkás *et al.* (2004) concluded that annual disking and plowing causes subsoil compaction at the depth of tillage within 3 years and that the compacted layer expanded both in surface and deeper layers after the 5^{th} year in Hungary and that soil quality deterioration by tillage-pans was improved by sub-soiling and maintained by planting soil-loosening catch crops and direct drilling. Others like Chatskikh and Olesen (2007) reported a 34 % increase in emissions under tilled soil compared to reduced tilled soil in Denmark, while Ellert and Janzen (1999) showed enhanced release of CO_2 immediately after tillage which was associated with the release of CO_2 stored in soil pores and from stimulated biological production. The CO_2 flux soon after soil disturbance has been related the degree of soil disturbance and to depth (Álvaro-Fuentes *et al.*, 2007). In other study of Calderón and Jackson (2014) concluded that Roto-tillage and disking increased the CO_2 efflux of the soil within 24 h after the tillage. The increase was higher in the disked soil, which was more than three times, and the CO_2 efflux of the control soil at 0.25 h after tillage. Calderón and Jackson (2014) explained this effect may be due to degassing of dissolved CO_2 since microbial respiration did not increase in tilled soils.

Carbone dioxide emissions under conservation tillage

Al-Kaisi and Yin (2005) found that in the initial periods after tillage the soil CO_2 emission might be governed by soil structural changes associated with pore structure and soil organic carbon substrate might not be the limiting factor controlling production. Over an intermediate to long term period (10- 100 days) enhanced biological production of CO_2 is the major driver of the increased emissions. Reduced turnover of soil organic matter under conservation tillage leads to decreased emission of CO_2 under long term conservation tillage.

In south-western Saskatchewan, Canada, there was a 20- 25% reduction in CO_2 flux under soils that had been zero tilled for 13 years compared to conventional respective of season. Zero tillage is reported to reduce the CO_2 emission rate by 0.6 Mg C ha^{-1} yr^{-1} compared to conventional tillage in long term experiment under maize (43 years) in the USA (Ussiri and Lal, 2009). In contrast, a long term study by Oorts *et al.* (2007) found on more than half of the sampling days, no-tillage exhibited larger CO_2 emissions and they attributed this to the achievement of equilibrium under long periods (32 years) of no-tillage. The authors attributed this larger CO_2 emission under no-tillage due to the decomposition of old weathered residues.

Tillage attributed to slower decomposition of surface left crop residues under zero tilled soil (Calderón and Jackson, 2014). In a long term tillage experiment maintained for 25 years, Bauer et al. (2006) found that irrespective of season, the CO_2 flux from conventional tillage was higher compared to conservation tillage. Zero tillage is reported to reduce the CO_2 emission rate by 0.6 Mg C ha^{-1} yr^{-1} compared to conventional tillage in long term experiment under maize (43 years) in the USA (Ussiri and Lal, 2009).

2.4.3.3 Irrigation

Many agricultural practices contribute to GHG emissions, including water management practices, such as irrigation. Irrigation alters the soil water status in the crop root zone, and can alter emissions of CO_2 to varying degrees. Conversely, CO_2 emissions from furrow and subsurface drip irrigation were similar (Kallenbach, Rolston and Horwath, 2010), probably because CO_2 emissions are more responsive to temperature than soil moisture fluctuations (Smith *et al.*, 2003; Schaufler *et al.*, 2010).

Calderón and Jackson (2014) found in their study that irrigation increased the CO_2 efflux of all N treatments but this result was most pronounced in the control soil YOLO silt loam soil (fine-silty, mixed, superactive, nonacid, thermic Mollic Xero-fluvent), which had an order of magnitude increase in CO_2 efflux after irrigation. An ancillary experiment carried out under similar conditions but with more frequent sampling done by (Calderón and Jackson, 2014) showed that increases in CO_2 efflux after irrigation were accompanied by increases in soil respiration.

2.4.3.4 Effects of plant residue and land-use change on soil GHG emissions

Due to related direct and indirect GHG emissions from C and N dynamics, agriculture influences global warming (Hellebrand, Kern and Scholz, 2003; Y. Y. Wang *et al.*, 2013). Deforestation and other land-use changes to increase the surface area for crop production further contribute to global warming. In addition, croplands generally stand for intensive agricultural

management (e.g., application of fertilizers and other chemicals, intensive tillage), enhancing GHG emissions. Cereals and oil crops (e.g., seed cotton, sorghum, wheat, barley, maize, rice, rapeseed/canola and millet) are the World's most prominent crops (FAO, 2014). Plant residues are being used as soil cover to decrease erosion, to improve soil quality (mulching) and to maintain soil humidity. This may influence soil emission rates.

The effect on soil emissions depends on the chemical properties of the residue cover. Yet, all types of plant residues (sugarcane trash, maize and sorghum straw, cotton residues and alfalfa) increased the cumulative CO_2 emission by about a factor of 3 (Muhammad *et al.*, 2011).

Various parameters enhance soil respiration. Changes in soil organic content and land-use management have a significant influence on respiration rates. Average CO_2 emission rates from wetlands exceed those from forestlands, grasslands, croplands and barren lands. The highest rates were registered at sites located over peat and drained organic soils, followed by sites with managed grasslands was recorded by (Oertel *et al.*, 2016).

It was suggested (Jin *et al.*, 2014; Xiao *et al.*, 2014) that land-use change may influence soil temperature and moisture and the types and amounts of C and N inputs via changes in the supply/availability of C and N via root exudates, root turnover and the decomposition of contrasting litter inputs and differences in vegetation productivity,

Land use change may also be associated with alterations in the number and diversity of microbial populations, which can have contrasting effects on the gaseous emissions of CO_2 and N_2O, as these are a result of independent soil and vegetation-related processes. CO_2 and N_2O emissions are likely to be positively related to the concentrations of soil C and N in mineral soils, as these are resources for microbial metabolism (Huang *et al.*, 2004).

2.4.4 Soil properties

In general, soil properties can affect Rs by altering the community productivity, assimilate allocation to belowground, and the quantity and quality of organic matter in soils (Chen *et al.*, 2014).

2.4.4.1 Soil organic matter content

Soil organic matter content also affects the soil respiration-moisture relationship by altering pore space, rates of microbial activity and water retention.

Rawls *et al.* (2003) showed that the effect of organic matter on soil water content can be complex, but generally it increases water retention in sandy and silty soils. Given its very large

specific surface area, the effect of soil organic matter on water availability, and thus on the moisture-respiration relation-ship, may be similar to that of clay. Consequently, it has a main influence through its effect on soil texture and pore space. Additionally, the different amounts of organic matter can affect the total available substrate for decomposition.

The interaction between substrate concentrations with water mediated diffusion will determine substrate availability for enzymes and decomposers and thus modify decomposition rates, as demonstrated in the case of Michaelis-Menten kinetics (Moyano, Manzoni and Chenu, 2013)

2.4.4.2 Soil pH-values

Soil pH is considered a master variable in soils as it affects many chemical processes. It specifically affects plant nutrient availability by controlling the chemical forms of the different nutrients and influencing the chemical reactions they undergo. The optimum pH range for most plants is between 5.5 and 7.5 (Flanigan and Saba, 2018). However, many plants have adapted to thrive at pH values outside this range.

The soil pH affects the microbial activity. Therefore, management practices such as liming influence soil emissions; additional carbonate can be released as CO_2 (Snyder *et al.*, 2009). Acidic soil conditions lead to lower soil emissions. CO_2 emissions were observed to be highest at neutral pH-values (Čuhel *et al.*, 2010).

Reth, Reichstein and Falge (2005) found that the spatial variation of soil CO_2 emission in the field correlated significantly with the soil pH and fine root mass, explaining up to 24% and 31% of the variability. Several studies showed significant effects of soil pH values on soil respiration (Sitaula, Bakken and Abrahamsen, 1995; Hall, Paterson and Killham, 1998; Andersson and Nilsson, 2001). Since, in particular, microbial activity increases with rising pH values (Ellis *et al.*, 1998).

2.4.4.3 Nutrients

Nutrient availability in the soil is paramount to microbial and plant respiratory processes. Hence, the natural N and C content in soil, as well as atmospheric deposition, manure or fertilizer applications play an important role (Weslien *et al.*, 2009; Shi *et al.*, 2014).

Soil nitrogen (N), phosphorus (P) and potassium (K) are important sources of micronutrients for plant growth and productivity, and they play an important role in terrestrial functions by influencing soil properties, plant growth and soil activities (Liu *et al.*, 2010). Soil N, P and K can individually or jointly affect terrestrial productivity (Li, Niu and Yu, 2016).

However, soils are characterized by high spatial and temporal variability due to climatic variables (Patil *et al.*, 2010), topography (Rezaei and Gilkes, 2005), vegetation types (Rodríguez *et al.*, 2009), soil texture (Gami, Lauren and Duxbury, 2009) and land use (Ross *et al.*, 1999)

Moreover, Phosphorus rather than N limits microbial metabolism of fresh plant-derived C in strongly-weathered tropical soils (Cleveland, Townsend and Schmidt, 2002; Kaspari *et al.*, 2008), despite being abundant in organic forms in these soils (Turner and Engelbrecht, 2011). However, microorganisms can overcome P limitation by acquiring P from organic compounds when provided with sufficient energy, such as following input of labile C (Olander and Vitousek, 2004; Nottingham *et al.*, 2012)

2.4.4.4 Soil pore space and soil texture

Soil pore space, which is strongly negatively and linearly related to soil bulk density, is an important variable affecting the diffusion of gases. Higher porosity increases soil air space at fixed volumetric water content and thus reduces oxygen limitations. soil porosity can also affected by soil texture (Moyano, Manzoni and Chenu, 2013).

Soil texture may influence the structure of the microbial community and the contribution of bacterial and fungal respiration rates, which may not conform to the same optimum water content of soil respiration (Davidson, Belk and Boone, 1998). In forests, soil organisms respired more at drier soil conditions. The soil textures were sand and sandy loam and at the grasslands and the wetlands (peat) organisms tended to respire more in wetter soil conditions. The soil texture at the grasslands was clay loam (Schaufler *et al.*, 2010)

Soil texture can influence soil CO_2 efflux through its effects on soil moisture (Saxton *et al.*, 1986), temperature (Lloyd and Taylor, 1994; Fang and Moncrieff, 2001), and nutrient availability (Reich, Walters and Ellsworth, 1997) all of which influence microbial and root activity. In addition, high soil bulk density, which generally increases with texture from clay to sandy soil, can impede root growth physically, but its growth-limiting bulk densities occur at lower densities for fine- than coarse-textured soils (Tuttle, Golden and Meldahl, 1988). Few studies have examined the relationship between soil CO_2 efflux and soil texture directly via field studies. In the laboratory, Bouma and Bryla (2000) examined soil carbon flux with citrus root stock grown in soil varying from 1 to 28% clay and found CO_2 release after watering to be reduced in the fine-textured soils. Soil texture may also influence soil CO_2 efflux rates through its effect on soil carbon residence times. Clay can trap organic carbon via bonding to colloid surfaces and the potential to form aggregates and sand can improve soil aeration (Dilustro *et al.*, 2005).

2.5. Measurement techniques used to quantify the GHGs emissions from soils

There are several methods for measuring GHG fluxes as an example, chamber method has been used extensively for measuring gas exchange between soil surfaces and the atmosphere (Delle Vedove *et al.*, 2007), and has the advantages of low cost and ease of use. Besides measuring soil respiration, it can also be applied for determining fluxes of soil respiration CO_2, CH_4 and N_2O. In general, trace gas emissions from soils are being directly measured in both field and laboratory (chamber techniques and micrometeorological methods), obtained through space and airborne measurements, and calculated with empirical and process-oriented models. Given the relevance of reliable soil respiration data for land-use management, chamber systems receive some more attention, since their output allows for more differentiated localized information (Oertel *et al.*, 2016).

2.5.1 Chamber systems

Flux chamber-based analysis is widely used in soil emission studies of CO_2, CH_4, N_2O and NO (Oertel *et al.*, 2012; Šimek, Hynšt and Šimek, 2014). A box or cylinder (PVC rings) (diameter from 20 cm^2 to x m^2 footprint) (Nagy *et al.*, 2011) is placed onto the soil surface so that the section of its base is open to the ground (Figure 2) and the top is covered, therefore the emitted gases accumulate in its chamber headspace. The change of mixing ratio can be analyzed with various gas sensors, e.g., gas chromatography (CO_2, N_2O, CH_4), IR-spectrometry including NDIR and FID with and without pumps (CO_2, CO, CH_4), chemiluminescence (NOx), Cavity-Ring-Down spectrometry (CO_2, CO, CH_4, N_2O, H_2S) or photoacoustics (CO_2, CO, CH_4, NO and N_2O). Problems of too high headspace with inhomogeneous gas concentrations inside the chamber can be prevented by decreasing the chamber height (Rochette, 2011) and by lower detection limits (Davidson *et al.*, 2002). Chamber systems can also be used to analyze isotopic ratios of C (Figure 3) and O species online in combination with a quantum cascade laser-based spectrometer (Kammer *et al.*, 2011). Such methods can be applied to quantify CH_4 oxidation since CH_4-oxidizing bacteria use ^{12}C methane (Börjesson, Samuelsson and Chanton, 2007). Delta ^{14}C of the emitted CO_2 serves to determine the age of the originating carbon source (Gorczyca, Kuc and Rozanski, 2013). C-isotopes can also be used to distinguish between plant root and microbial respiration (Pausch *et al.*, 2013).

Figure 2. Closed chamber systems; a. (Licor 6400, LiCor, Inc. Lincoln, NE, USA), b. EGM-4 (PPSystems, Amesbury, USA) in our study site (Kartal) during the flux measurement.

Diel patterns in $\delta^{13}C$ may also be related to biases in measuring methods (Midwood and Millard, 2011; Fassbinder, Griffis and Baker, 2012). Balogh *et al.* (2017) measured continuously the $^{13}CO_2$ concentration of the CO_2 efflux of the different soil components in open system by cavity-ring-down spectroscopy (Picarro G1101-i gas analyzer) and the Keeling-plot approach was also used to calculate the isotopic signals of the sources.

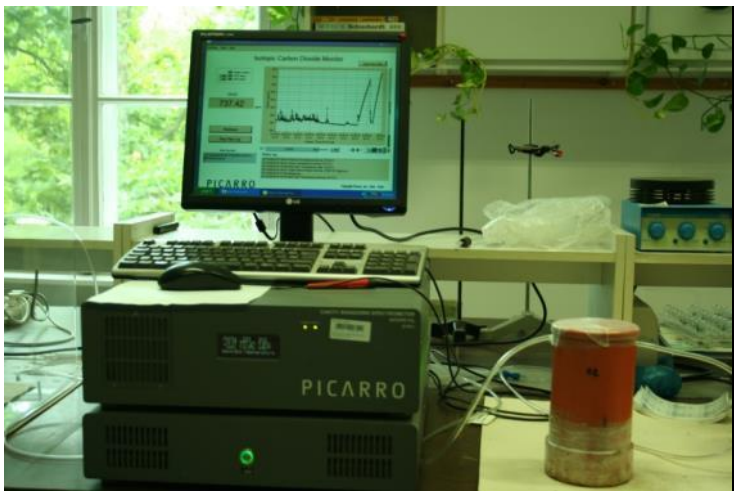

Figure 3.Closed chamber system (Picarro G1101-i gas analyser) during the the flux measurement in the laboratory of the Department of Plant Physiology and Plant Ecology, Gödöllő.

Chamber systems can be divided into closed and open chambers, with closed chambers being subdivided into closed static and closed dynamic ones (Kutzbach *et al.*, 2007). Closed dynamic chambers may also be referred to as non-steady state flow-through chambers. There is still no standardized chamber system, which may inhibit direct comparison of data sets from different research groups (Pumpanen *et al.*, 2004). All chamber systems should be equipped with auxiliary sensors to register soil emission-influencing parameters. Sensors for air temperature, pressure and relative humidity should be installed inside and outside the chamber to log ambient conditions and to register the differences from within the chamber. Burrows *et al.* (2005) mentioned that a photosynthetic active radiation (PAR) sensor needs to be additionally installed outside of the chamber, which is indispensable for net ecosystem exchange (NEE) measurements, so a transparent chamber is used in this case. All chambers have to be installed on a collar (PVC) in order to prevent gas leakage from the chamber to the atmosphere. To minimize the influence of the collar on the soil structure and plant roots, the collar should, if possible, be embedded to a depth of a few centimeters (Heinemeyer and McNamara, 2011). Bahn *et al.* (2012) suggested that the collars should be installed at least 24 h prior to the first measurement to avoid the effects of soil disturbances.

Pumpanen *et al.* (2004) mentioned that some of the chambers may work better without a collar, yet this is not recommended for use on forest soils. Transparent chambers are used to measure NEE (Wang *et al.*, 2013) and also above short canopy, for measurements of the net flux including the effect of photosynthesis and soil exhalation (Farkas *et al.*, 2011), while opaque ones serve the determination of ecosystem respiration and of other gases (Sanz-Cobena *et al.*, 2014). (Xu *et al.*, 2006) mentioned that the opaque material also insulates against temperature increases inside the chamber that would lead to influence soil emissions and pressure changes.

A rapid change (few minutes) between transparent and opaque mode is possible with some chamber systems (Oertel *et al.*, 2015). In such a configuration, NEE and ecosystem respiration can be measured with one system. If gas production in different soil depth is of interest, gas concentration profiles can be assessed (Chirinda *et al.*, 2014). Samples taken with syringes from specific soil depths may be analyzed in the laboratory by gas chromatography (Reich, Walters and Ellsworth, 1997; Petersen *et al.*, 2011). Gas sensors may also be directly installed at specific soil depth for automatic and continuous measurements (Tang *et al.*, 2003).

2.5.1.1 Closed chambers

Closed static chambers are most common for the analysis of CH_4 and N_2O fluxes (Pihlatie *et al.*, 2013). These chambers also offer an absorption method for CO_2 analysis, where CO_2 is trapped in an alkaline solution. This permits fluxes to be measured over longer times and replicates at several measurement points without the need for additional sensors (Yim, Joo and Nakane, 2002). Yet, this method systematically underestimates CO_2 fluxes (Nay, Mattson and Bormann, 1994) and is rarely used. In closed dynamic chamber systems, gases accumulating in the chamber are analyzed either externally and pumped back into the chamber (Heinemeyer and McNamara, 2011) or are being analyzed inside the chamber with a compact NDIR-sensor that continuously monitors the atmospheric CO_2 concentrations (Oertel *et al.*, 2015). Automatic chambers may have a moveable lid, comparable to the hinged lid of a traditional beer mug, which enables gas exchange (Pape *et al.*, 2009). An additional fan in all chamber types intermixes the inside air to maintain a constant and homogeneous level of increasing emitted gases (Christiansen *et al.*, 2011).

CO_2 fluxes require the shortest accumulation times 2-4 min (Caprez, Niklaus and Körner, 2012). This requires fast IR-spectrometers that analyze CO_2 fluxes in less than 10 s. During a measurement, the CO_2-mixing ratio may change from several tens to hundreds of ppmv. Methane measurements take about 60-90 min with sampling intervals of about 20 min, using a gas chromatograph with a manual chamber (Fiedler, Höll and Jungkunst, 2005). The accumulation time for N_2O measurements lies between 30 and 90 min (Hayakawa *et al.*, 2009; Yao *et al.*, 2009). On average, 5-30 min are needed to accumulate NO (Yan *et al.*, 2013).

Rella *et al.* (2013) mentioned that cavity ring-down spectroscopy (CRDS) exists for monitoring systems, where CO_2, CH_4, and N_2O are analyzed from one sample, similar to gas chromatography. Yet, CRDS is faster, measuring every 8 s compared to 3-4 min by gas chromatography (GC), achieves higher precision and does not need additional equipment such as gas generators or gas bottles, thus providing better portability. However, high acquisition costs are involved.

Myklebust, Hipps and Ryel (2008) compared the NEE measured with a dynamic chamber system and with eddy covariance and found no relevant deviations during the non-growing season and wind speeds above 0.2 m s^{-1}. Wang *et al.* (2013) found 4% higher fluxes for NEE on an agricultural site measured with a static chamber system compared to values from eddy covariance. Especially on agricultural sites, comparisons of measurements between eddy covariance and chamber systems are still needed.

Field measurements and modeling for the same plots deliver different results. Pumpanen *et al.* (2004) tested different chambers under constant conditions with a calibration system. According to this study, closed chambers tended to underestimate CO_2-fluxes by 10%. Closed static chambers, sampled with syringes, underestimated fluxes by up to 35% and decreased errors with shorter accumulation times. Nevertheless, entire ecosystem fluxes, e.g., for CO_2, cannot be measured since bigger plants and trees cannot be included inside chamber systems. Here, eddy covariance, remote sensing or modeling is required.

2.5.1.2 Open chambers

Another type of chamber system is the open dynamic chamber. Its two openings draw in ambient air and generate a continuous gas flow (Kutsch, Bahn and Heinemeyer, 2009). Gas concentrations are analyzed at the air inlet and outlet of the chamber. The gas flux is calculated by the difference of the concentrations at both ends. Consequently, there is no accumulation times needed, since the flux is analyzed continuously. Continuous measurement systems do not need mechanical parts. Problems due to high headspace mixing ratios do not occur (Balogh *et al.*, 2007).

Balogh *et al.* (2007) mentioned that open Chambers are suitable for hot and dry conditions in summer with low gas exchange rates. Closed chamber systems require longer accumulations times under such conditions, leading to pressure gradients and temperature. Obviously, open dynamic chambers are technically more sophisticated and more expensive as compared to closed systems. For this reason, economically priced closed dynamic chambers are still the most common systems (Pumpanen *et al.*, 2004).

Nagy *et al.* (2011) was developed and calibrated an automated open system for measurement of soil CO_2 efflux (Rsc) against known fluxes and tested in the field (Bugac, Hungary). Small chamber size (5cm in diameter) of the chamber system made it possible to use the chambers also in vegetation gaps, thereby avoiding the necessity of removing shoots, the disturbance of the spatial structure of vegetation and the upper soil layer.

Nagy *et al.* (2011) found that the continuously operated automatic open chamber system and the gradient system makes possible the detection of situations when the eddy system underestimates R_{eco}, gives the lower limit of underestimation (chamber system) and helps in quantifying the downward flux component of soil respiration (gradient method) between the soil layers. The correlation between chamber fluxes and gradient fluxes was strong, gradient fluxes were generally larger than the flux from chambers. Calibration of gradient flux system by chamber effluxes is proposed by (Nagy *et al.*, 2011)

2.5.2 Data evaluation

Christiansen *et al.* (2011) mentioned that soil flux for all gases can be calculated by linear and non-linear (exponential) regression, using the slope of the concentration change inside the chamber headspace. The linear model is easier to handle and works best for short chamber closure times, ideal for CO_2 (Forbrich *et al.*, 2010). Therefore, it is widely used and least biased for curves with a convex-upward shape (Venterea *et al.*, 2012). These authors found that linear regression is more sensitive to relative flux changes and is useful for studies with changing experimental parameters. The lowest detection limits for flux calculations are gathered with linear regression (Parkin, Venterea and Hargreaves, 2012). Yet, soil fluxes are significantly underestimated in contrast to the exponential model (Kutzbach *et al.*, 2007) especially for curves with a convex-downward shape (Venterea *et al.*, 2012). Burrows *et al.* (2005) explain this with increasing plant stress during measurements. The exponential model is suitable for longer closure times with few data points, e.g., when using a gas chromatograph (Forbrich *et al.*, 2010).

2.5.3 Laboratory experiments on soil CO_2 efflux

Schaufler *et al.* (2010) found that laboratory approaches help when the influence of single parameters (e.g., soil temperature, soil water content or nutrient availability) on soil emissions shall be assessed. Single parameters can be changed, while others are kept constant. Soils from different climate zones can be investigated under controlled temperature and humidity conditions (Schaufler *et al.*, 2010). As an example, Gritsch, Zimmermann and Zechmeister-Boltenstern, (2015) analyzed soil monoliths from nine stations across Europe, representing different land-use types. The authors could clearly show dependencies between CO_2 emissions with soil temperature and moisture. Climate chambers that allow full control of temperature and humidity, as well as light conditions, are used for such experiments.

Many laboratory incubation studies have used sieved and homogenized or undisturbed soil (cores) material. While undisturbed sampling does not negatively influence soil structure and microbial life (Schaufler *et al.*, 2010; Petersen *et al.*, 2013), the heterogeneity among soil cores

demands a larger sample size. Gritsch, Zimmermann and Zechmeister-Boltenstern, (2015), for instance, took 33 undisturbed samples per site. Problems lie in destroying roots during sampling and in maintaining constant physical soil core conditions during transport. Influencing parameters can be observed better with homogenized soil material, which is a widespread approach (Laville *et al.*, 2009; Oertel *et al.*, 2011). Yet, soil structure is destroyed in the lab and before sieving soil material needs to be air-dried, which inadvertently influences microbial activity. Small field chamber systems can be used both in the laboratory and on lysimeters in the field, while some research groups use chambers, specially designed for laboratory use (Schaufler *et al.*, 2010; Yao *et al.*, 2010; Jäger *et al.*, 2011)

2.5.4 Micro-meteorological methods

The eddy covariance method is a direct micrometeorological approach (Figure 4). It uses vertical turbulences to analyze the turbulent heat and gas exchange between soil surface and atmosphere (Nagy *et al.*, 2011). A 3-D ultrasonic anemometer and a gas analyzer attached to a tower or mast of at least a 2-m height are needed for this method (Myklebust, Hipps and Ryel, 2008). The most commonly analyzed gases are CO_2, CH_4 and N_2O, yet substances like carbonyl sulphides or volatile organic compounds can be determined too (Asaf *et al.*, 2013). Measurements may run continuously and incorporate areas of up to several square kilometers (Nagy *et al.*, 2011). Eddy covariance integrates plants and trees, and thus completely covers soil, biosphere and atmosphere to determine NEE. The method does not work properly if very low near-ground turbulent mixing occurs. This leads to an under-estimation of fluxes (Papale *et al.*, 2006). This also applies if the system is installed within a forest (Kutsch, Bahn and Heinemeyer, 2009). It is recommendable to perform measurements on levelled ground, above or within low-density vegetation (Baldocchi, 2003). Du *et al.* (2014) mentioned that data post-processing is complex and measurement gap filling is important to calculate fluxes.

Figure 4. Eddy-covariance (EC) station since October 2017, located in our study site (Kartal) (47.658°N, 19.532°E, 153 m a.s.l.).

The open-path Fourier Transform Infrared spectroscopy (FTIR) is another near-ground micro-meteorological method, using an instrument that can be mounted on a tower or a pole. A radiation source emits the entire IR-spectrum simultaneously (Griffith *et al.*, 2012). The gases modify the response signals that are received by a telescope along the pathway. Such pathways may have standard lengths of 100–500 m (Griffiths, Shao and Leytem, 2009); (Kelliher *et al.*, 2002) measured CO_2 and N_2O on a 97-m-long path. Methane, H_2O and other gases as well as C-isotopes can also be analyzed by FTIR (Griffith *et al.*, 2012).

2.5.5 Continuous monitoring

Chamber systems are suitable for continuous monitoring, except when winter conditions yield higher snow levels. Eddy covariance systems have well-known problems at night and during periods of low turbulence (Papale *et al.*, 2006). Kelliher *et al.* (2002) mentioned that the open-path FTIR method can be applied during night time and in periods without turbulence. Remote sensing and airborne data depend on the orbit of the satellite or the flight frequency and track of the aeroplane, yet the measuring network is rather sparse over oceans and the tropics. Consequently, Hungershoefer *et al.* (2010) concluded that more remote sensing data are needed to fill this gap, although it is still challenging to distinguish between sinks and sources.

However, continuous measurements need large and expensive infrastructure, installed in a certain position, therefore portable chamber systems are preferred in most studies dealing with soil CO_2 efflux.

2.5.6 Spatial measurements

Remote sensing from satellites may deliver information on GHG soil emissions in two different ways. One approach is to estimate tropospherical, near-surface CO_2 and CH_4 concentrations based on the measurement of the intensity of the reflected sunlight in small wavelength bands in the visible and short-wavelength IR portion of the spectrum (Oertel *et al.*, 2016). Earlier earth observation missions of the European Space Agency (ESA) like ERS-1 and ENVISAT carried low resolution scanning imaging absorption spectrometers for atmospheric cartography (ERS-GOME; ENVISAT-SCIAMACHY) (Frankenberg, Platt and Wagner, 2005) with a precision of 1-2 % (Schneising *et al.*, 2008). Simultaneously, the Japan Aerospace Exploration Agency (JAXA) operated the GOSAT system with a thermal and near-infrared sensor for observations of carbon (TANSO), which is operational until present (Kuze *et al.*, 2009). The OCO-2 (Orbiting Carbon Observatory) (Figure 5), a NASA satellite with a precision of 1-2 ppm for CO_2, can cover the variability of CO_2 and CH_4 sinks and sources with high spatial and temporal resolution (Boesch *et al.*, 2011). The continuation of the global time series of CO_2 and CH_4 concentrations after the lifetime of GOSAT and OCO-2 is being planned with the Carbon Monitoring Satellite (Carbon Sat).

Oertel *et al.* (2016) mentioned that the change of land cover types and mapping the spatial distribution that represent sources or sinks for CO_2 and CH_4 is the alternative to direct estimations of GHG concentrations from remote sensing systems. Coarse to medium resolution remote sensing data deliver a globally consistent and objective source of information for a spatially explicit mapping of the distribution of potential C stocks in terms of land cover type maps. However, some studies (Herold *et al.*, 2008; Pflugmacher *et al.*, 2011) found that there is still considerable uncertainty in distribution of the relevant land cover types (e.g., grassland, forests, barren land, cropland, wetland) and in the spatial agreement of the area and hence of the globally stored C. These uncertainties are attributed to a number of limitations that are determined by either the technical specification of the sensor (wavelength, spectral and spatial resolution) or the derived data products (e.g., land cover maps). Differences between land cover maps have important implications on modeling global emissions. Thus, the choice of a map might introduce a significant bias in any regional to global carbon balance model.

Fóti *et al.* (2014) found that spatial patchiness became less robust and the correlations generally decreased as soil moisture content was high and that explanatory variable of N_{opt} was also SWC, with negative correlation between them. Fóti *et al.* (2014) concluded that the sampling could be optimized on the basis of the easily measurable actual SWC, which determines both the optimal number of Rs measurements and the minimum distances between individual samples in semi-arid ecosystems.

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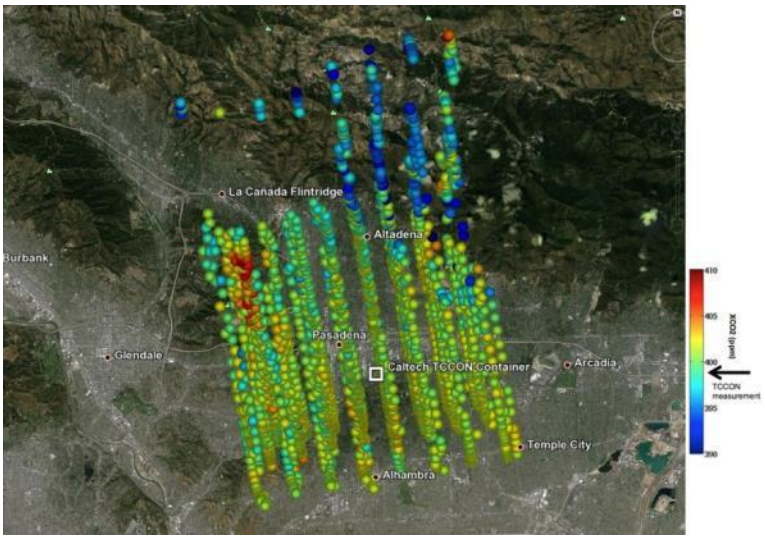

Figure 5 .remote sensing: Orbiting Carbon Observatory-2 (OCO-2) of CO_2 levels over Pasadena CA on 5th of September, 2014. Each coloured dot depicts a single measurement of CO2 made during a 5-min satellite flight over the area. Over the heart of Pasadena, a level of 402 ppmv of CO_2 was recorded. Image source: NASA/JPL-Caltech, http://www.jpl.nasa.gov/spaceimages

2.6. Strategies for GHG emissions mitigation in agriculture

Many agricultural practices can potentially mitigate greenhouse gas (GHG) emissions, the various prominent of which are improved cropland and grazing land management and restoration of degraded lands and cultivated organic soils (Smith *et al.*, 2008). Lower, but still significant mitigation potential is provided by water and rice management, livestock management and manure management, land use change and agroforestry. The global technical mitigation potential from agriculture (excluding fossil fuel offsets from biomass) by 2030, considering all gases, is estimated to be approximately 5500–6000 Mt CO_2-eq. yr.

Often a practice to mitigate the GHG will affect more than one gas, by more than one mechanism, sometimes in opposite ways, so that the net benefit depends on the combined effects on all gases (Schils *et al.*, 2005). In addition, the temporal pattern of influence could vary among

practices or gases for a given practice; some emissions are decreased indefinitely, other decrements are temporary (Marland *et al.*, 2003; Six *et al.*, 2004). The impacts of some mitigation options are presented in the table 2.

Table 2. A list of some proposed measures for mitigating GHG emissions from agricultural ecosystems (Smith *et al.*, 2008).

Mitigation effects				
Measure	Examples	CO_2	CH_4	N_2O
Cropland management	Agronomy	+		±
	Nutrient management	+		+
	Tillage/residue management	+		±
	Water management (irrigation, drainage).	±		+
	Rice management		+	
	Agroforestry			±
	Set-aside, land-use change (LUC)	+	+	±
		+		+
Grazing land management/ pasture improvement	Grazing intensity	±		±
	Increased productivity (e.g. fertilization)	+		+
	Nutrient management			
	Fire management	+		±
	Species introduction (including legumes)	+		±
		+		±
Management of organic soils	Avoid drainage of wetlands	+	-	±
Restoration of degraded lands	Erosion control, organic amendments, nutrient amendments	+		±

(+) denotes reduced emissions or enhanced removal (positive mitigative effect); (-) denotes increased emissions or suppressed removal (negative mitigative effect); (±) denotes uncertain or variable response.

2.6.1 Cropland management

Smith *et al.* (2008) mentioned that the croplands offer many opportunities to impose practices that reduce net emissions of GHGs (Table 2).

2.6.1.1 Agronomy

Improved agronomic practices that augment yields and generate higher inputs of residue C can lead to increased soil C storage (Follett, 2001). Some of such practices examples; using improved crop varieties; extending crop rotations, notably those with perennial crops which allocate more C below-ground; and avoiding or reducing use of bare (unplanted) fallow (West and Post 2002; Freibauer et al. 2004; Lal 2004). Adding more nutrients, when deficient, can also upgrade soil C gains (Alvarez, 2005). Emissions can also be reduced by adopting less intensive cropping systems, which reduce reliance on pesticides and other inputs (and therefore the GHG cost of their production; (Paustian *et al.*, 2004)).

2.6.1.2 Tillage/residue management

Advances in weed control methods and farm machinery now allow many crops to be grown with minimal tillage (reduced tillage) or without tillage (no till). These practices are now increasingly used throughout the world. Since soil disturbance tends to stimulate soil C losses through enhanced decomposition and erosion, reduced- or no-till agriculture often results in soil C gain, though not always (Ogle *et al.*, 2003).

2.6.1.3 Nutrient management

Cassman *et al.* (2003) found that nitrogen applied in manures and fertilizers is not always used efficiently by crops. Improving this efficiency can decrease emissions of N_2O, generated by soil microbe largely from surplus N and it can indirectly decrease emissions of CO_2 from N fertilizer manufacture (Schlesinger, 1999). Adjusting application rates based on precise estimation of crop needs (e.g. precision farming); using slow-release fertilizer forms or nitrification inhibitors (which slow the microbial processes leading to N_2O formation); avoiding time delays between N application and plant N uptake (improved timing); placing the N more precisely into the soil to make it more accessible to crops roots (Cole *et al.*, 1997; Robertson and Grace, 2004; Monteny, Bannink and Chadwick, 2006) those are practices that improve N use efficiency.

2.6.1.4 Water management

About 18% of the world's croplands now receive supplementary water through irrigation (Millennium, 2005). Extending this area or using more effective irrigation measures can enhance C storage in soils through enhanced yields and residue returns (Follett, 2001; Lal, 2004). However, some of these gains may be offset by CO_2 from energy used to deliver the water (Schlesinger, 1999; Mosier *et al.*, 2005) or from enhanced decomposition of SOM.

2.6.1.5 Land cover (use) change

One of the most efficient techniques of diminishing emissions is to allow or encourage the reversion of cropland to another land cover, typically one similar to the local vegetation (Smith *et al.*, 2008). The diversion can occur over the entire land area ('set-asides') or in localized spots such as grassed waterways, field margins or shelterbelts (Follett, 2001; Freibauer *et al.*, 2004). Such land cover change often increases storage of C; for example, converting arable cropland to grassland typically results in the accrual of soil C owing to lower soil disturbance and reduced C removal in harvested products.

2.6.2 Management of grazing land and pasture improvement

Grazing lands occupy much larger areas than croplands (Smith *et al.*, 2008), but are usually managed less intensively. Some examples of practices to reduce GHG emissions and enhance removals are cited.

2.6.2.1 Grazing intensity

The intensity and timing of grazing can influence the growth, C allocation and flora of grasslands, thereby affecting the amount of C accrual in soils (Conant, Paustian and Elliott, 2001; Freibauer *et al.*, 2004; Reeder *et al.*, 2004; Conant *et al.*, 2005). Carbon accrual on optimally grazed lands is often greater than on ungrazed or overgrazed lands (Liebig *et al.*, 2005). The effects are inconsistent, however, owing to the many types of grazing practices employed and the diversity of plant species, soils and climates involved (Derner, Boutton and Briske, 2006).

2.6.2.2 Augment productivity (including fertilization) and species introduction

Smith *et al.* (2008) reported that for croplands, C storage in grazing lands can be improved by a variety of measures that promote productivity. For instance, alleviating nutrient deficiencies by fertilizer or organic amendments increases plant litter returns and, hence soil C storage (Conant, Paustian and Elliott, 2001). Adding nitrogen, however, may stimulate N_2O emissions (Conant *et al.*, 2005), thereby offsetting some of the benefits. Irrigating grasslands, similarly, can promote soil C gains (Conant, Paustian and Elliott, 2001), though the net effect of this practice depends also on emissions from energy use and other related activities on the irrigated land (Schlesinger, 1999). Introducing grass species with higher productivity or C allocation to deeper roots has been shown to increase soil C. (Soussana *et al.*, 2004).

2.6.3 Management of organic soils

Emissions on drained organic soils can be reduced to some extent by practices such as avoiding row crops and tubers, avoiding deep ploughing and maintaining a more shallow water

table, but the most important mitigation practice, probably, is avoiding the drainage of these soils in the first place, or re-establishing a high water table where GHG emissions are still high (Freibauer *et al.*, 2004). Another strategy which is the use of composting and anaerobic digestion of agroindustry by-products are common treatments that can improve the properties of the organic matter and can also provide additional overall GHG reductions (Dolores *et al.*, 2013). The composting process has relatively low associated GHG emissions (Pardo *et al.*, 2015) and can lead to moderate to high SOC sequestration rates when used as soil amendments (Aguilera *et al.*, 2013). The composted material will lower the soil pH, reducing the decarbonation process in soils developed over calcareous materials (common in the Mediterranean basin). Anaerobic digestion of agro-industry by-products reduces overall GHG emissions through the generation of biogas (Sanz-Cobena *et al.*, 2017).

2.6.4 Restoration of degraded lands

Several studies (Batjes, 1999; Lal, 2004; Foley *et al.*, 2005) concluded that a large fraction of agricultural lands have been degraded by erosion, organic matter loss, excessive disturbance, salinization, acidification or other processes that curtail productivity. Often the C storage in these soils can be at least partly restored by practices that reclaim productivity including: revegetation (e.g. planting grasses); improving fertility by nutrient amendments; applying organic substrates such as manures, biosolids and composts; reducing tillage and retaining crop residues; and conserving water (Paustian *et al.*, 2004).

3. MATERIAL AND METHODS

3.1. Field measurements

3.1.1 Site description

The study was performed from November 2017 to November 2019 in cropland near Kartal (47.658°N, 19.532°E, 153 m a.s.l.) which is located in the middle part of Hungary. The site has a running eddy-covariance (EC) station (figure 6) since 2017 for CO_2/H_2O gas exchange and meteorological measurements. Gödöllő Experimental Farm Ltd. has the land management rights of the site and provided management data. The average annual temperature was 11.75 °C, 12.94 °C and 12.91 °C and the annual precipitation sum was 620, 552 and 694 mm in 2017, 2018 and 2019, respectively.

Figure 6. The study site and the eddy-covariance (EC) station.

The soil is chernozem type brown forest soil (WRB, 2015: chernozem) with 54.9% sand, 28.05% clay and 17.05% loam, having the following properties. The amount of $CaCO_3$ of samples investigated was 1.73%. Although the amount of humus (3.6%) of the soil is good, the phosphorus and the potassium contents are moderate (AL-P_2O_5: 160 mg/kg, A-K_2O: 387 mg/kg), and the NH_4^+-N and NO_3—N are: 4.5 mg/kg, 8.8 mg/kg, respectively.

Regarding the pH, it is slightly acidic pH (H_2O): 6.26 which can be attributed to the effect of long term of fertilizer application (Székely, 2004).

Management data during the study period are shown in Table 3 contained soil tillage, spraying, sowing, harvesting and fertilizer application timings to the different crop rotations.

Crop rotation of the measured field: 2017-2018 winter wheat, 2018-2019 rapeseed, 2019 sorghum, and 2019-2020 winter wheat. Crops were separated by fallow periods, with no cover crop used between them.

Table 3. Agricultural management practices during two years-long study period in Kartal site.

Study years	Crops type	Seedbed preparation date	Sowing Date	Fertilization date	Nitrogen application rate (kg N/ha)	Harvesting amount and date	Tillage date
2017	Winter wheat	02.10.2017	03.10.2017	01.10.2017	100 Kg/ha CAN 27%	- 14.07.2018	-
2018	rapeseed	31.08.2018	10.09.2018	15.03.2018 29.08.2018	140 Kg/ha Nikrol 30% 200 kg/ha NPK 15-15-15	7.04 t/ha 02.04.2019	01.08.2018
2019	Sorghum	26.04.2019	03.05.2019	03.05.2019 04.10.2019	200 kg/ha MAS 27% 100 kg/ha MAS 27%	9.38 t/ha 30.09.2019	-

3.1.2 Field design and soil CO_2 exchange measurements

The soil CO_2 efflux measurements were made from November 2017 to November 2019. Ten PVC rings (10.2 cm of diameter and 5 cm high) were installed one month before the flux measurement (Figure 7), the living weeds and the litter in the PVC ring were removed from the soil surface to avoid soil disturbance and ground vegetation respiration (Han *et al.*, 2014). PVC rings were inserted approx 2.5 cm into the ground, leaving 2.5 cm above the ground to measure CO_2 efflux at each point.

Figure 7. Ten closed PVC rings installed in the study site.

Fluxes of CO_2 were measured about bi-weekly/monthly during a two-year-long study period. PVC rings were left in the field for the entire two-year measurement period, except during the farm operations and they were kept free of any plants for the entire study period.

CO_2 efflux was measured between 10:00 and 12:00 h as the most suitable time of the day for measurements (Burri *et al.*, 2018). Measurements were made by closed chamber systems: Licor 6400 (LiCor, Inc. Lincoln, NE, USA) in 2017 and EGM-4 (PPSystems, Amesbury, USA) in 2018 and in 2019. PVC rings (10.2 cm in diameter and 5 cm high) were inserted approx. 2.5 cm into the ground, leaving 2.5 cm onto the soil surface so that the section of its base is open to the ground and the top is covered with the chamber of Licor 6400 or EGM-4, therefore the emitted gases accumulated in its chamber headspace. the CO_2 gas accumulated in the chamber are analyzed either externally and pumped back into the chamber and the soil respiration was calculated automatically. There is an attached thermometer in the two instruments for the soil temperature measurement CO_2 fluxes require the shortest accumulation times, it takes from 2 to 4 min.

Net ecosystem exchange of CO_2 (NEE) was measured by eddy-covariance (EC) technique. The EC system at the Kartal site has been measuring the CO_2 and sensible and latent heat fluxes continuously since October 2017. It consists of a CSAT3 sonic anemometer (Campbell Scientific, USA) and a Li-7500 (Licor Inc, USA) open-path infra-red gas analyzer (at the height

of 2 m, anemometer direction: north), both connected to a CR1000 data-logger (Campbell Scientific, USA) via an SDM (synchronous device for measurement) interface.

Additional data used in the present study included air temperature and relative humidity (HMP35AC, Vaisala, Finland), precipitation (ARG 100 rain gauge, Campbell, UK), volumetric soil moisture content (CS616, Campbell, UK) and soil temperature (105T, Campbell, UK). Fluxes of sensible and latent heat and CO_2 were processed by EddyPro® (Webb, Pearman and Leuning, 1980) using double rotation, linear detrending and WPL correction (Reichstein *et al.*, 2005). Gap-filling and flux partitioning were performed by the REddyProc online data processing tool (Nagy *et al.*, 2011).

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3.1.3 Additional measurements

Soil temperature (T_s) was measured outside the PVC rings concurrently during the soil CO_2 efflux measurements in the top 5 cm of soil surface using a thermometer unit attached to the LICOR-6400 or to the EGM-4.

Soil moisture was recorded in the top 7.5 cm, where most of the gas diffusivity from the soil to the atmosphere is likely to occur (Hosen, Tsuruta and Minami, 2000). SWC was measured by time domain reflectometry (FieldScout TDR300 Soil Moisture Meter, Spectrum Technologies, IL-USA).

Leaf area index was measured by an AccuPar LP-80 ceptometer (Decagon Devices, USA) at each measurement campaign over each plot.

VIgreen (VIgreen index) was derived from red, green, blue (RGB) values of photographs made by a commercial digital camera (Canon Eos 350D) from the measured plots. VIgreen is the normalized difference of reflected green and red light (Gitelson *et al.*, 2002):

Equation 1. VIgreen index

$$VIGreen = \frac{Green - Red}{Green + Red}$$

where, VIgreen is a dimensionless index, Green and Red are the component values of a digital image. VIgreen was calculated in R (R Core team, 2019).

Bulk density was calculated from the compactness of the topsoil layer measured by a penetrometer (Eijkelkamp, The Netherlands).

3.2. Soil characteristics analysis

Soil organic matter (SOM), the contents of total nitrogen (TN), pH_{KCl} and PH_{H2O}, Calcium carbonate ($CaCO_3$%), Nitrate (NO_3^- mg/kg), and Ammonium (NH_4^+ mg/kg) were measured before the establishment of the different laboratory experiments. The soil characteristics results are shown in table 4.

Table 4. Soil characteristics

Experiment number	SOM	TN mg/kg	pH_{KCl}	PH_{H2O}	$CaCO_3$%	NO_3^- mg/kg	NH_4^+ mg/kg
1	7.4 (1) 7.7 (2)	677.6(1) 1797.3(2)	6.5 (1) 6.5 (2)	6.4(1) 6.3(2)	0.0 (1) 0.1 (2)	10.5(1) 14.0(2)	5.0(1) 4.5(2)
2	7.5	2189.3	6.5	6.4	0.1	12	5
3	7.2	621.6	6.5	6.6	0.3	7.5	6.5

3.3. Lab measurements

3.3.1 Sampling of soil

Soil from the top 15 cm layer was collected from the same field and transported to the lab, the analyzed soil profile was restricted to the topsoil layer (0-15 cm). Because most labile organic C and most easily accessible nutrients are located within the topsoil, it is the layer with highest microbial activity and correspondingly high GHG production/consumption (Risk *et al.*, 2008).

Before establishing the laboratory experiments, the soil was air-dried, visible roots, large stones and organic residues were removed and the soil was passed through a 2-mm mesh size (figure 8a) then mixed thoroughly; PVC tubes (10.2 cm in diameter and 20 cm height) (figure 8b) were used as pots filled up to 15 cm with about 1.6 kg of soil to achieve a bulk density of 1.30 g cm^{-3}. The top 5 cm of the tube was used as a soil respiration chamber during the measurements. Then pots were brought to different soil water content (see below) and were incubated for two weeks before starting the measurement.

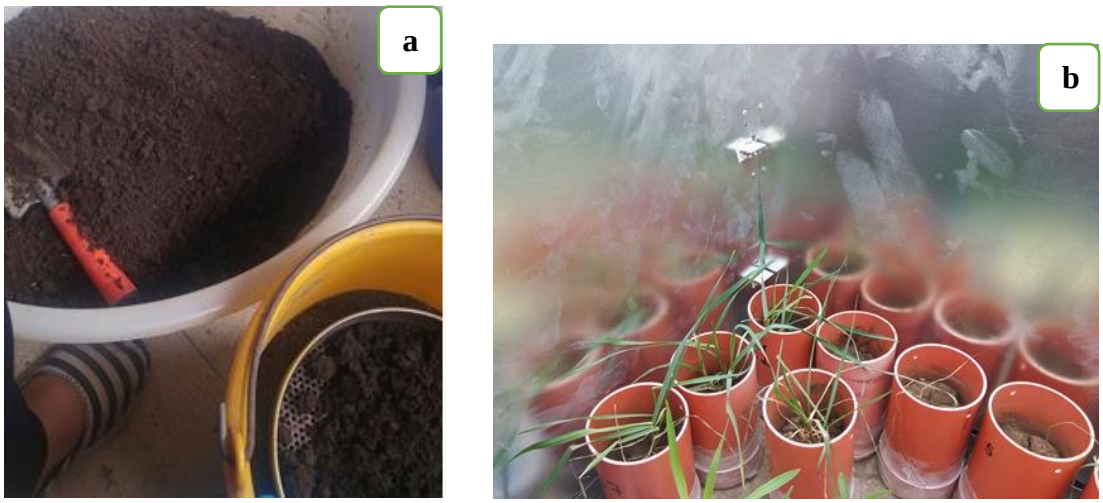

Figure 8. a: The use of 2.2-mm mesh size, b: PVC tubes with soil and wheat plants during incubation time in room temperature.

The soil samples were stored in plastic tubes after collection (Figure 9) for analysis of soil physicochemical properties. Another amount of soil sample was placed in sterile tubes and brought back to the laboratory for storage at 4 ℃ and -20 °C for soil microbial count determination, BIOLOG EcoPlate measurement and metagenomics analysis.

Figure 9. Collection of soil samples for microbial investigation and soil charasteristics

3.3.2 Lab design and soil CO_2 exchange measurements

Three successive laboratory experiments using the different treatments (SWC and N fertilization) in the presence and absence of plant were carried out in the following order**:**

3.3.2.1 Experiment 1:

Our manipulation experiment was divided into two periods, the first contained a series of 27 pots; 18 pots were planted with wheat plants and 9 pots were bare soil, while the second contained a series of 30 pots: bare soil (9 pots) and the other pots were planted with wheat plants. NH_4NO_3 fertilizer was applied on the surface of the soil at the beginning of the study period with different level of treatments (N0, N50 and N100) for the first series and (N0, N75 and N150) for the second one. Two levels of SWC was applied (20% and 25%) (Table 5).

The CO_2 efflux measurements were done weekly during 4 weeks for the first series and 5 weeks for second one.

Table 5.Experimental settings of the lab experiment 1.

Plant presence	SWC %	The amount of NH_4NO_3
First series		0 kg/ha
Planted soil	20%	50 kg/ha
Bare soil		100 kg/ha
Second series		0 kg/ha
Planted soil	25%	75 kg/ha
Bare soil		150 kg/ha

3.3.2.2 Experiment 2:

Our lab experiment was done using the same method which was used in the first experiment; we decided to increase the frequency of the measurements of the other experiments.

This experiment contained around 125 pots divided into planted soil with maize plants (63 pots) and bare soil (60 pots). Different soil moisture was set for the measurement series and these moisture levels were binned into two different categories during the data analysis: below 30% (15, 20 and 25%) and above 30% (35 and 40%). Different levels of N fertilizer (N0, N75 and N150) of ammonium nitrate (NH_4NO_3) were applied on the surface of the soil (Table 6).

The CO_2 efflux measurement were done often daily during long laboratory study period.

Table 6. Experimental settings of the lab experiment 2.

Plant presence	SWC %	The amount of NH_4NO_3
Planted soil	<30% (15, 20 and 25%) >30% (35 and 40%)	0 kg/ha 75 kg/ha 150 kg/ha
Bare soil	<30% (15, 20 and 25%) >30% (35 and 40%)	0 kg/ha 75 kg/ha 150 kg/ha

3.3.2.3 Experiment 3:

Our lab experiment contained 36 pots divided into planted soil with maize plants (18 pots) and bare soil (18 pots). Two levels of soil moisture were set for the measurement series. Different levels of N fertilizer (N0, N75 and N150) of nitrate ammonium (NH_4NO_3) were applied on the surface of the soil. Beside the effect of these factors, we aimed to study the effect of glucose addition on soil CO_2 efflux. First portion of D (+) glucose monohydrate ($C_6H_{12}O_{6.}$ $.H_2O$), (250 mg glucose kg^{-1} soil) was added to our bare and planted soil at 251 h after fertilization and the second portion was added for bare soil at 445 h after fertilization. The CO_2 efflux were measured often daily during 4 weeks long laboratory study period (Table 7).

Table 7. Experimental settings of the lab experiment 3.

Plant presence	SWC %	The amount of NH_4NO_3	Glucose addition ($C_6H_{12}O_6$)
Planted soil	20% 40%	0 kg/ha 75 kg/ha 150 kg/ha	1st portion at 215 h
Bare soil	20% 40%	0 kg/ha 75 kg/ha 150 kg/ha	1st portion at 215 h 2nd portion at 445 h of gas measurement

These measurements were conducted in a controlled environment under 12/12 h day/night periods, 20 °C of air temperature and the soil water content of each pot was controlled and was adjusted one day before gas efflux measurement.

Closed chamber technique was used for measuring the emission of carbon dioxide and its isotopic composition by a Picarro G1101-i gas analyser. Each sample was measured for 20 minutes, CO_2 efflux was calculated using the slope of the concentration change during this period.

CO_2 efflux was calculated by the following equation:

Equation 2. Calculation of soil CO_2 efflux.

$$F = \frac{n*\Delta C}{A}$$

Where n represents the number of mols (µmol) in the volume of the closed system, ΔC is the concentration change of the carbon dioxide (µmol mol^{-1} s^{-1}) and A is the area of soil in the PVC tube used in the lab experiment (m^2).

Keeling plots were constructed by plotting the $\Delta^{13}C$ of CO_2 in any given gas sample obtained at each collar with the inverse of the CO_2 concentration of each gas sample. To ensure linearity, only those y-intercepts from linear regressions with $r^2>0.20$ were used to calculate isotopic composition of the emitted CO_2 (Trueman and Gonzalez-Meler, 2005; Nickerson and Risk, 2009).

3.3.3 Cumulative gas effluxes

The cumulative emissions were calculated based on the measurements of lab experiment 2 using the following formula:

Equation 3. Calculation of cumulative emissions

$$T = \sum_{i=1}^{n} \left[\frac{(X_i + X_{i+1})}{2} \times (t_{i+1} - t_i) \times 24 \times \frac{3600}{1000000} \times 44 \right]$$

Where, T (g CO_2 m^{-2}) is the cumulative CO_2 flux, X (µmol CO_2 m^{-2} s^{-1}) is the average daily CO_2 flux rate, i is the i^{th} measurement, and ($t_{i+1} - t_i$) is the number of days between two adjacent measurements.

3.4. Microbiological method used

From the two-year long study period five soil samples were choosen (S1: 15th of June 2018, S2: 27th August 2018, S3: 26th of September 2018, S4: 25th of April 2019 and S5: 26th of June 2019) for doing the microbiological measurements which was mentioned above. These samples were selected on the basis of the phenological stages of the plants and the agricultural management practices.

3.4.1 Classical method

Microbiological counts were expressed as a number of colony-forming units (CFUs) per g of dry soil. 1 g of fresh soil was taken and suspended in 9 ml of sterile water. Total number of microorganisms was determined by the dilution method on agarized soil extract (Figure 10). Soil bacteria were cultured and counted by using nutrient medium inoculated with soil diluent at 10^{-3}. Soil actinomycetes were cultured and counted by using starch casein agar (SCA) inoculated with soil diluent of 10^{-3}. Soil ammonificans were cultured and counted by using Frazier culture medium inoculated with soil diluent of 10^{-3}. Soil fungi were cultured and counted by using Bengal red medium containing gentamicin inoculated with soil diluent at 10^{-2}. 25 µl of soil diluent was inoculated in each culture medium for the microbiological counts.

Denitrifying bacteria were enumerated by MPN technique using Alexander and Clark media. Each sample was inoculated in 25 tubes for 5 appropriate successive dilutions. All assays were performed in triplicate and all tubes were incubated at 30 °C. After that Hoskins table was used to determine the most probable number of viable cells per milliliter (for 3-3 inoculations per dilution).

Figure 10. The results of microbiological counts.

3.4.2 BIOLOG EcoPlate

Preparation of Sample Solution and Plate inoculation

Firstly, 1 g of fresh soil was taken and suspended into 9 ml of 0.85% stroke- physiological saline solution. followed by shaking for 20 min at 20 °C (Gałązka, Grzęda and Jończyk, 2019), and the suspension was then left to settle at room temperature for 10 mn. The five soil samples were separately diluted to a 10^{-3} gradient and each sample was processed in triplicate (Ge *et al.*, 2018) (Figure 11a). Inoculation was accomplished by pipetting 120 µL of samples to each well of the BIOLOG EcoPlate (Figure 11b). Then, the microplate was placed in its bag to avoid desiccation and incubated at 25 °C in dark for 4, 24, 48, 72, 96, 120, 144, 168, 192 and 216 h,

and the OD_{492} nm absorbance was measured every 24 h (Gałązka, Grzęda and Jończyk, 2019) by BOECO-Germany BMR-100 (Figure 11c). The average well color development (AWCD) was calculated for each group of substrates and the final result is shown in Figure 11d.

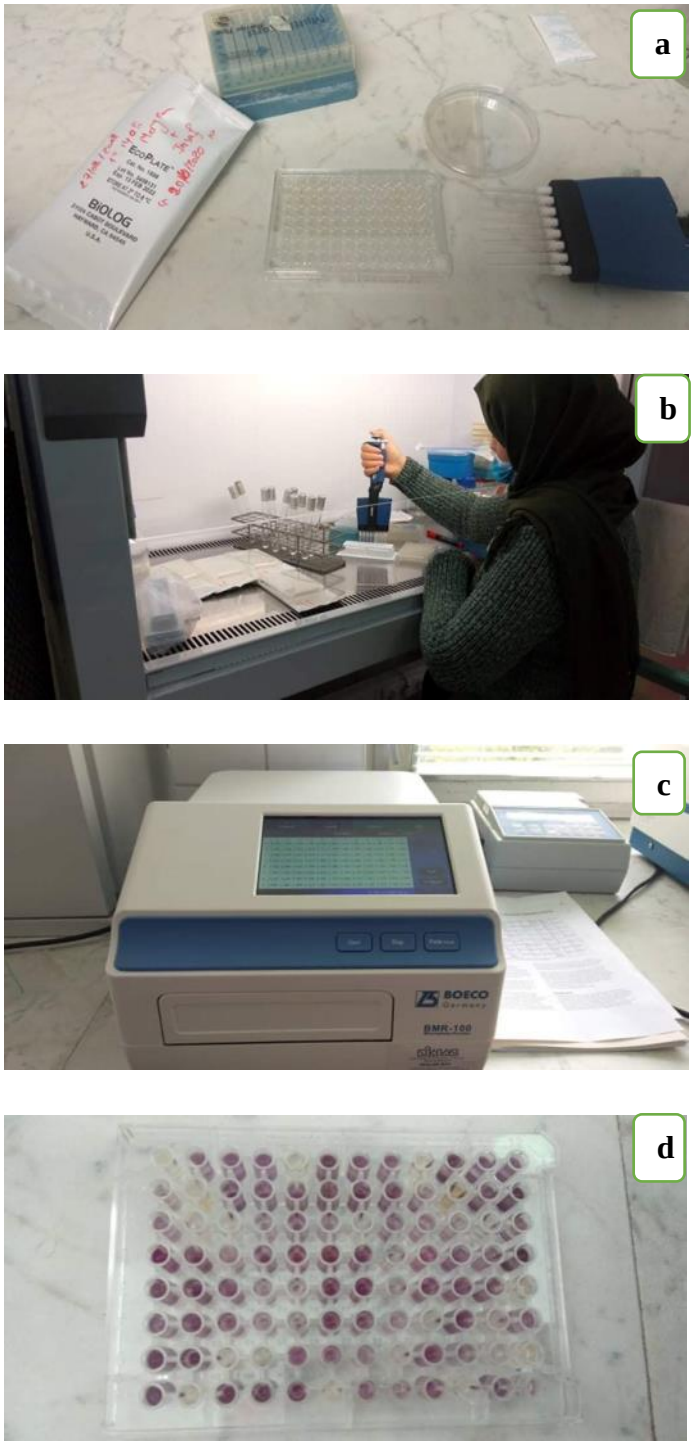

Figure 11. a: The BIOLOG EcoPlate No.1506, b: Inoculation of soil dilluted by pipetting 120 µL of samples to each well of the BIOLOG EcoPlate, c: Absorbance measurement by BOECO-Germany.BMR-100, d: Result of BIOLOG EcoPlate after 168 h of incubation.

Determination of Average Well-Color Development Values

Metabolism of the substrate in particular well-results in Formosan production, producing chroma change in the tetrazolium dye (Preston-Mafham, Boddy and Randerson, 2002). The capability of microorganisms to utilize different carbon sources in microbial communities was measured by average well-color development (AWCD) (Garland and Mills 1991). Samples with larger variation were thought to have higher carbon source utilization capability and tend to have higher microbial abundance (Garland, 1997). The calculation formula for the AWCD is:

Equation 4. averege well-color developement (AWCD).

$$AWCD = \sum_{i=1}^{n}(Ci - R)/n$$

Where, *Ci* is the absorbance value of each reaction well at 492 nm, *R* is the absorbance value of the control well and n is the number of wells. (Ci–R) less than 0.06 of wells are calculated as zero (Classen *et al.*, 2003).

Calculation of Metabolic Functional variables

Diversity Indices

Zak et al. (1994) proposed that the calculation method based on functional diversity indices of BIOLOG EcoPlate could investigate the diversity of communities. Moreover, Keylock (2005) and Strong (2016) extended the concept of evenness to characterize the utilization levels and utilization patterns of microorganisms by carbon source.

(1) Shannon-Wiener diversity index (H´) (Keylock 2005; Spellerberg 2008)

Equation 5. Shannon-Wiener diversity index.

$$H' = \sum Pi \ln Pi$$

Equation 6. Ratio of the absorbance

$$Pi = (Ci - R)/\sum(Ci - R)$$

where, *Pi* represents the ratio of the absorbance value in the i^{th} (1 to 31) well to the total absorbance values of all wells.

(2) Shannon evenness index (E) (Keylock, 2005)

Equation 7. Shannon evenness index

$$E = H'/\ln S$$

Where, *S* represents the total number of utilized carbon sources (31 carbon sources), the number of wells that vary in color.

(3) Simpson diversity index (D)

Equation 8. Simpson diversity index.

$$D = 1 - \sum P_i^2$$

The above indices reflected the metabolic functional diversity of microbial communities, which was similar to the measurements of diversity indices in general ecology.

Principal Component Analysis

A BIOLOG EcoPlate is a 96-well microplate that contains 31 common carbon sources from altogether six compound groups-that is, carboxylic acids, carbohydrates, amino acids, polymers, miscellaneous, and amines/amides - plus a blank well as a control (Table 8), all these replicated thrice to control variation in inoculum densities (Sofo and Ricciuti, 2019). Each EcoPlate is filled with a dilution of one soil suspension, thus representing one soil sample.

Table 8. The 31 kinds of carbon substrates found in the BIOLOG EcoPlates.

Chemical guild	Plate number	Substrates	Chemical formula
Miscellaneous	B1	Pyruvic acid methyl ester	$C_4H_6O_3$
	G2	Glucose-1-phosphate	$C_6H_{13}O_9P$
	H2	D,L-α-Glycerol phosphate	$C_3H_9O_6P$
Polymers	C1	Tween 40	–
	D1	Tween 80	–
	E1	α-Cyclodextrin	$C_{36}H_{60}O_{30}$
	F1	Glycogen	$(C_6H_{10}O_5)_n$
Carbohydrates	G1	D-Cellobiose	$C_{12}H_{12}O_{11}$
	H1	α-D-Lactose	$C_{12}H_{12}O_{11}$
	A2	Methyl-D-glucoside	$C_7H_{14}O_6$
	B2	D-Xylose	$C_5H_{10}O_5$
	C2	i-Erythritol	$C_4H_{10}O_4$
	D2	D-Mannitol	$C_6H_{14}O_6$
	E2	N-Acetyl-D-glucosamine	$C_8H_{15}NO_6$
Carboxylic acids	F2	D-Glucosaminic acid	$C_6H_{13}NO_6$
	A3	D-Galactonic acid latone	$C_6H_{10}O_6$

	B3	D-Galacturonic acid	$C_6H_{10}O_7$
	C3	2-Hydroxy benzoic acid	$C_7H_6O_3$
	D3	4-Hydroxy benzoic acid	$C_7H_6O_3$
	E3	γ-Hydroxy butyric acid	$C_4H_8O_3$
	F3	Itaconic acid	$C_5H_6O_4$
	G3	α-Keto butyric acid	$C_4H_6O_3$
	H3	D-Malic acid	$C_4H_6O_5$
Amino acids	A4	L-Arginine	$C_4H_{14}N_4O_2$
	B4	L-Asparagine	$C_4H_8N_2O_3$
	C4	L-Phenylalanine	$C_9H_{11}NO_2$
	D4	L-Serine	$C_3H_7NO_3$
	E4	L-Threonine	C4H9NO_3
	F4	Glycyl-L-glutamic acid	$C_7H_{12}N_2O_5$
Amines/amides	G4	Phenylethylamine	$C_8H_{11}N$
	H4	Putrescine	$C_4H_{12}N$

3.4.3 Metagenomics analysis

3.4.3.1 DNA extraction and metagenome analysis

DNA was extracted from soil samples (100±1 mg) using Quick-DNA Fecal/Soil Microbe Microprep Kit (ZYMO Research, CA, USA) following the manufacturer's instructions. The yield and purity of DNA extracts were quantified using an Implen Nanophotometer P300 (Implen GmbH, München, Germany). Purified DNA from five samples per sampling time (MI1: 15/06/2018, MI2: 27/08/2018, MI3: 26/09/2018, MI4: 25/04/2019, and MI5: 26/06/2019) were pooled and used as a template for sequencing analysis. The abundance of the bacterial and fungal communities of soil samples were estimated using high-throughput sequencing on Illumina MiSeq platform at UD-GenoMed Ltd. (Debrecen, Hungary). The V3-V4 region of 16S rRNA gene (in the case of bacteria) and the ITS1 region (in the case of fungi) were amplified from the microbial DNA extracted from each sample with the following primers: 16S forward: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3', 16S reverse: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3', ITS forward: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGGTCATTTAGAGGAAGTAA-

3', ITS reverse: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGCGTTCTTCATCGATGC-3'. The next steps were similar in both cases. 12.5 ng DNA and the KAPA HiFi Hot Start Ready Mix (KAPA Bio-systems, Wilmington, Massachusetts, US; Roche AG, Switzerland) was used to perform 25 cycles of PCR amplification, with denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s. Post-amplification quality control was performed by on an Agilent Bio-analyzer (Agilent Technologies, Santa Clara, CA, USA). MagSi-NGS^{Prep} Plus (Magtivio B.V., The Netherlands) magnetic beads was used to purify the amplicons away from the free primers and primer dimer species. For the Index PCR the Nextera XT Index Kit was used (Illumina, San Diego, CA, USA) with 502, 503, 504, and 701, 702, 703, 704, 705, 706 index primers. To perform the PCR reaction the KAPA HiFi Hot Start Ready Mix was used with the following parameters; 8 cycles with denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s. Before the library quantification MagSi-NGS^{Prep} Plus (Magtivio B.V., The Netherlands) magnetic beads was used to clean up the PCR products. For the library validation 1 µl of the diluted final library was run on a Bioanalyzer DNA 100 chip on an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Next, each library was normalized, pooled and loaded onto the Illumina MiSeq platform for 2x250 bp paired-end sequencing.

16S rRNA gene and ITS1 paired-end amplicon reads were processed using the Frogs pipeline (Escudié *et al.*, 2018). Briefly, forward and reverse reads were filtered and merged using vsearch (Rognes *et al.*, 2016) with the parameters: min amplicon size: 44; max amplicon size: 550; mismatch rate: 0.15). Merged sequences were clustered using swarm (Mahé *et al.*, 2014). Chimera sequences were removed using *remove_chimera.py* from the Frogs pipeline. Taxonomic assignment was performed using BLAST (McGinnis and Madden, 2004) against SILVA_SSU_r132_March2018 database (Quast *et al.*, 2012) for ribosomal small-subunit RNA and UNITE Fungi 8.2 database (Abarenkov *et al.*, 2010) for the fungal internal transcribed spacer region.

3.5. Data Processing and Modelling

Data processing and statistical analysis were done in R (R Core team, 2018). Gaussian error propagation was used to calculate propagated uncertainties of the cumulative sums and for the averages and model parameters.

Three different soil respiration models were used during the data processing to describe the response of the different CO_2 fluxes to the main biotic and abiotic drivers.

In the Lloyd and Taylor model (model 1) soil temperature is the only driving variable:

Equation 9. Lloyd and Taylor model

$$F = a \times e^{\left(b \times \left(\frac{1}{56.02} - \frac{1}{(Ts - 227.13)}\right)\right)}$$

Where, F is the soil CO_2 efflux (μmol CO_2 m^{-2} s^{-1}), T_s is the soil temperature at 5 cm in Kelvin, a and b are the model parameters.

Model 2 additionally includes SWC (Balogh *et al.*, 2011):

Equation 10. Lloyd and Taylor model with SWC (model 2)

$$F = a \times e^{\left(b \times \left(\frac{1}{56.02} - \frac{1}{(Ts - 227.13)}\right)\right) + \left(-0.5 \times \left[\log\left(\frac{SWC}{c}\right)\right]^2\right)}$$

where, *SWC* is the volumetric soil water content (%) and c is a model parameter.

Model 3 is extended model 2 by adding VIgreen as a driving variable:

Equation 11. Model 2 by adding VIgreen.

$$F = a \times e^{\left((d \times VIGreen) + b \times \left(\frac{1}{56.02} - \frac{1}{(Ts - 227.13)}\right)\right) + \left(-0.5 \times \left[\log\left(\frac{SWC}{c}\right)\right]^2\right)}$$

where, *VIgreen* is the vegetation index and d is a model parameter.

4. RESULTS AND DISCUSSION

4.1. Field experiment

4.1.1 Meteorological and environmental conditions during the study period

The maximum T_s (38.5 °C) were observed in 26th June 2019 and the highest SWC (57.6%) was observed in 8th November 2019. The minimum T_s (1.1 °C) was observed in 18th December 2017 and the lowest SWC (4.1%) on 22nd January 2019, while the maximum T_a (34.6 °C) was observed on 12th August 2019 and the minimum T_a (–11.6 °C) was observed on 28th February 2018.

Weather data (precipitation) were obtained from the EC station, located beside the study site. The annual sum of precipitation in 2018 was lower (552 mm) than it was in 2017 (620 mm), while it was the highest in 2019 (694 mm).

The values of the VIgreen measured during 2018-2019 varied between –0.06- 0.34 and –0.06-0.26, respectively. It was lower than 0 when no vegetation was present in the field (fallow periods), while it was rapidly growing after sowing and germination. The highest VIgreen values were related to the peak green biomass of the crops, observed on 16 April 2018 in wheat (0.3) and 26 June 2019 in sorghum (0.2) (Figure 12, middle panel).

While the values of LAI were equal to 0 when no vegetation was present in the field, the highest leaf area index was observed during the last stages of crop growth, on 16th May 2018 it was 5.0 $m^2 m^{-2}$ and on 15th August 2019, it was 5.7 $m^2 m^{-2}$ (Figure 12, middle panel).

4.1.2 Seasonal variation of soil respiration

Soil respiration values were low during winter, increased in spring and reached their maximum during the summer periods of 2018 and 2019 and started to decrease at the beginning of autumn (Figure 12). The highest emissions of 7.04±0.44 μmol CO_2 m^{-2} s^{-1} were detected immediately after soil loosening in the fallow period in 27th of August 2018 at an intermediate soil water content of 26% and soil temperature of 23 °C.

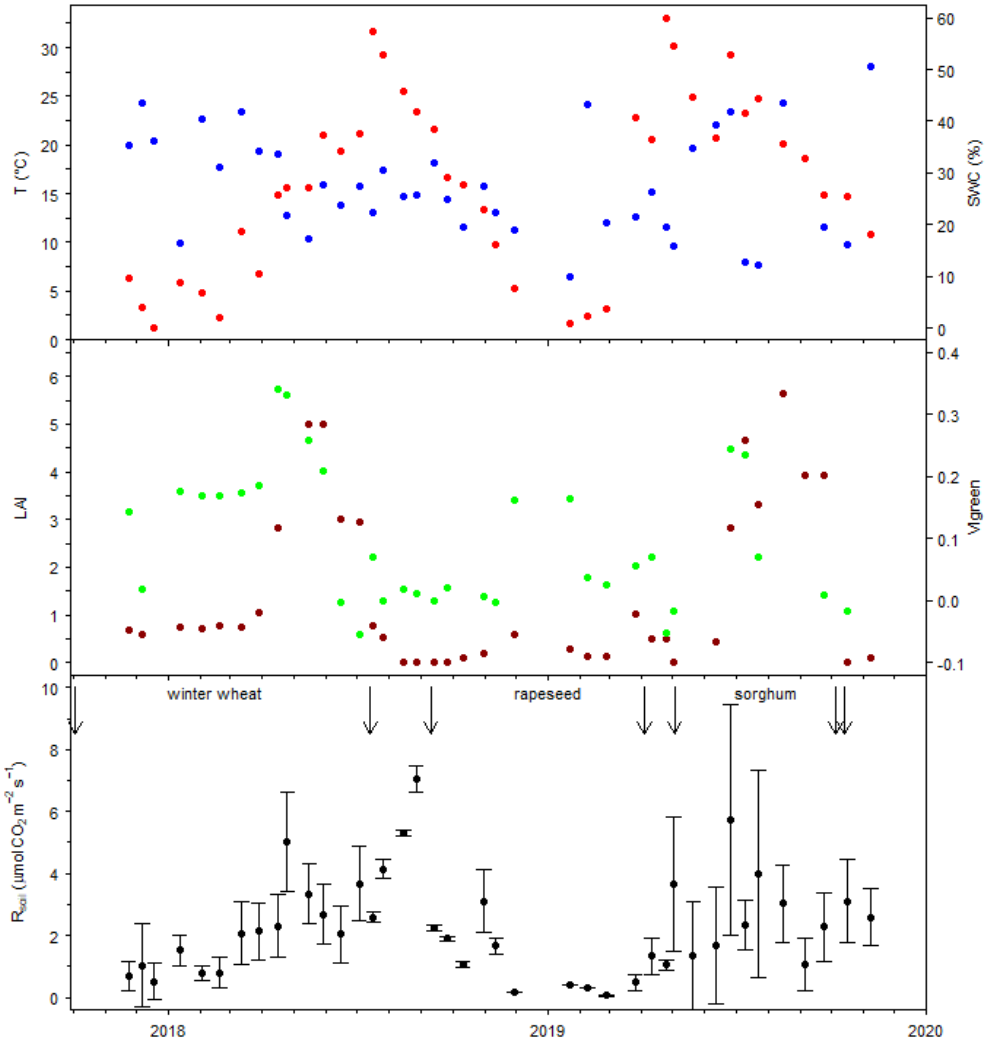

Figure 12. Top panel: Seasonal variations of soil moisture (SWC, %, blue dots) in the 0–7.5 cm soil layer, 5 cm depth soil temperature (T_s, °C, red dots). Middle panel: seasonal variations of leaf area index (LAI, m^2 m^{-2}, brown dots) and VIgreen index (VIgreen, green dots). Lower panel: crop rotation of the measured field (Winter wheat, Rapeseed, Sorghum), and soil respiration (Rs, whiskers showing standard deviation) during the two-year-long study period. Downward-facing arrows indicate the timing of sowing and harvesting in the site.

Soil respiration is typically related to air or soil temperature, soil water content and in more recent cases to substrate supply (Lloyd and Taylor, 1994; Balogh *et al.*, 2011). During the tillering stage the air and soil temperature gradually increases, plants grow quickly, soil

microbial activities are enhanced and root exudate production increases, providing suitable conditions for soil respiration (Tang *et al.*, 2018). Chengfang *et al.* (2020) suggested that the response of Rs to temperature is highly correlated with species diversity and hydrological changes, Furthermore, Schaufler *et al.* (2010) concluded that intermediate soil moisture conditions (between 20% and 60% WFPS) produced the highest CO_2 emissions. Photosynthesis was also proposed as one of the controlling variables in soil respiration (Tang, Baldocchi and Xu, 2005; Zhang, Lei and Yang, 2013).

The second-highest emission of 5.72±3.72 µmol CO_2 m^{-2} s^{-1} was observed in 26th of June 2019 a few weeks after sorghum sowing and N fertilizer application, accompanied by higher soil water contents (42%) due to a heavy rainfall before the day of the measurement. The ample water availability in the soil, plant activity (VIgreen, 0.3) and high soil temperature (29 °C) all resulted in a peak in soil CO_2 emission rate (Figure 12).

According to previous studies the impacts of N addition on CO_2 efflux varied widely with the level of N addition resulting in contradictory viewpoints concerning whether N applied to soils (regardless of its forms) increases soil CO_2 production or not (Johnston, Poulton and Coleman, 2009; Ramirez, Craine and Fierer, 2010). In addition, a previous study suggested that increased N supply significantly stimulated CO_2 emission and these conditions generally promoted autotrophic plant respiration of above- and belowground parts (Chen, Hooper and Lin, 2011), as well as rhizosphere respiration by microbes due to the accelerated decomposition of soil organic matter (Nakano, Nemoto and Shinoda, 2008). Therefore, these conditions are suitable for greater root respiration and more priming for the microbes (Moyano, Manzoni and Chenu, 2013).

Soil respiration decreased to 0.17±0.006 µmol CO_2 m^{-2} s^{-1} on 30th of November 2018. Accompanied by 19% of SWC and 5 °C of soil temperature, this lower efflux was due to the lack of vegetation in the field because the sowing of rapeseed at the beginning of autumn in 2018 wasn't successful. Kuzyakov (2006) mentioned that the vegetation may contribute strongly to the total CO_2 efflux by root and rhizo-microbial respiration. Another possible reason was the low temperature (Smith *et al.*, 2018), low temperatures slow down soil respiration by lowering rates of C cycling via autotrophic and heterotrophic respiration (Bond-Lamberty and Thomson, 2010; Melillo *et al.*, 2011). However, temperature and plant biomass were good proxies for variations in both autotrophic and heterotrophic capacity for soil respiration (Flanagan and Johnson, 2005; Smith *et al.*, 2018).

While it decreased substantially in the winter to 0.06±0.007 µmol CO_2 m^{-2} s^{-1} in 26th of February 2019 with 20% of SWC and at a temperature of 3 °C (Figure 12). This lower efflux was due to the low temperature and to the fact that the autotrophic respiration was generally very low or zero because there was no vegetation growing in the study site. However, the heterotrophic respiration (soil microorganisms: bacteria and fungi and other micro-organisms) could maintain both catabolic (CO_2 production) and anabolic processes (biomass synthesis) under frozen conditions (Drotz *et al.*, 2010). Thus, a gaseous exchange between the atmosphere and soil does not stop even in frozen soil, resulting in the accumulation of CO_2 during winter and its release into the atmosphere during spring thaw events (Burton and Beauchamp, 1994; Drotz *et al.*, 2010).

Soil respiration showed a positive correlation with soil temperature R=0.57, but no other investigated variable showed a significant correlation with soil respiration (Figure 13). Soil temperature was found to be the principal factor influencing soil respiration on both diurnal and longer time scales (Balogh *et al.*, 2019), it is used in the majority of Rs models (Lloyd and Taylor, 1994; Daly *et al.*, 2008; Zhang, Lei and Yang, 2013) due to its general effect on soil microclimate conditions and the biological activity of below-ground organisms (Yuste *et al.*, 2003; Dhital *et al.*, 2019). The eventual influence on soil respiration by the variation of soil temperature as observed in the present study was similar to previous studies (Shen, Li and Fu, 2015; Bao *et al.*, 2016; Feng *et al.*, 2017).

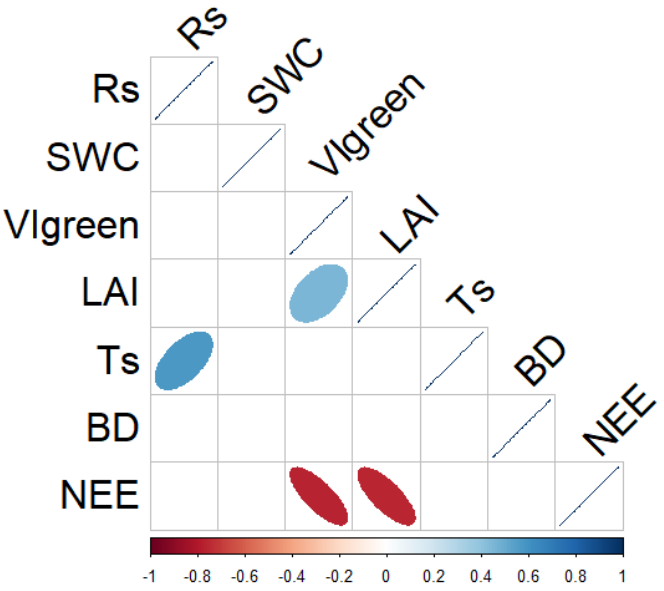

Figure 13. Correlation plot between soil respiration and SWC (soil water content), VIgreen (VIgreen index), LAI (leaf area index), T_s (soil temperature), BD (bulk density of the soil) and NEE (net ecosystem exchange of CO_2). Only statistically significant ($p < 0.05$) correlations are presented

Using an exponential model (Model 1, Lloyd and Taylor (1994), Eq. 9) between CO_2 efflux and soil temperature, the goodness-of-fit was r^2=0.4 (Table 9). Since there is no one single widely accepted model type that can describe the relationship between soil CO_2 efflux and soil water content (Davidson, Belk and Boone, 1998), using Lloyd and Taylor soil respiration model extended by a log-normal function of soil water content (Eq. 10) would allow to include SWC in the modelling and this way the goodness-of-fit value had slightly improved (r^2=0.45).

Furthermore, using soil respiration model extended by a log-normal function of soil water content and by an exponential function of VIgreen (Eq. 11) was apt to represent better the response of soil respiration to these factors at our site with r^2= 0.54 (Table 9).

Table 9. r^2 values for soil respiration and the three drivers (Ts, SWC and VIgreen), and model coefficients for model 1, 2 and 3. Statistical significance levels of the coefficients and model fitting were p-value <0.001 in all cases.

	r^2	a	b	c	d
Model 1	0.40	0.85	237.33	—	—
Model 2	0.45	1.54	242.31	71.66	—
Model 3	0.54	1.27	247.45	66.07	0.11

Fitted parameters of the three soil respiration models (model 1, 2 and 3, table 9) show that Model (3) where T_s, SWC and VIgreen were included was the best fit because the r squared value improved with the increasing number of variables. The log-normal shape of soil moisture-respiration response was proposed before (Balogh *et al.*, 2011; Moyano, Manzoni and Chenu, 2013). It originated from the Michaelis Menten kinetics of the response of respiration to substrate and oxygen availability (Davidson *et al.*, 2012).

The reflected green and red lights of the surface obtained by commercial digital camera (Canin Eos 350D) were used to calculate VIgreen, which changed with the different phenological stages of the vegetation during the seasons (Nagai *et al.*, 2014). Muraoka *et al.* (2013) conducted an experiment to prove that VIgreen will change according to the season, therefore it can be incorporated into soil respiration models (Huang *et al.*, 2012).

Relationship between soil respiration and ecosystem respiration

We also aimed to quantify the share of soil respiration in total ecosystem respiration (R_{eco}). Beside soil respiration R_{eco} has another major part, which is the respiration of the above-ground autotrophic (plant shoots) and heterotrophic (animals) components. While soil respiration is the largest component within R_{eco} (Claire L. Phillips *et al.*, 2017), the respiration of the above-ground parts of the plants can be significant as well. During the field work we measured soil respiration in different phenological stages, even when no plants were present (fallow periods). Therefore, we had a wide range of plant activity and aboveground respiration component.

R_{eco} was calculated by partitioning NEE (Nagy *et al.*, 2011) , therefore it was measured continuously during the study. For the analysis, we selected the R_{eco} values measured in the same time (half-hour frequency) when manual soil respiration measurements were conducted.

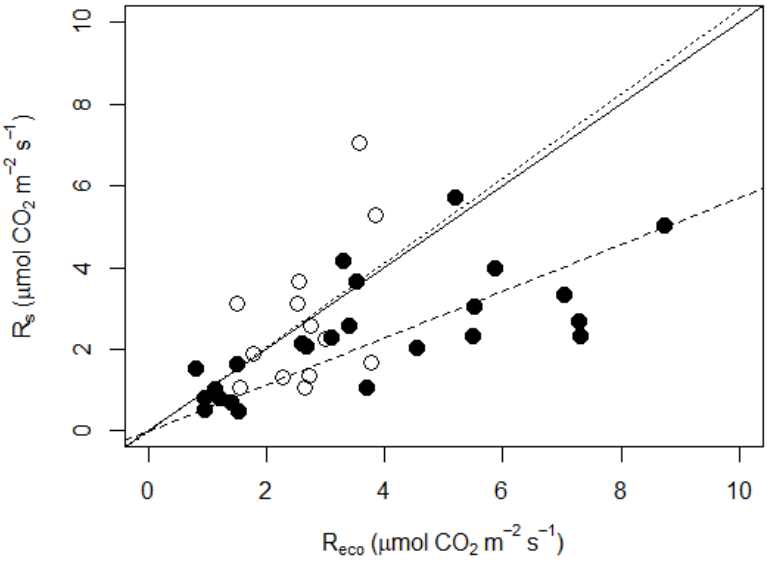

Figure 14. Average soil respiration as a function of ecosystem respiration during the study period, November 2017-November 2019, Kartal. Full circles represent measurements when leaf area index was greater, than 0.5 (LAI>0.5), while open circles represent measuring occasions when no, or small amount of plant biomass were present in the field (LAI<0.5). Solid line is 1:1 line, while dotted line is the linear regression between the variables when LAI<0.5, and dashed line represent the regression when LAI>0.5.

Figure 14 shows the regressions between Rs and R_{eco}. The slope of the linear regression for the whole dataset was 0.65 ($p<0.001$, not presented in Figure 14), but we split our dataset into two parts: when LAI was significant (LAI>0.5, full circles) and when there was no or very small plant biomass (LAI<0.5). Both regressions are significant ($p<0.001$), but the slope of the regressions is different: the slope was 0.57 in the first case and 1.03 in the latter. Therefore, the

share of soil respiration in total ecosystem respiration was 57% on average when crops were present in the field, while ecosystem respiration originated from soil respiration, when crops were not present in the field. These results are similar to findings of Zhang *et al.* (2015), Myklebust, Hipps and Ryel (2008) and Claire L Phillips *et al.* (2017)

4.2. Microbiological results

4.2.1 Microbial diversity

The dynamics of soil microorganism development expressed by changes in the number of the particular groups of bacteria and fungi settled in this environment, i.e., the total number of bacteria, fungi and many others, is a measurable indicator of the biological life of soil (Gałązka, Grzęda and Jończyk, 2019)**.**

As variables in the analysis, the following activities were chosen: total number of bacteria (actinomyces, ammonificans and denitrifying bacteria), total number of fungi (Figure 15), and indicators of soil functional metabolism evaluation on the example of BIOLOG EcoPlate analysis.

The highest total bacteria number in our soil which was collected from Kartal was (5×10^6 CFU g^{-1} soil) in S1 (Figure 15) in the summer of 2018 (15^{th} of June 2018), while the lowest total bacteria number was in S2 (27^{th} August 2018) with (2.9×10^5 CFU g^{-1} soil). Also, the highest number of fungi was found in the same year and in the same sample, S1 (Figure 15) with 45×10^2 (CFU g^{-1} soil) and the lowest number was found in S4 and S5 (25^{th} of April 2019 and 26^{th} of June 2019, respectively) with (15×10^2 CFU g^{-1} soil).

The highest number of denitrifying bacteria present in the soil collected from Kartal was found in S1 with 2300 cell/ml, while there were zero denitrifying bacteria in S3 and S5 (26^{th} of September 20 and 26^{th} of June 2019) (Figure 15). Deutzmann *et al.* (2014) found that denitrifying bacteria have been found to play a significant role in the oxidation of methane (CH_4) (where methane is converted to CO_2 and water and energy)

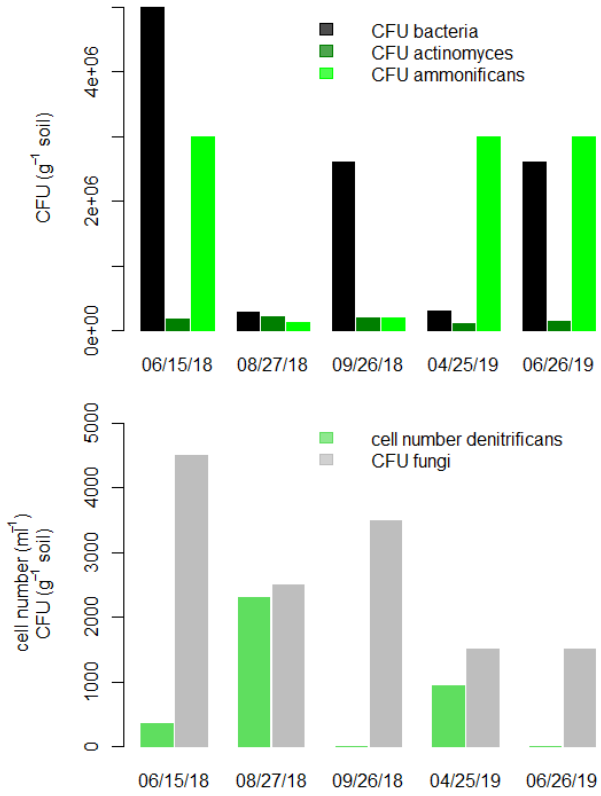

Figure 15 . Microbial diversity (Bacteria population, actinomyces, ammonificans,fungi and denitrificans) in 5 soil samples (S1: 15th of June 2018, S2: 27th of August 2018, S3: 26th of September 2018, S4: 25th of April 2019 and S5: 26th of June 2019) during two years long study period (2018-2019)

Soil sample (S1: 15th of June 2018) were characterized by relatively high populations of microorganisms (bacteria, fungi and denitrifying bacteria). The CO_2 efflux, the soil temperature and SWC of this sampling date were; 2.04 µmol CO_2 m^{-2} s^{-1}, 19.37 °C and 23.63% respectively.

Microorganisms are present in all ecosystems and due to their rapid responses to physical and chemical changes, they can be used as bio-indicators of environmental quality (Sofo and Ricciuti, 2019). Furthermore, Gryta, Frąc and Oszust, (2014) mentioned that microorganisms play an important role in many biological processes in order to circuit elements in the ecosystem and the decomposition of organic matter.

It is important to assess the entire populations and the whole ecosystem because, in this way, it is possible to obtain the most likely reflection of the natural environmental conditions. The enzymatic activity of the microorganism populations is strictly correlated with its composition (Garland and Mills 1991). Changes in enzymatic activity could be the indicator of the changes occurring in the microorganism populations under a wide range of conditions.

4.2.2 AWCD of all carbon sources in soil microbial communities within 216 h incubation time.

Garland and Mills (1991) concluded that the degree of carbon source oxidation was proportional to the metabolic capability of corresponding microbes in general, which could be characterized by AWCD. The AWCDa of the five soil microbial communities are shown in (Figure 16). Our results showed that the AWCDa of all soil samples displayed an apparent lag phase in the first 24 h. Then the average absorbance started to increase significantly, showing that the five soil microbial communities were able to metabolize organic substrates in BIOLOG EcoPlates.

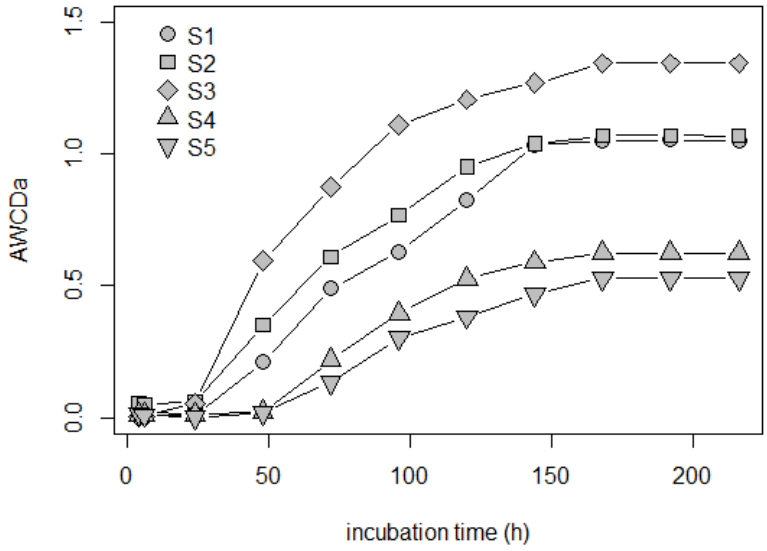

Figure 16 . The Average well color development of all carbon sources in soil microbial communities within 216 (h) incubation time. (S1: 15th of June 2018, S2: 27th of August 2018, S3: 26th of September 2018, S4: 25th of April 2019 and S5: 26th of June 2019)

The metabolic activity analysis was from 4 h to 216 h (Figure 16), but the period selected was from 24 h to 168 h (7days), the slopes of AWCDa curves within this period represented the average metabolic rates of the microbial communities (Kong, Wang and Ji, 2013). However, some studies showed the time of 72 h or 96 h is the more reasonable time since fungi will spread after 96 h inoculation (Cai *et al.*, 2010), while Jia, Dong and Zhou (2013) claimed that 144 h or 168 h is better for bacteria and fungi respectively.

The increased rate of AWCDa was slower after 144 h (6 days). Meanwhile, the AWCDa reached the tops of 168 h (7days) and the metabolic utilization capability of microbial communities served to be stable (Tian-Yuan *et al.*, 2014), stating that all cultivable microorganisms enable to steadily use carbon sources during the stable period (Miyake *et al.*, 2016). Among the five soil samples, the metabolic rate of S3 was faster than S1, S2, S4 and S5 (Figure 16). The AWCDa of S3 increased from 0 to around 1.34 after 168 h (7days), this higher metabolite rate might be due to management practices, sowing of rapeseed (figure 12) and NPK 15-15-15 fertilization application (table 3) before one month of soil sampling of S3 and also due to abiotic drivers which will be discussed below.

S5 showed the lowest metabolic rate of the substrates in the BIOLOG EcoPlate, and the AWCDa increased to around 0.52 when it got to the stable, which indicated that the utilization of substrates by S5 was less efficient than the others, it could be due to the higher soil water content (41.86%) in the sampling date of this sample because the tolerance to water stress varies significantly across soil microorganisms (Lennon *et al.*, 2012)

Moyano, Manzoni and Chenu (2013) found a low tolerance corresponding to complete metabolic inactivity at ca. ⁻1.5 MPa is found in strains of bacteria (spiral bacteria), while the highest tolerance of over ⁻60 MPa has been observed in fungal species (yeasts, ascomycete and xerophilic fungi). However, other study show that the response of microbial activity to water potential is very similar across soils of different properties and under different climates (Schaufler *et al.*, 2010).

Li, Ou and Chen (2014) mentioned that soil moisture affect the activity of microorganism and plant roots and the diffusion of gases through the soil pores, and also affect the change of the substrate supply and plant growth

This illuminated that biotic (presence of plants) (Balogh *et al.*, 2019) and abiotic (soil temperature and moisture) (Risk, Kellman and Beltrami, 2002) drivers, agricultural management practices (sowing and N fertilization) (Al-Kaisi, Kruse and Sawyer, 2008) and the different season of sampling during the two years long study period (Gałązka, Grzęda and Jończyk, 2019) had an obvious effect on promoting metabolic activity of microorganisms in soil samples. Besides, in the stable period, there were significant differences in the AWCDa among four soil microbial communities ($p < 0.05$) except between S1 and S2 there was no significant differences, and the order was S3 > S2 > S1> S4 >S5 (26th of September 2018 > 27th of August 2018 > 15th of June 2018 > 25th of April 2019 > 26th of June 2019), which suggested that soil properties, biotic

drivers, management practices and abiotic drivers (soil temperature and soil water content (related to soil texture conditions)) (Schaufler *et al.*, 2010) were, the higher metabolic capability of soil microbial community was.

Nevertheless, the AWCDa of S3, S2 and S1 were 1.34, 1.07 and 1.05, respectively with 17 °C, 25 °C and 19 °C of soil temperature and 24%, 25% and 23% of soil water content (Figure 12, top panel) respectively. Soil temperature was found to be the principal factor influencing soil respiration (Yuste *et al.*, 2003; Balogh *et al.*, 2019), due to its general effect on soil microclimate conditions and the biological activity of below-ground microorganisms (Yuste *et al.*, 2003; Dhital *et al.*, 2019) which affect the metabolic capability of corresponding microbes, meanwhile, moisture in soils is essential for both plant growth (Huxman *et al.*, 2004) and soil microbial activity.

4.2.3 Metabolism of different biochemical categories of substrates

Tian-Yuan *et al.* (2014) reported that the BIOLOG Eoplates were assigned into six categories, including carboxylic acids, carbohydrates, amino acids, polymers, miscellaneous, and amines/amides according to the biochemical properties of carbon sources. The 31 kinds of carbon substrates found in the BIOLOG EcoPlates were described in Table 8. The AWCD of different types of carbon sources were classified and analyzed in the experiment. Figure 17 showed the Average well color development of carboxylic acids, carbohydrates, amino acids, polymers, miscellaneous, and amines/amides. Our results indicated that the utilization of six types of carbon sources by microbes presented an increasing trend with the prolongation of incubation time.

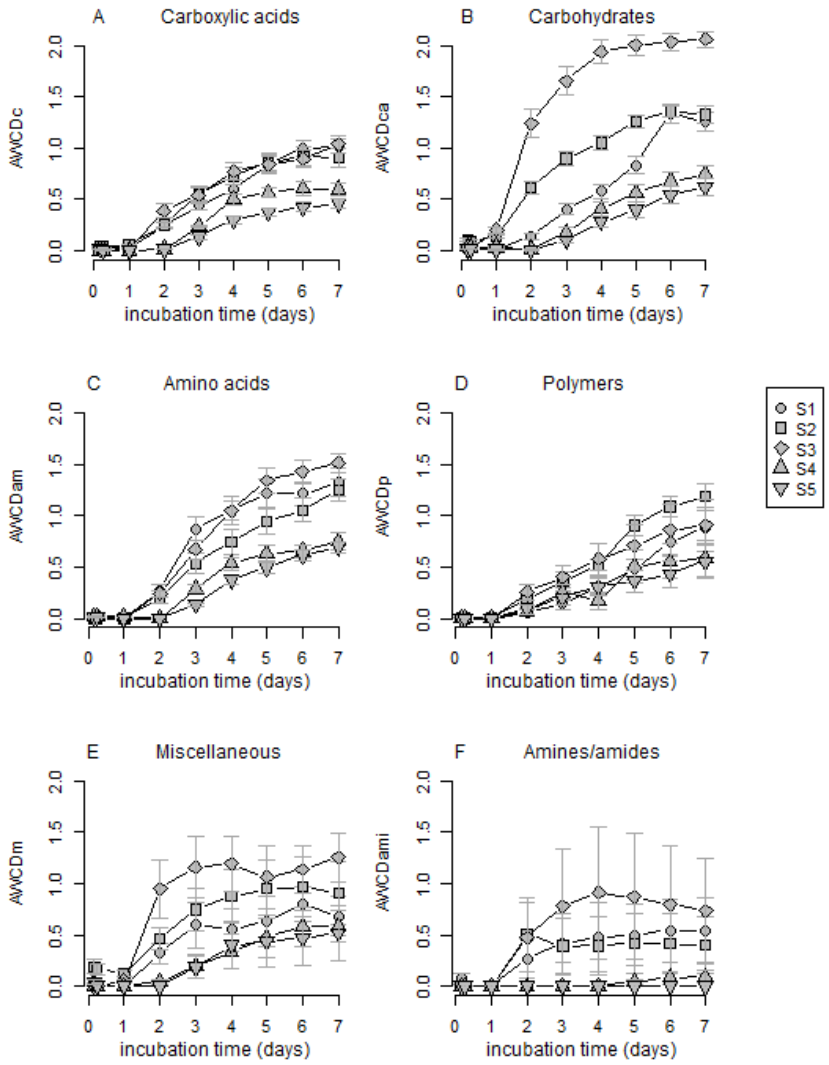

Figure 17. The Average well color development of six types of carbon sources in five soil microbial communities, including carboxylic acids (A), carbohydrates (B), amino acids (C), polymers (D), miscellaneous (E), and amines/amides (F).

For miscellaneous, amines/amides and polymers there was no significant difference in the utilization among the five soil microbial communities. The utilization of carbohydrates, carboxylic acids and amino acids by the soil microbial communities, however, differed significantly ($p < 0.05$). For carbohydrate, there were significant differences between (S1, S3, S4 and S5) also between (S2, S3, S4, and S5) and between (S3, S4, S5). For carboxylic, acids there were significant differences between (S1, S4 and S5) and between (S3, S4 and S5). For amino acids, there were significant differences between (S3, S4 and S5), and the utilization capability order was S3 > S2 > S1> S4 > S5 (26th of September 2018 > 27th of August 2018 > 15th of June 2018 > 25th of April 2019 > 26th of June 2019), thereby clarifying that the microbial community of soil samples increased the utilization of carbohydrate, carboxylic acids and amino acids during the sampling period.

For different microbial communities, the capacity utilization of six-type carbon sources was different. The current study revealed that carbohydrate was characterized by the highest metabolic activity, while the lowest activity was determined for amines/amides. The average well color development of carbohydrates was the highest, and the lowest was amines/amides, whereby illustrating that carbohydrates were the carbon sources with the highest degree of metabolic utilization, and the lowest degree of metabolic utilization was amines/amides. Our results were similar to the findings reported by previous researches, the utilization of carbohydrates was relatively higher than other substrates among the six types of carbon sources, whereas the lowest utilization substrates differed from microbial communities (Kong, Wang and Ji, 2013; Tian-Yuan *et al.*, 2014; Lopes *et al.*, 2016; Ge *et al.*, 2018). Generally, the metabolic rates of carbon sources were determined by calculating a single value (AWCD) at a single time point through BIOLOG EcoPlates, which demonstrated and gave a clear comparison of soil microbial communities.

4.2.4 Comparison of metabolic functional diversity indices

Zhang, Lei and Yang (2013) reported that the metabolic functional diversity of microbial communities was actually reflected by functional diversity indices. The Shannon diversity index (H´), Shannon evenness index (E), and Simpson index (D) of five soil microbial communities were showed in table 10. Shannon diversity index (H´) is greatly influenced by the species richness of communities (Sun *et al.*, 2012). A higher diversity index indicated that the soil microbial community metabolic functional diversity was larger (Strong, 2016).

Table 10. Comparison of metabolic functional diversity indices of the soil microbial communities

Sample	Shannon diversity (H´)	Shannon evenness (E)	Simpson diversity (D)
1	2.633±0.067	0.782±0.002	0.955±0.001
2	3.198±0.056	0.960±0.002	0.955±0.001
3	3.195±0.057	0.939±0.002	0.957±0.001
4	2.879±0.084	0.931±0.004	0.937±0.003
5	2.902±0.079	0.953±0.004	0.939±0.003

Data in the table are mean ± SD, n = 5. Using T student test of diversity indices separately

Zhang, Lei and Yang (2013) mentioned that the higher the Shannon evenness index (E) was, the more evenly the individuals divided. Simpson (1949) proposed the Simpson index (D) which is greatly reflected by the most common species. Student t-test was separately used in

these indices. The results (Table 10) clearly indicated that two indices except for the Simpson index (D) of the soil microbial communities had a significant difference ($p < 0.05$).

The Shannon diversity index of S2 was highest, followed by sample S3, S5, S4 and S1, while the Shannon evenness index of S2 was the highest again, followed by S5, S3, S4 and S1, indicating that the species richness and evenness of microorganisms in soil were relatively high in those periods (S1: 15th of June 2018, S2: 27th of August 2018, S3: 26th of September 2018, S4: 25th of April 2019 and S5: 26th of June 2019). However, as shown in table 10, there was no significant difference in the Simpson index (D), which manifested that the most common species of the five soil microbial communities were similar (Magan, 2006).

Indices like AWCD, H´, E, D and S (data not shown) calculated based on results measured OD is very useful to describe activity and diversity of microorganism population (Gryta, Frąc and Oszust, 2014). The BIOLOG EcoPlate has been found to be a good indicator of reflecting changes of metabolic activity and/or potential functional versatility of microbial communities exposed to abiotic conditions (Kapanen *et al.*, 2013). The AWCD reflects the oxidative ability of microorganisms developed in Biolog, and it may be used as an indicator of microbial activity. Additionally, the calculation of the richness index is also sensitive enough to evaluate microbial activity. High value of richness index indicates a high number of oxidized C substrates (Gryta, Frąc and Oszust, 2014).

4.2.5 Metagenomics results

Relative abundances of soil procaryotics in five soil samples

The soil metagenomes obtained at 0-15cm depth and five time points (MI1: 15/06/2018, MI2: 27/08/2018, MI3: 26/09/2018, MI4: 25/04/2019, and MI5: 26/06/2019) throughout two years long study period from agricultural field, which received different agricultural management practices, provided new insights into the functional and community dynamics of indigenous microbial communities.

The figure 18 represents the relative abundances of top 10 phyla in our bacterial soil samples, where the most abundant phyla comprised Actinobacteria, Proteobacteria, Acidobacteria and Firmicutes. Meanwhile the less abundant phyla obtained in our study were Nitrospirae, Gemmatimonadetes, Bacteroidetes and Chloroflexi.

This distribution was almost the same in the five soil samples and there were no big differences between their percentigies.

Our results revealed remarkable composition stability for these microbial communities in their functional, taxonomic, and individual population components during the sampled period.

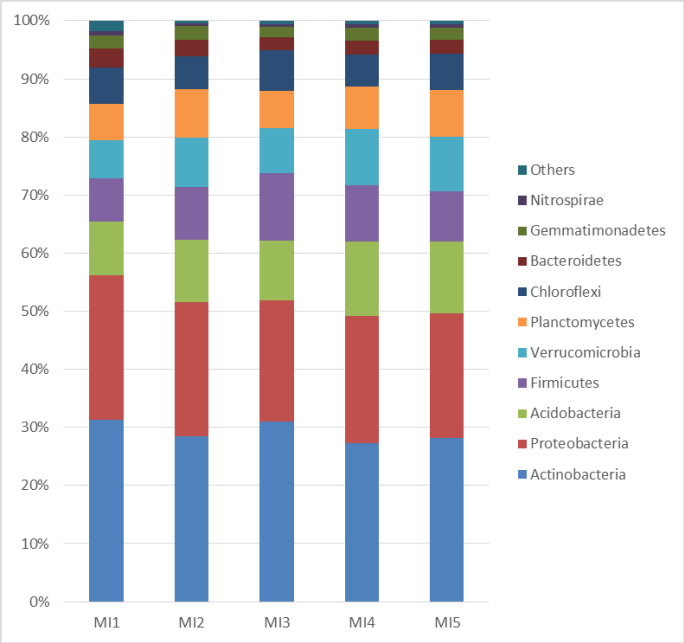

Figure 18. Relative abundances of soil prokaryotic for the top 10 phyla in the five soil samples (MI1—MI5)

In the class level (Figure 19) Alphaproteobacteria, Actinobacteria and Thermoleophilia were the most abundant classes in all five soil samples which belongs to the same phylum; Actinobacteria, followed by Bacilli which belong to Firmicutes phylum. Other classes were present in all the five samples which belong to Proteobacteria like Gammaproteobacteria and Deltaproteobacteria, where Deltaproteobacteria was less abundant compared with Gammaproteobacteria

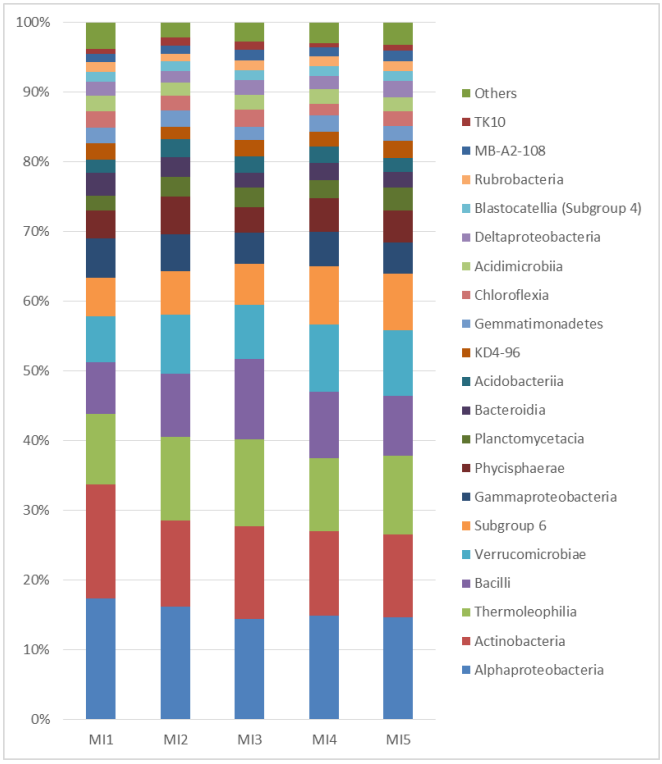

Figure 19. Relative abundances of soil prokaryotic for the top 20 classes in the five soil samples (MI1—MI5)

Among the relative abundances of top 20 species in the five soil samples. Seven species belong to Proteobacteria phylum (*Archangium gephyra sp, Sphingomonas sp. Lysobacter sp. Microvirga sp. Sorangium cellulosum, beta proteobacterium WX53, Aetherobacter rufus*), five species belong to actinobacteria phylum (*Geodermatophilaceae bacterium URHB0062, Mycobacterium sp. Streptomyces sp. Actinoallomurus sp. Luedemannella sp*), where all of them belong to the same class; Actinomycetia (Figure 20)

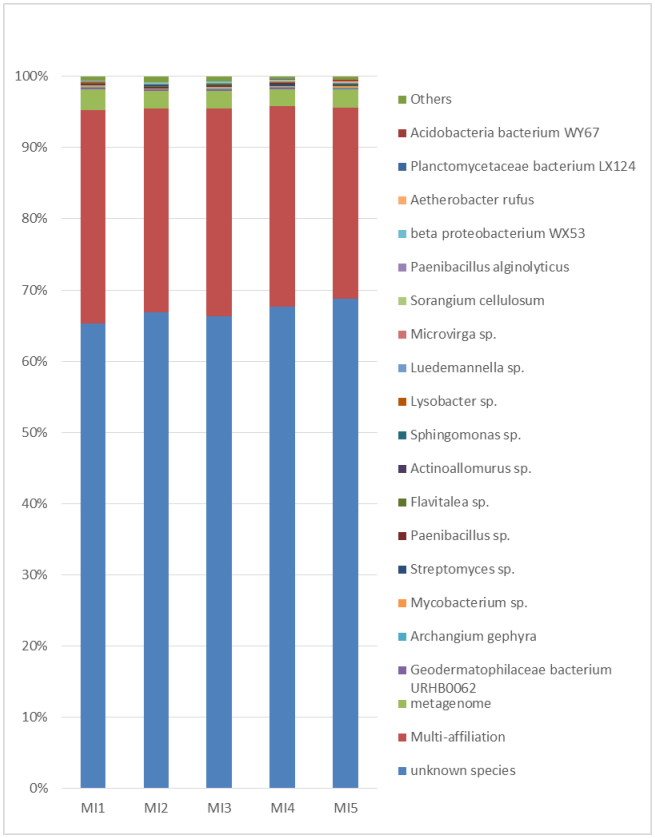

Figure 20. Relative abundances of soil prokaryotic for the top 20 species in the five soil samples (MI1—MI5)

Relative abundances of soil fungal in five soil samples

The figure 21 represents the relative abundances of soil fungal for top 10 phyla in our five soil samples, where the most abundant phyla comprised Ascomycota, Basidiomycota and Mortierellomycota. The cited phyla were higher in MI2, MI1 and MI5, respectively. While the less abundant phyla obtained in our study were; Blastocladiomycota in MI3, Zoopagomycota in MI1, and Olpidiomycota in MI1, respectively

This distribution was different among the five soil samples and there were significant differences between their percentigies.

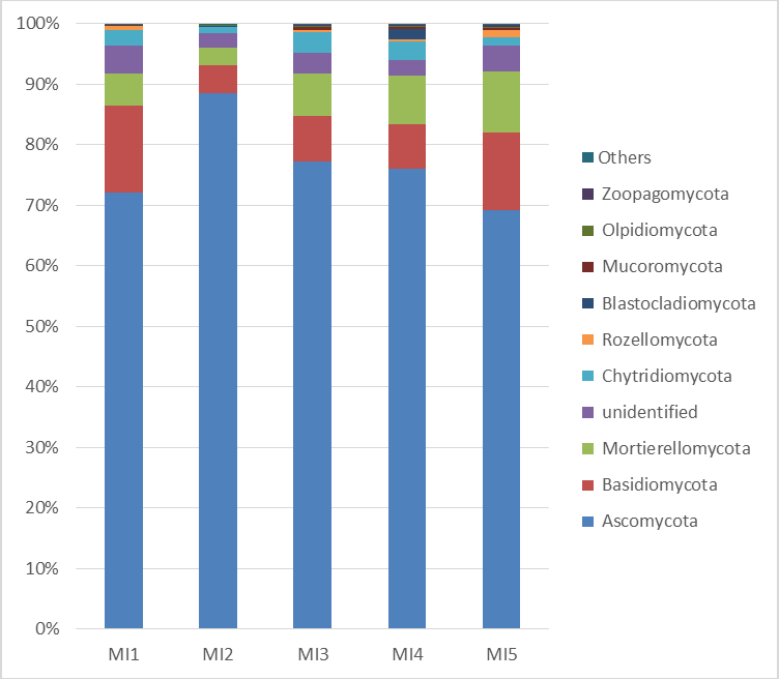

Figure 21. Relative abundances of soil fungal for the,top 10 phyla in the five soil samples (MI1—MI5)

In the class level (Figure 22), Sordariomycetes, Dothideomycetes, Eurotiomycetes were the most abundant classes in all five soil samples which belongs to the same phylum; Ascomycota, followed by Agaricomycetes class which belongs to Basidiomycota phylum. Other classes were present in all the five samples which belong to Chytridiomycota phylum like; Rhizophlyctidomycetes, Spizellomycetes and Chytridiomycetes. In which Chytridiomycetes was less abundant compared with two other classes.

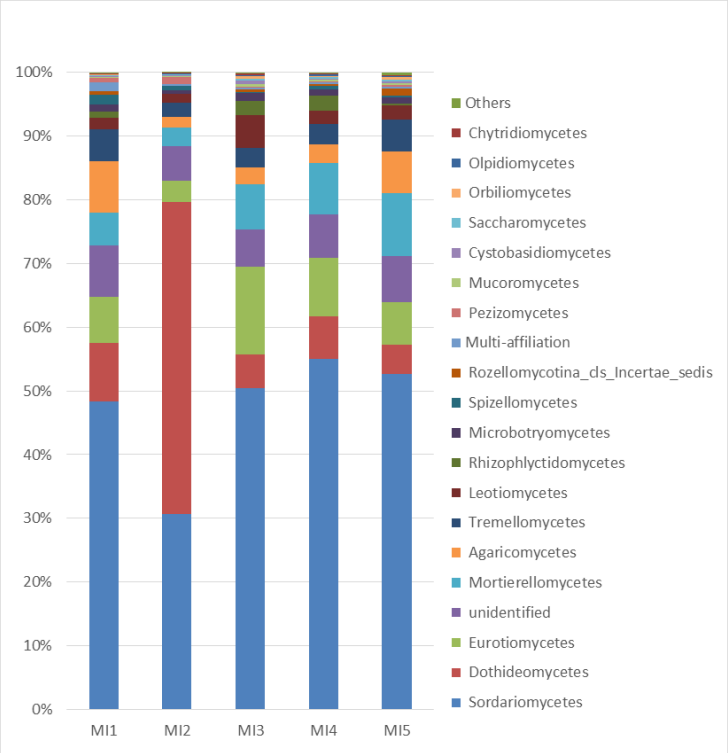

Figure 22.Relative abundances of soil fungal for the top 20 species in the five soil samples (MI1—MI5)

Among the relative abundances of top 20 species in the five soil samples. Fourteen species belong to Ascomycota phylum *(Verticillium_dahliae, Sclerostagonospora_sp, Fusarium_sp, Stachybotryaceae_sp, Lasiosphaeriaceae_sp, Talaromyces_euchlorocarpius, Acrostalagmus_luteoalbus, Aspergillus_clavatonanicus, Chaetomium_angustispirale, Plectosphaerella_oligotrophica,, Penicillium_sp, Schizothecium_sp, Trichoderma_atroviride, Acremonium_furcatum*). One species belong to Chtridiomycota phylum (*Rhizophlyctis_rosea*) and the other one (*Mortierella_elongata*) belong to opisthokonta phylum (Figure 23)

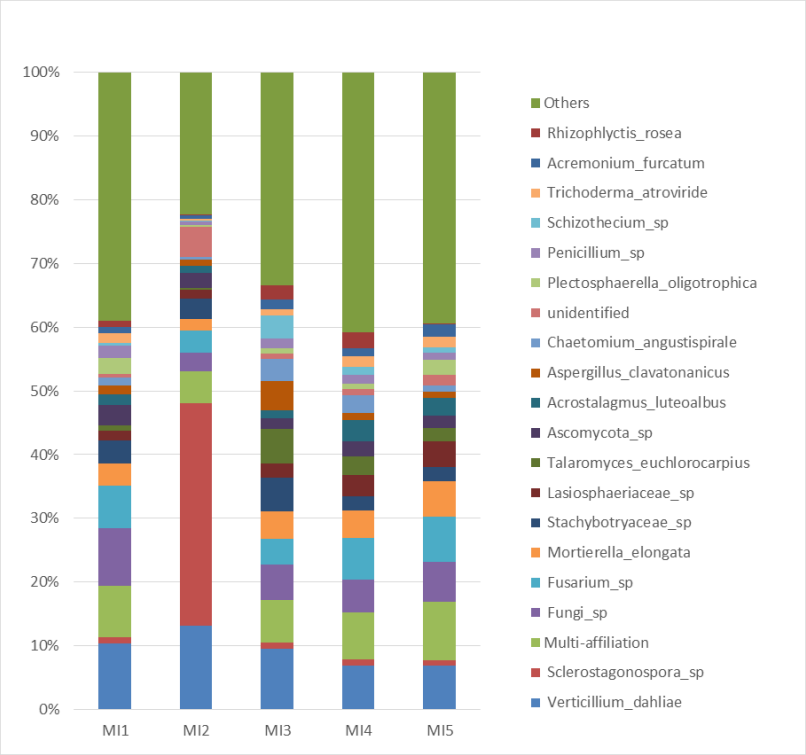

Figure 23. Relative abundances of soil fungal for the top 20 species in the five soil samples (MI1—MI5)

Soil is one of the most complex and challenging environments for microbiologists. In fact, although it contains the largest microbial diversity on the planet, the majority of these microbes are still uncharacterized and represent an enormous unexplored reservoir of genetic and metabolic diversity (Mocali and Benedetti, 2010). Metagenomics, the study of the entire genome of soil biota, currently represents a powerful tool for assessing the diversity of complex microbial communities, providing access to a number of new species, genes or novel molecules that are relevant for biotechnology and agricultural applications (Freedman *et al.*, 2016). Moreover, this technology in soil have mainly focused on bacteria, although recent improvements to genomic databases have allowed for metagenomic insights into the fungal community as well (Hesse *et al.*, 2015). According to our result we found that the taxonomic membership and functional capacity of bacterial and fungal community are almost the same among the five soil samples.

In fact, the total number of prokaryotic cells on earth has been estimated at 4-6 × 10^{30} including 10^{6}-10^{8} individual genomes belonging to different species (Mocali and Benedetti, 2010). Agricultural management practices such harvesting (Cardenas *et al.*, 2015), tillage application (Carbonetto *et al.*, 2014) and nitrogen (N) fertilization (Orellana *et al.*, 2018) can affect the structure and composition of soil microorganisms which promote microorganisms by decreasing soil organic matter stability and therefore increasing nutrient availability.

4.3. Laboratory experiments

4.3.1 First experiment

CO_2 efflux cources during 4 and 5 weeks laboratory study period under different traitments and two levels of soil moisture in the presence/absence of plants

This experiment contained two different series with different levels of SWC and different amount of treatments, CO_2 efflux of the samples was measured weekly.

20% of soil moisture and three levels of NH_4NO_3 (N0, N50 and N100) was applied in the first series. Figure 24 shows that the CO_2 efflux in the first week was almost the same in all treatments (N0, N50, N100) in bare soil at lower soil moisture (20%) with 0.49±0.15, 0.39± 0.11, 0.53±0.21 µmol CO_2 m^{-2} s^{-1}, respectively, while in planted soil the CO_2 efflux in N100 was higher than N0 and N50 with 0.70 ±0.86, 0.31±0.01, 0.30±0.4 µmol CO_2 m^{-2} s^{-1}, respectively.

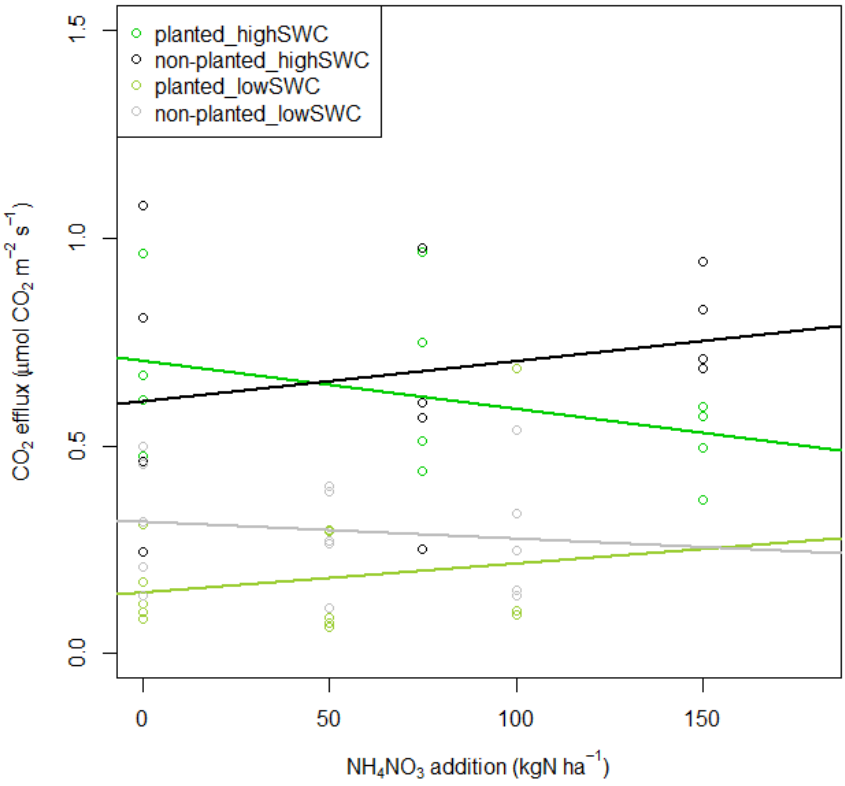

Figure 24. CO_2 efflux of different treatments (N0, N50, N75, N100 and N150) under two levels of soil moisture (20 and 25%) in planted and non-planted (bare soil) during four-five weeks long laboratory study period.

In the second week of gas measurement , the CO_2 efflux in bare soil at lower soil moisture (20%) was around two times higher than planted soil, with 0.45±0.47, 0.40±0.06, 0.34±0.06

µmol CO_2 m^{-2} $s^{-1}$and 0.17±0.08, 0.29±0.08, 0.15±0.11 µmol CO_2 m^{-2} s^{-1}, respectively. There was no effect of N treatments.

In the third week of gas measurement, the same phenomenon was observed as the first and the second week, at lower soil moisture the CO_2 efflux in bare soil was around three times higher than in planted soil in all treatments with 0.32±0.1, 0.27±0.04, 0.25±0.05 µmol CO_2 m^{-2} s^{-1} and 0.08±0.06, 0.06 ±0.04, 0.10±0.05 µmol CO_2 m^{-2} s^{-1}, respectively.

In the fourth week of gas measurement, the efflux decrease at lower soil moisture in both soils bare and planted with 0.13±0.13, 0.11±0.10, 0.15±0.06 µmol CO_2 m^{-2} $s^{-1}$and 0.12±0.06, 0.07±0.03, 0.09±0.07 µmol CO_2 m^{-2} s^{-1}, respectively.

We repeated the experiement with slight changes in the treatments. 25% of soil moisture and three levels of NH_4NO_3 (N0, N75 and N150) was applied in the second series. In the first week and at higher soil moisture (25%) the CO_2 efflux was significantly higher, the efflux in bare soil was higher than planted soil (Figure 25) in all treatments (N0, N75, N150) with 0.81± 0.07, 0.57±0.04, 0.71±0.11 µmol CO_2 m^{-2} $s^{-1}$and 0.67±0.07, 0.51±0.08, 0.3 ±0.11 µmol CO_2 m^{-2} s^{-1}, respectively, and there was no obvious effect of NH_4NO_3 in both planted and bare soil.

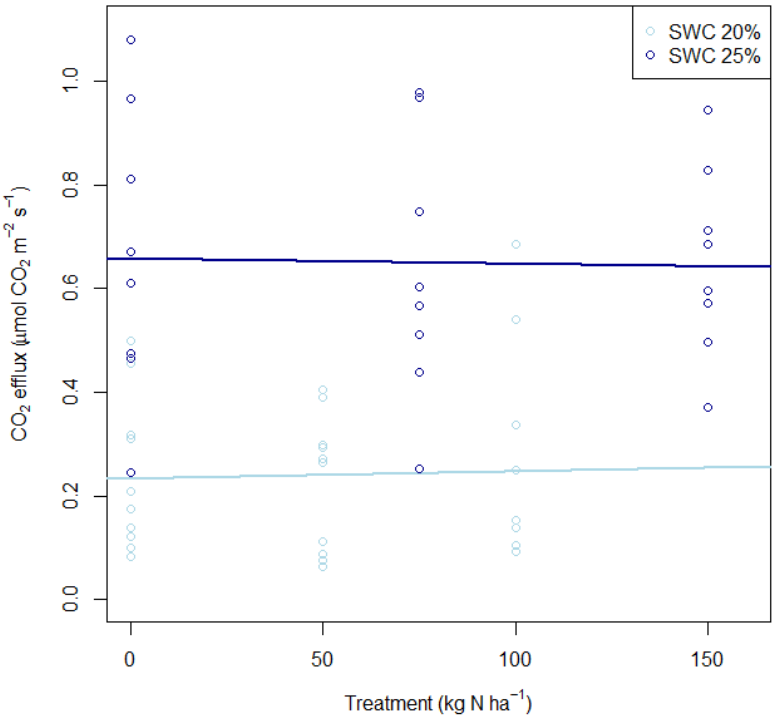

Figure 25. CO_2 efflux (µmol CO_2 m^{-2} s^{-1}) under higher and lower soil moisture and four levels of N fertiliztion (N0, N50, N100 and N150) during the gas measurement.

In the second week of measurement, at higher soil moisture the efflux was higher in planted soil than in bare soil in (N0 and N75): 0.61±0.09, 0.97±0.02 µmol CO_2 m^{-2} $s^{-1}$and 0.46± 0.26, 0.60±0.06 µmol CO_2 m^{-2} s^{-1}. But it was lower in N100: 0.59±0.19 µmol CO_2 m^{-2} s^{-1}in planted soil and higher in bare soil by 0.94±0.42 µmol CO_2 m^{-2} s^{-1}.

In the third week, the efflux increased significantly in bare and planted soil to 1.08 ± 0.84, 0.98±0.18, 0.83±0.20 µmol CO_2 m^{-2} s^{-1} and 0.97±0.23, 0.74±0.06, 0.57±0.07 µmol CO_2 m^{-2} s^{-1}respectively, and there was no effect of fertilization in both bare and planted soil within the different N treatments (Figure 26).

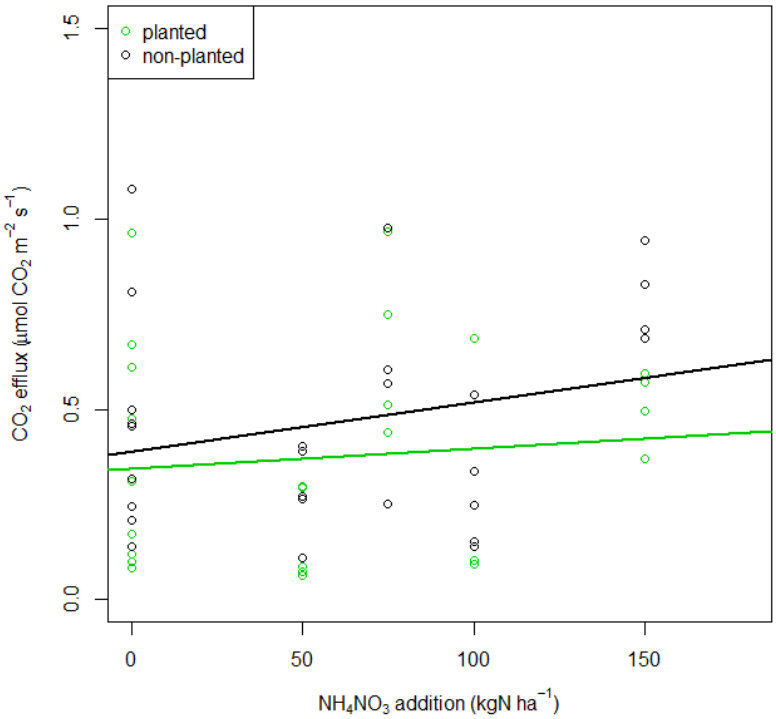

Figure 26. The CO_2 efflux (µmol CO_2 m^{-2} s^{-1}) in planted and non-planted (bare soil) under different NH_4NO_3 treatments during long laboratory study period

In the fourth week at higher soil moisture the efflux increased in both soils (bare and planted) with 1.08±0.84, 0.98±0.18, 0.83±0.20 µmol CO_2 m^{-2} s^{-1} and 0.97±0.23, 0.74±0.06, 0.57 ±0.07 µmol CO_2 m^{-2} s^{-1}, respectively.

At the end of this experiment (fifth week) no significant changes were observed, the CO_2 efflux was higher in bare soils than planted soils in both series and no effect of N treatments was found.

The separate effect of the different levels of SWC (20% and 25%), N treatment (N0, N50, N75, N100, N150) and plant presence which was discussed in this expirement are shown in figures 25 and 26.

From the results, we can conclude that there was no effect of plant presence on the CO_2 efflux during four and five weeks of measurement. Although it was surprising, we must note that the plants were small during the first weeks having small root respiration. Also, soil CO_2 efflux was highest in the zero N application treatment so there was no effect of different treatments in this experiment which was the same with some of studies Liu *et al.* (2017) and He *et al.* (2018) who indicated that N addition alone exerts no obvious effect on Rs. Meanwhile, the soil CO_2 efflux was significantly higher at the higher soil moisture level (25%) as we mentioned below, moisture in soils is essential for both plant growth (Huxman *et al.*, 2004) and soil microbial activity, thus affecting carbon inputs as well as the decomposition of litter and soil organic matter, and hence heterotrophic respiration and carbon outputs (Davidson, Janssens and Lou, 2006; Moyano, Manzoni and Chenu, 2013).

4.3.2 Second experiment

4.3.2.1 Cumulative CO_2 efflux course with different levels of N treatment in the presence/absence of plants

Lab measurements were aimed at quantifying the effect of the presence/absence of plants, the effect of soil moisture and the effect of different N addition (0, 75, and 150 kg N ha^{-1}) on the cumulative CO_2 efflux.

The data implied that more than three weeks after N fertilization (on days 21^{st} after fertilization), the cumulative CO_2 efflux in bare soil at N75, N150 were 0.63±0.01 g CO_2 $m^{-2}$and 0.90±0.02 g CO_2 m^{-2}, respectively (Figure 27, left panel), which is higher than that in the N0 treatment (0.60±0.02 g CO_2 m^{-2}). However, much higher cumulative CO_2 effluxes were observed in planted soil samples in all treatments N0, N75 and N150: 1.05±0.02 g CO_2 m^{-2}, 1.26±0.03 g CO_2 $m^{-2}$and 1.30±0.03 g CO_2 m^{-2}, respectively. These results suggest that N addition had a slight positive effect on soil respiration: cumulative efflux was 1.3 times higher in N75 and N150 than in N0 in planted samples, while CO_2 efflux of N150 treatment was 1.5 times higher than N0 in bare soil samples.

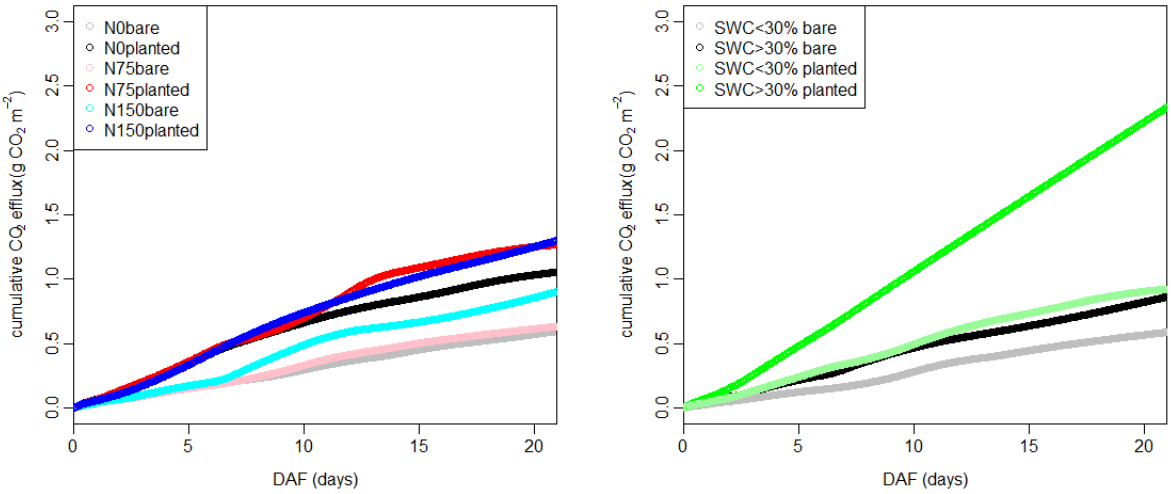

Figure 27. Cumulative CO_2 efflux (g CO_2 m^{-2}) courses across the 3 weeks long laboratory study period. CO_2 effluxes are separated by N treatments (left panel, 0, 75 and 150 kg N ha^{-1}) and by soil water content (right panel, >30% and <30%) in bare and planted soil.

The efflux values from planted soil N0, N75 and N150 were around twice as high as N0, N75 and N150 in bare soil, respectively. The difference between planted and bare soil in our study was due to the activity of plants resulting in root respiration and the priming effects of root exudates on soil microbes (Kuzyakov and Larionova, 2005), which, in turn, improved soil nutrient content, (Manzoni, Joshua P. Schimel and Porporato, 2012; Savage, Davidson and Tang, 2013), and accelerated the decomposition of soil organic matter (Nakano, Nemoto and Shinoda, 2008). The CO_2 emission in our present study was found to have a positive correlation with the stand age of the plant and with N fertilizer rates: as the plants grew and more N was added more CO_2 was emitted. A previous study suggested that N addition stimulated CO_2 emission by promoting autotrophic plant respiration (above and below ground parts) (Chen, Hooper and Lin, 2011) as well as heterotrophic respiration (rhizospheric respiration) by microbes due to the accelerated decomposition of SOM which was discussed above. Other studies also found a significant increase in soil respiration in unplanted and N fertilized soil compared to the unfertilized soil (Schaufler *et al.*, 2010; Shen, Li and Fu, 2015; Smith *et al.*, 2018).

4.3.2.2 Relationship between the cumulative CO_2 efflux and soil water content

Pearson's correlation of CO_2 efflux and soil moisture indicated that soil moisture was well correlated with CO_2 emission (R= 0.43). Figure 27, right panel shows that the cumulative CO_2 efflux increased with increasing SWC, the efflux was significantly (almost three times) higher

(by as much as 2.3 ± 0.05 g CO_2 m^{-2}) in planted soils at higher soil moisture levels (>30% and after three weeks of N fertilization) than at the lower soil moisture levels (<30%) by 0.92 ± 0.01 g CO_2 m^{-2}, and, similarly, three times higher than in bare soil at higher SWC by 0.86 ± 0.02 g CO_2 m^{-2}. Therefore, the effects of plant presence and soil moisture on soil respiration had similar magnitude.

In bare soil the cumulative CO_2 efflux was also significantly lower (by as much as 0.59 ± 0.03 g CO_2 m^{-2}) at the lower soil moisture level (<30%) than at higher soil moisture level (>30%) (by 0.86 ± 0.02 g CO_2 m^{-2}) (Figure 27, right panel). Similarly to our results, increased CO_2 fluxes were observed in soils with higher SWC under maize in other studies as well (Tang, Baldocchi and Xu, 2005; Talmon, Sternberg and Grünzweig, 2011). This higher efflux at higher soil water content in the presence of plants indicate greater root and rhizosphere respiration and increased SOM decomposition and thus more CO_2 emission (Moyano, Manzoni and Chenu, 2013).

The influence of moisture content on soil CO_2 efflux is complex through its effect on respiratory activity of roots and microbes (Vargas and Allen, 2008) and gas transport through the soil (Fang and Moncrieff, 1999). Generally, soil CO_2 efflux increases as soil moisture increases but soil moisture content can significantly reduce soil CO_2 efflux at its highest (wet soil) by blocking CO_2 transport because of low soil effective porosity (Vargas *et al.*, 2011; Balogh *et al.*, 2019), and at its lowest (dry soil) (Bardgett *et al.*, 2005; Wan *et al.*, 2007; Wang *et al.*, 2016), by limiting respiration substrate availability and thereby it reduces soil respiration (Webb, Pearman and Leuning, 1980; Wu *et al.*, 2010; Whitaker *et al.*, 2014; Dhadli, Brar and Black, 2015). However, soil respiration is more responsive to the combined effect of soil water content and soil temperature (Yuste *et al.*, 2003; Zhang, Lei and Yang, 2013). In our study the higher soil moisture levels (35% and 40%) could enhance respiration rates and no negative effect of high soil moisture was observed.

4.3.3 Third experiment

Carbon dioxide efflux dynamics with different levels of N fertilization and glucose addition in the presence/absence of plant.

This experiment was aimed to quantify the effect of glucose addition (carbon source) on the CO_2 efflux together with the effect of the biotic (presence of vegetation) and abiotic (SWC and N fertilization) drivers which were mentioned before.

Figure 28, left panel shows that the CO_2 efflux before fertilization (–200, –100 and 0 h) was almost three times higher in planted soil with maize than in bare soil with values of

2.81±1.87, 0.73±0.58 and 1.81±1.08 µmol CO_2 m^{-2} s^{-1} for planted soil and 1.08±1.00, 0.27±0.17 and 0.68±0.91 µmol CO_2 m^{-2} s^{-1} for bare soil, respectively.

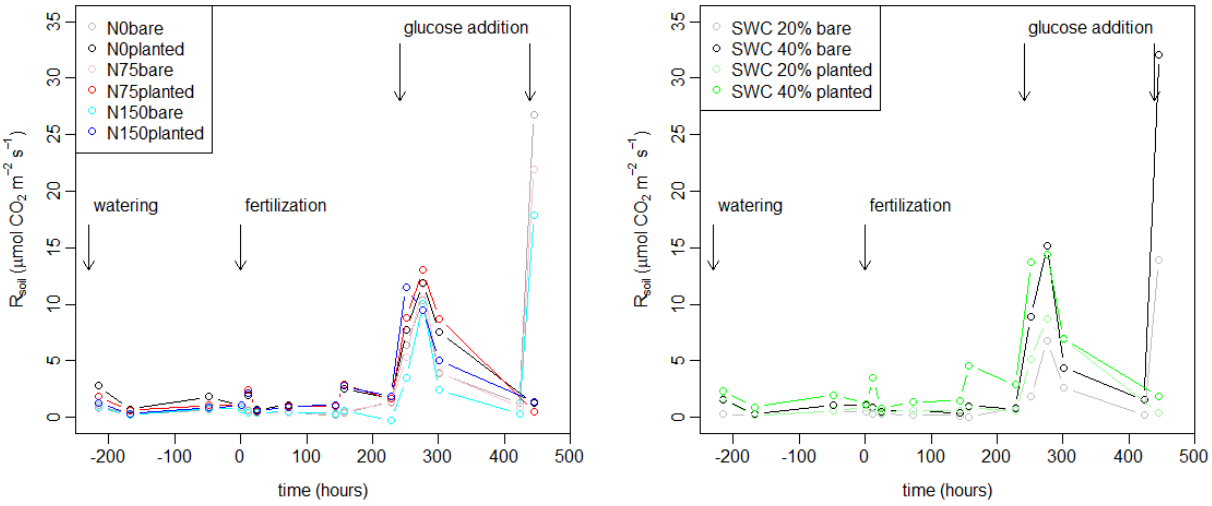

Figure 28. The CO_2 efflux (µmol CO_2 m^{-2} s^{-1}) dynamic during 4 weeks long laboratory study period. CO_2 effluxes are separated by N fertilization (left panel, 0, 75 and 150 kg N ha^{-1}) and by soil water content (right panel, 20% and 40%), addition of glucose in the25 the251 h in bare and planted soil and at 445 h in bare soil.

Two hours after fertilization, we could see that the CO_2 efflux in planted soil was still higher than bare soil but there were no significant differences between the three treatments (N0, N75 and N150) in both planted and bare soil, with values of 1.04±0.31, 1.05±0.35 and 1.03± 0.12 µmol CO_2 m^{-2} s^{-1} and 0.88±0.85, 0.69±0.30 and 0.70±0.44 µmol CO_2 m^{-2} s^{-1}, respectively.

After 12 hours of fertilization, the efflux increased, and it was higher in N75 in both bare and planted soil compared with N0 and N150. It was three times higher in planted soil in all treatments (N0, N75 and N150) than it was in bare soil with 1.94±0.70, 2.42±2.23 and 2.14±2.15 µmol CO_2 m^{-2} s^{-1} and 0.62±0.42, 0.70±0.30 and 0.48±0.53 µmol CO_2 m^{-2} s^{-1}, respectively.

The CO_2 efflux was almost stable after the period of 12 h from fertilization until 157 h. Then it started to increase again, it was six times higher in planted soil in all treatments (N0, N75 and N150) as compared to bare soil 2.53±1.54, 2.91±2.86, 2.78±2.55 µmol CO_2 m^{-2} s^{-1} and 0.42 ± 0.62, 0.50±0.72, 0.54±0.56 µmol CO_2 m^{-2} s^{-1} respectively, but there were no significant differences between the fertilizer treatments.

At 251 h the first portion of glucose was added for both bare and planted soil, the CO_2 efflux increased significantly and reached higher values, it was higher in planted soil than in bare soil in all treatments 7.70±3.80, 8.81±4.32, 11.50±7.45 µmol CO_2 m^{-2} s^{-1} and 6.38±4.42, 5.34±2.18, 3.44±2.88 µmol CO_2 m^{-2} s^{-1}, there was a fertilizer effect in planted soil especially with N150 but there was no effect in bare soil. The effluxes continue to increase and reached higher values, than start to decrease after 276 h in both bare and planted soil.

At 445 h the second portion of glucose was added to bare soil samples. In the graph (Figure 28, left panel) we can see that the CO_2 efflux in bare soil increased significantly and reached higher values in the all three treatments (N0, N75 and N150) with 26.78±12.80, 21.95±10.55 and 17.89±10.42 µmol CO_2 m^{-2} s^{-1} respectively.

From those results, we found that there was a positive effect of vegetation on CO_2 efflux with their activity resulting in root respiration and the priming effects of root exudates on soil microbes (Kuzyakov and Larionova, 2005), which is the same case in the previous experiment.

N application can have a significant effect on soil C pools, plant biomass production, and microbial biomass C processing (Al-Kaisi et al. 2008). According to our results, there was no clear effect of N applied in both bare and planted soil samples similarly to some studies indicating that N addition alone exerts no obvious effect on Rs (Liu and Wang, 2017; He *et al.*, 2018). However, in some of the samples we found an effect of N treatment which stimulated CO_2 efflux by promoting autotrophic plant respiration (above and below ground parts) (Chen, Hooper and Lin, 2011) as well as heterotrophic respiration (rhizospheric respiration) by microbes due to the accelerated decomposition of SOM which was discussed above. But, glucose addition had a much larger positive effect.

Carbon dioxide efflux dynamics with two levels of SWC (20% and 40%) and glucose addition in the presence/absence of plant

Figure 28, right panel shows that the CO_2 efflux increased with increasing SWC and the CO_2 efflux before fertilization (–200, –100 and 0 h) was significantly higher in planted soils at higher soil moisture levels (40%) by 2.33±1.05, 0.91±0.40, 1.94±1.65 µmol CO_2 m^{-2} s^{-1}, respectively than it was at lower soil moisture levels (20%) by 1.60±0.73, 0.18±0.09 and 0.55±0.14 µmol CO_2 m^{-2} s^{-1}, respectively.

In bare soil the CO_2 efflux was also significantly higher at higher level of SWC (six and two times higher) by 1.54±0.50, 0.31±0.11, 1.04±0.78 µmol CO_2 m^{-2} s^{-1}, respectively than it was

at lower SWC level (20%) by 0.25±0.14, 0.17±0.26 and 0.54±1.01 µmol CO_2 m^{-2} s^{-1}, respectively (Figure 28, right panel).

Two hours after fertilization the CO_2 efflux in planted soil at higher SWC was almost the same as in bare soil with 1.21±0.24 and 1.03±0.71 µmol CO_2 m^{-2} s^{-1}, respectively.

The CO_2 efflux increased rapidly and reached a higher value when the first portion of glucose was added (251 h), Rs was higher in planted soil than in bare soil at both lower and higher SWC, it was three times higher in planted comparing to bare soil at lower SWC by 5.09±2.45 and 1.85±1.00 µmol CO_2 m^{-2} $s^{-1}$and two times higher at higher SWC by 13.71±4.00 and 8.85±1.97 µmol CO_2 m^{-2} s^{-1}. After peaking at 276 h the CO2 efflux started to decrease both in bare and planted soil at higher and lower soil moisture levels The CO_2 efflux in bare soil increased speedily after the adding of the second portion of glucose (445 h) and reached higher values, it was two and half times higher at higher SWC than at lower SWC with 32.11±6.56 and 13.89±6.43 µmol CO_2 m^{-2} s^{-1}Rs rates, respectively

Moisture in soils is essential for both plant growth (Huxman *et al.*, 2004) and soil microbial activity, thus affecting carbon inputs as well as the decomposition of litter and soil organic matter, and hence heterotrophic respiration and carbon outputs (Davidson, Janssens and Lou, 2006). According to our results, we can conclude that there was a positive relationship between CO_2 efflux and soil moisture, soil CO_2 efflux increases as soil moisture increases. Therefore, the effects of plant presence and soil moisture on soil respiration had a similar magnitude. Higher efflux at higher soil water content in the presence of plants indicate greater root and rhizosphere respiration and increased soil organic matter decomposition and thus more CO_2 emission (Moyano, Manzoni and Chenu, 2013). In this experiment, the higher soil moisture levels (40%) could enhance respiration rates and no negative effect of high soil moisture was observed.

There was a positive effect of glucose addition in both bare and planted soils (Figure 28, right panel) at the two levels of SWC. At 40% of SWC glucose additions with low and high rates of N fertilization (N0, N75, and N150) significantly increased CO_2 emissions, rather than reducing these.

The glucose additions enhanced soil respiration rates at higher soil moisture in both soils bare and planted which is similar to the findings of Sanchez-Martin *et al.* (2008) .

We hypothesized that amending our soil with glucose addition, the most abundant sugar in rhizodeposits (Derrien, Marol and Balesdent, 2004) would: (1) increase soil C decomposition (positive priming), (Pegoraro *et al.*, 2019), (2) sustain higher rates of soil C decomposition over

the long-term because microbes will readily use substrates as an energy source following each pulse.

Glucose often produces a rapid response in microbial activity (Bernal *et al.*, 2016) and leads to rapid metabolic changes in a wide variety of fast-growing bacteria that utilize it as a substrate (Hungate *et al.*, 2015). Studies show that glucose was readily used by microbes as an energy source to produce enzymes that assist in the decomposition of organic molecules that are resistant to microbial degradation (Schimel and Weintraub, 2003; Fontaine *et al.*, 2007; Bernal *et al.*, 2016). Some studies have investigated the implication of tundra shrub expansion on SOM decomposition by adding glucose or low molecular weight C to soil and found no effects of priming (Rousk, Michelsen and Rousk, 2016; Lynch *et al.*, 2018).

The CO_2 efflux (µmol CO_2 m^{-2} s^{-1}) and its isotopic signal difference between planted soil with maize and bare soils.

In Figure 29 we can see that the soil CO_2 efflux before fertilization in planted soil with maize was slightly higher than in bare soil with values of 1.97±0.96, 0.55±0.47, 1.25±1.30 µmol CO_2 m^{-2} $s^{-1}$and 0.89±0.75, 0.24±0.21, 0.79±0.93 µmol CO_2 m^{-2} s^{-1}, respectively, also the natural isotopic abundance of Rs ($\delta^{13}CO_2$) before fertilization was higher in planted soil with maize than bare soil with values of –20.24±2.58, –19.28±5.00, –19.89±3.10 and –22.19±6.37, –20.79±0.24, –23.61±4.95 ‰, respectively

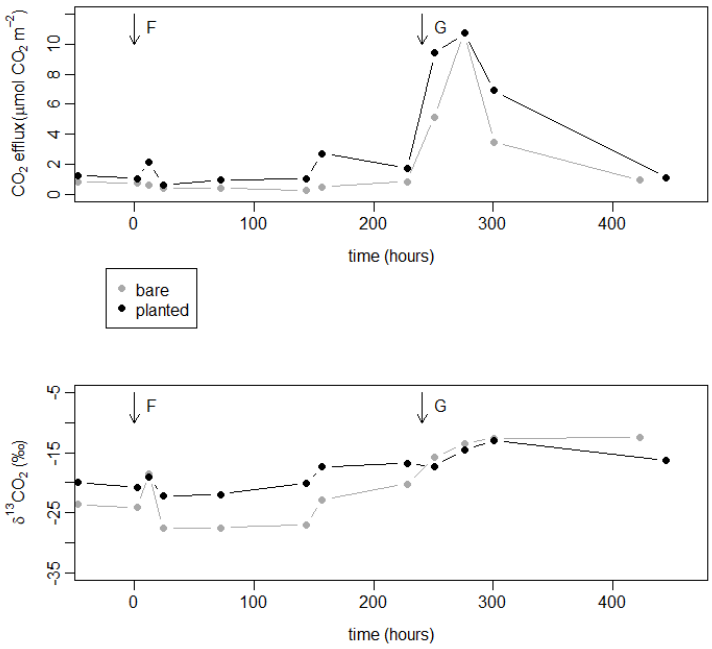

Figure 29. The CO_2 efflux (µmol CO_2 m^{-2} s^{-1}) and its $\delta^{13}CO_2$ ‰ difference between planted soil with maize and bare soils in time with N fertilization (F) and glucose additionin the 251 h (G) during 4 weeks long laboratory study period

After 12 h of N fertilization, the efflux increased in planted soil from 1.04±0.25 µmol CO_2 m^{-2} s^{-1} to 2.16±1.73 µmol CO_2 m^{-2} s^{-1} and there was no increment in bare soil, while the $\delta^{13}CO_2$ ‰ of planted and bare soil increased from –20.84±3.06 to –19.08±2.73 and –24.10±5.00 to –18.62±4.62‰.

CO_2 efflux was almost stable after the period of 12 h from fertilization in both soils until 157 h, then the efflux started to increase again, it was two times higher in planted soil compared with bare soil at 228 h (figure 29) with values of 1.70±2.11 µmol CO_2 m^{-2} s^{-1} and 0.83±1.84 µmol CO_2 m^{-2} s^{-1}, respectively. $\delta^{13}CO_2$ of planted soil and bare soil Rs c increased and reached

higher values: from 24 h to 228 h of fertilization it increased from –27.60±5.01‰ to –20.20±7.81‰ in bare soil and it was higher in Rs of planted soil increasing from –22.23±3.30 to –16.75±7.09‰.

251 h after fertilization the glucose was added for both bare and planted soil. Soil CO_2 effluxes increased significantly and reached higher values, it was two times higher in planted soil than in bare soil with 9.40±5.48 µmol CO_2 m^{-2} s^{-1} and 5.14±3.89 µmol CO_2 m^{-2} s^{-1}, respectively than it decreased significantly to reach 1.11±0.91 µmol CO_2 m^{-2} $s^{-1}$for planted soil and 0.94±0.70 µmol CO_2 m^{-2} s^{-1} for planted soil. Meanwhile, the $\delta^{13}CO_2$increased in bare soil when the glucose was added from –20.20±7.81‰ to –15.84±2.76‰, but it decreased in planted soil from –16.75 ±7.09‰ to –17.34 ±2.52‰. After that, the $\delta^{13}CO_2$ increased again in both bare and planted samples and reached similar values (–12.44±1.00‰ and –13.00 ±1.16‰) in bare soil and in planted soil, respectively

According to these results, we found a positive effect of plant presence, glucose addition and N fertilization on CO_2 efflux and on its stable isotope signal ($\delta^{13}CO_2$ ‰). The $\delta^{13}C$ of CO_2 respired by roots and other rhizosphere components may also be affected by utilization of fast or slow turnover carbon pools (Schnyder and Lattanzi, 2005) or allocation between growth vs. maintenance (Ocheltree and Marshall, 2004). The $\delta^{13}C$ of CO_2 respired by heterotrophic soil microorganisms depends on the substrates within soil organic matter utilized for decomposition. The artificial addition of glucose has changed the isotopic signal in our experiments, it increased in both planted and bare soils.

We separated the isotopic signals of Rs by the different treatments (Figures. 30-32). Isotopic signatures of soil respiration are a useful tool for estimating the contribution of its main components (Carbone *et al.*, 2011) and for tracing the transfer of C in ecosystems (Johnson *et al.*, 2002; Carbone and Trumbore, 2007; Högberg *et al.*, 2008) and thus have the potential to provide insights into the coupling of photosynthetic assimilation and soil respiratory fluxes. Plants contain less ^{13}C than the atmosphere due to processes discriminating against ^{13}C during CO_2 fixation (Farquhar, O'Leary and Berry, 1982). Maize is a C4 plant so it has a slightly higher delta value than soil and C3 plants (–18- –14‰). Natural abundance techniques make use of the fact that different carbon pools in the environment can have different ratios of carbon isotopes; for example, (Staddon, 2004) mentioned that the $\delta^{13}C$ of C3 plants (e.g. wheat) ranges from 25‰ to 35‰, whilst that of C4 plants (e.g. maize) ranges from 10‰ to 20‰. The difference in ^{13}C signatures of biological material occurs as a result of differing discrimination against ^{13}C in different biochemical pathways (Ehleringer, 1991; Lajtha, 1994).

Total soil organic matter is generally enriched in ^{13}C relative to leaf litter and becomes progressively more enriched with depth (Ehleringer, Buchmann and Flanagan, 2000). Carbon dioxide produced during decomposition can be depleted (Fernandez, Mahieu and Cadisch, 2003) or enriched (Boström, Comstedt and Ekblad, 2007) in ^{13}C relative to bulk soil organic matter. Total soil respiration tends to be a few ‰ enriched in ^{13}C relative to site-specific bulk leaf $\delta^{13}C$ (Bowling, Pataki and Randerson, 2008). We used a C4 species planted in a soil with mixed origin (both C3 and C4 crops were grown in the last years). Figure 30 shows that the medians $\delta^{13}CO_2$ of planted soil with maize higher than in bare soil by –19.79‰ and –23.77‰, respectively and there was a significant difference between them. Maize is C4 plant and it has a slightly higher delta value than soil and C3 plants.

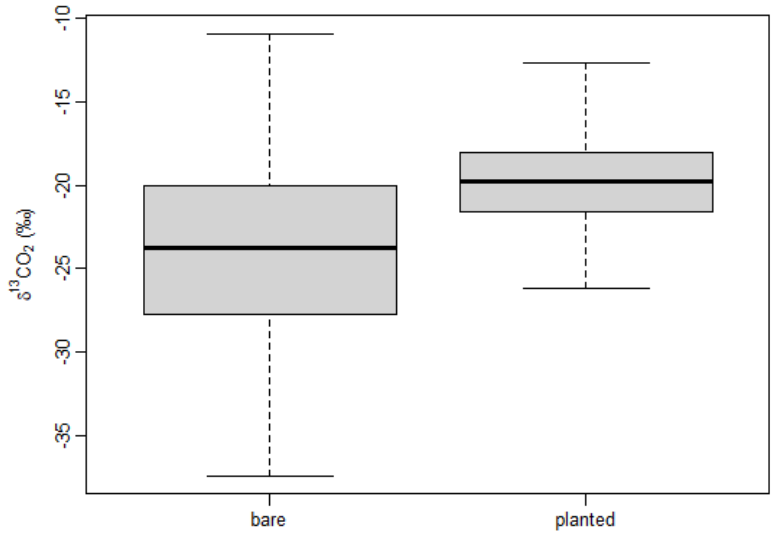

Figure 30. Isotopic signals of soil samples distinguished by the presence of maize plants (planted) and the absence of maize plants (bare).

Figure 31 shows that soil water content (two levels: 20% and 40 %) also affected the isotopic signal of soil respiration, $\delta^{13}CO_2$ increased with increasing SWC, the $\delta^{13}CO_2$ was significantly higher at higher soil moisture levels (40%) with median of –20.2‰, than in lower soil moisture level (20%) with median of –22.65‰ (Figure 31). According to the results, there was a significant difference in isotopic signals between the two levels of soil moisture. Water shortage can change the isotopic signal of soil respiration. Balogh *et al.* (2016) found that the autotrophic component is more sensitive to soil drying than the heterotrophic one in dry grasslands. In their study, the isotopic signal increased during drought, but the obtained values were measured in C3 grassland. In our study, the isotopic signal increased with increasing SWC

suggesting that C4 plant (maize) was more active at higher soil moisture level. Therefore, these results are similar to those measured in the grassland also supporting the hypothesis that heterotrophic respiration component is the less sensitive part of soil respiration during drought.

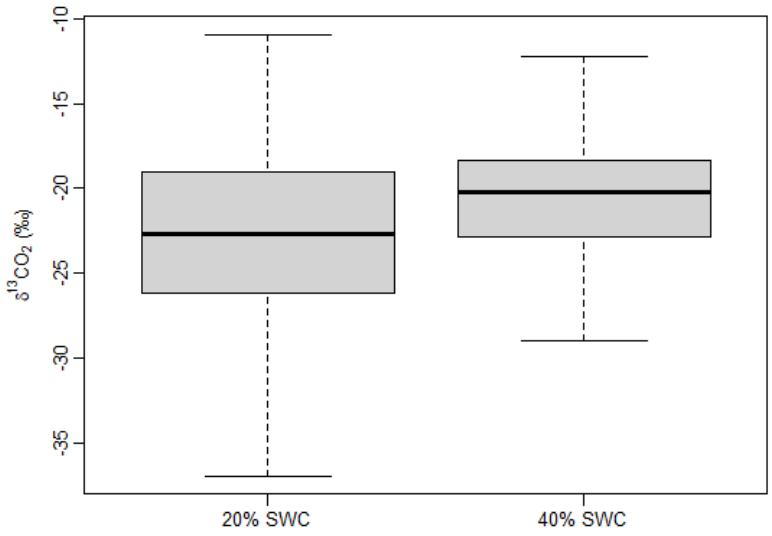

Figure 31. Isotopic signals of soil samples distinguished by SWC (20% and 40%).

We found no effect of fertilization on $\delta^{13}CO_2$ (Figure 32) as there were no significant differences between the treatments, medians: –20.55‰ (N0), –21.89‰ (N75), –21.75‰ (N150).

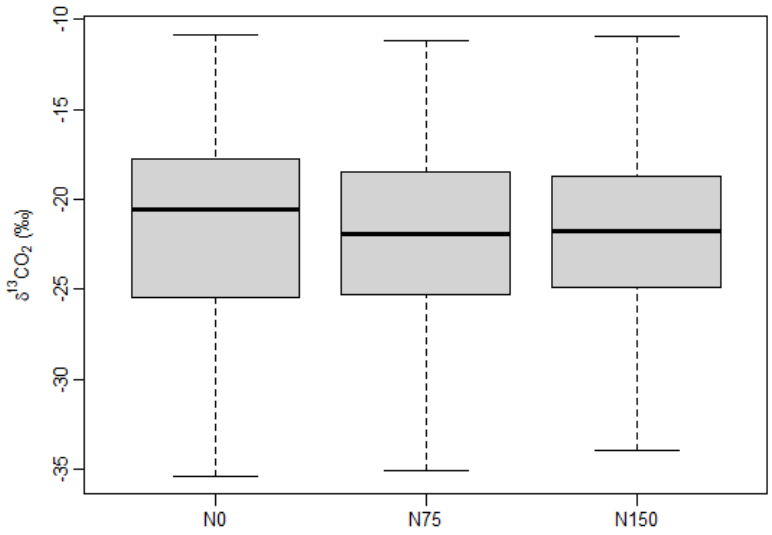

Figure 32. Isotopic signals of soil samples of the three levels of NH_4NO_3 addition (N0, N75, and N150)

5. NOVEL SCIENTIFIC RESULTS

Field data of CO_2 emission from a temperate cropland soil located in the middle part of Hungary (Kartal) under conventional management (tillage, sowing, harvesting, loosening, fertilization) during different crops (winter wheat, sorghum, rapeseed, winter wheat) with parallel laboratory experiments on the same soil were performed during a two-years-long study period.

We found the following results:

1. We described the temporal variation of soil CO_2 efflux in a conventionally managed agricultural soil in Hungary. We found that soil respiration had 57% share on average in total ecosystem respiration during crop periods, while it had 100% share on average during fallow periods.
2. We described the response of soil respiration to temperature, soil moisture and plant activity by using three different soil respiration models. According to our results; Model (3) (with soil temperature, soil moisture and VIgreen) was the best fit because the r squared value (from 0.40 to 0.54) improved with the increasing number of variables

3. We found that the impacts of N addition on CO_2 efflux varied with the level of N addition. Based on the field and lab data, we found a positive effect of fertilization on the CO_2 efflux of the soil, NH_4NO_3 stimulated CO_2 efflux by promoting autotrophic plant respiration as well as heterotrophic respiration.

4. We found that CO_2 efflux increased with increasing SWC. The efflux was significantly higher in both planted soils and in bare soils at higher soil moisture levels than at the lower soil moisture levels in all experiments and in the field as well. According to our results, higher soil moisture levels (35% and 40%) could enhance respiration rates and no negative effect of high soil moisture was observed in this soil.

5. We found that the vegetation could contribute strongly to the total CO_2 efflux by root and rhizo-microbial respiration, therefore the presence of plants and their growth can explain the temporal variations in CO_2 efflux due to root biomass and its activity in croplands. According to our result a positive correlation with the stand age of the plant was found: as the plants grew more CO_2 was emitted.

6. Glucose additions, as easily decomposable carbon source enhanced soil respiration rates independently on soil moisture and plant presence. It was two times higher in planted soil comparing with bare soil. Glucose additions had stronger effect on soil CO_2 efflux than N fertilization by producing a rapid response in microbial activity and leads to rapid metabolic changes in a wide variety of fast-growing bacteria that utilize it as a substrate

7. Indices like AWCD, H´, E and D was very useful to describe the activity and diversity of microorganism population. The BIOLOG EcoPlate has been found to be a good indicator of reflecting changes of metabolic activity and/or potential functional versatility of microbial communities exposed to abiotic conditions. The AWCD reflects the oxidative ability of the microorganisms developed in Biolog, and it may be used as an indicator of microbial activity.

6. CONCLUSION

Field and laboratory experiments were performed during a two-year-long study period (From November 2017 to November 2019) to quantify the different effects of principal biotic and abiotic drivers on soil CO_2 efflux and to investigate the temporal dynamics of CO_2 efflux from the soil surface. We found that the highest CO_2 emission rates occurred during summer and the lowest rates during the snow-covered winter period, and that soil temperature, soil water content, agricultural management practices and plant growth were the principal drivers playing a major role in the carbon cycle at this temperate cropland site.

We aimed to separate the effect of these drivers on CO_2 efflux in our laboratory study and we found that the CO_2 efflux in the N application was higher than that it was in zero-N treatment in both planted and bare soil in in most cases but sometimes there was no obvious effect of N treatment, therefore the presence of plants and their growth could explain the temporal variations in CO_2 efflux due to root biomass. On the other hand, significant positive correlations between CO_2 efflux and soil moisture were found, as soil moisture increases soil CO_2 efflux increases indicating that soil water content was the main factor limiting the rate of CO_2 emission from the soil. We found in one of our experiments that the higher soil moisture levels (40%) could enhance respiration rates and no negative effect of high soil moisture was observed. There was a positive effect of glucose addition also on CO_2 efflux in both bare and planted soils under different levels of SWC. At 40% of SWC; glucose additions with low and high rates of N fertilization (N0, N75, and N150) significantly increased CO_2 emission, rather than reducing it.

According to the microbiological results of the current study, the microbial counts showed that the highest total bacteria number, the highest number of fungi and the highest number of denitrifying bacteria in our soil which was collected from Kartal was found during the summer of 2018.

For the average well color development measurement, the AWCDa of all soil samples displayed an apparent lag phase in the first 24 h. Then the average absorbance started to increase significantly, showing that the five soil microbial communities were able to metabolize organic substrates in BIOLOG EcoPlates, and concerning the six compound groups (carboxylic acids, carbohydrates, amino acids, polymers, miscellaneous, and amines/amides - plus a blank well as a control) exist in the BIOLOG EcoPlate, the current study revealed that carbohydrate was characterized by the highest metabolic activity, while the lowest activity was determined for amines/amides.

We also studied the isotopic signal of the respired CO_2 and the effect of the major factors on that. We found that soil moisture and plant presence had a significant positive effect on $\delta^{13}C$, while N addition had no effect on that.

7. SUMMARY

Global warming has become a severe problem that cannot be overlooked. Climate change is accelerating and greenhouse gas (GHG) concentrations are increasing together with global temperatures towards serious levels. Soil respiration is a major component of carbon cycling in agricultural systems. In fact, soil respiration and its components are under the control of a complex set of biotic (plant growth and presence) and abiotic driving forces (SWC, soil temperature, and fertilization). As cropland soils are one of the main sources of greenhouse gases into the atmosphere, a study of the temporal dynamics of soil respiration has great significance. We performed field and laboratory experiments during a two-year-long study period (from November 2017 to November 2019) to quantify the different effects of principal biotic and abiotic drivers on soil CO_2 efflux and to investigate the temporal dynamics of CO_2 efflux from the soil surface.

Based on the field measurement, the temporal change of Rs was studied using a closed chamber IRGA system about bi-weekly/monthly during two years in 10 positions. The highest CO_2 emission rates occurred during summer and the lowest rates during the snow-covered winter period. Soil respiration model including soil temperature (T_s), soil water content (SWC) and the incorporation of VIgreen (plant growth and functioning) gave the higher goodness-of-fit value (r^2=0.54) than the simple temperature response. According to our field results, different variables including T_s, SWC, VIgreen and agricultural management practices played a principal role in the carbon cycle of the investigated cropland. Based on the measured values, we determined the share of soil respiration in total ecosystem respiration during crop season (57%) and during fallow periods (100%).

We aimed to separate the effect of these drivers on CO_2 efflux in our laboratory study and we found: (1) according to our first experiment we found that soil CO_2 efflux was highest in the zero N application. Moreover, soil CO_2 efflux was significantly higher at the higher soil moisture level (25%). (2) In the second experiment the cumulative CO_2 efflux in the N application was higher than that it was in zero-N treatment in both planted and bare soil, therefore the presence of plants and their growth could explain the temporal variations in cumulative CO_2 efflux due to root biomass. On the other hand, significant positive correlations between CO_2 efflux and soil moisture were found, indicating that soil water content was the main factor limiting the rate of the CO_2 emission from the soil in our laboratory study. In the third experiment, the higher soil moisture levels (40%) could enhance respiration rates and no negative effect of high soil moisture was observed. There was also a positive effect of glucose addition in both bare and planted soils at the two levels of SWC. At 40% of SWC, glucose additions at low and high rates

of N fertilization (N0, N75, and N150) significantly increased CO_2 emissions, rather than reducing these. We also found that isotopic signal of soil respiration was affected by the treatments (soil moisture, plant presence), except N addition, where no significant differences were found.

For the microbiological results obtained in the current study, the microbial counts showed that the highest total bacteria number, the highest number of fungi and the highest number of denitrifying bacteria in our soil which was collected from Kartal was found in the summer of 2018 indicating a temporal course in the development of the soil microbial community. For the average well color development measurement, the AWCDa of all soil samples displayed an apparent lag phase in the first 24 h. Then the average absorbance started to increase significantly, showing that the five soil microbial communities were able to metabolize organic substrates in BIOLOG EcoPlates. Concerning the six compound groups (carboxylic acids, carbohydrates, amino acids, polymers, miscellaneous, and amines/amides - plus a blank well as a control) existing in the BIOLOG EcoPlate, the current study revealed that carbohydrate was characterized by the highest metabolic activity, while the lowest activity was determined for amines/amides.

8. ÖSSZEFOGLALÁS

A globális felmelegedés napjaink legnagyobb környezeti problémája. Az egyre gyorsuló klímaváltozás fő okozója az üvegházhatású gázok koncentrációjának növekedése az atmoszférában. A globális szénforgalom egyik legnagyobb CO_2 árama a talajlégzés (Rs). A talajlégzést több különböző környezeti tényező és biotikus faktor befolyásolja (hőmérséklet, talajnedvesség, növényi aktivitás), ez is megnehezíti vizsgálatát. A szántóföldi talajok fontos forrásai az üvegházhatású gázoknak, emiatt is fontos ezen talajok CO_2 kibocsátásának vizsgálata. Kutatómunkánk során terepi és laboratóriumi vizsgálatokat végeztünk a talajlégzés időbeli dinamikájának, illetve a meghatározó faktorok és a talajlégzés ráta közötti összefüggések feltárásának céljából.

Az Rs időbeli dinamikájának vizsgálatát szántóföldi méréssorozatban vizsgáltuk két éven keresztül (2017-2019) egy zárt rendszerű gázcseremérő kamrával, IRGA technikával. A méréseket kétheti-havi gyakorisággal végeztük 10 térbeli ismétlésben. A legnagyobb Rs értékeket a nyári időszakban, a legalacsonyabbat a téli, hóborította időszakban mértük. A mérési adatokra sikeresen illesztettük a talajhőmérséklet, talajnedvesség és VIgreen indexen alapuló talajlégzés-modellt (r^2=0.54). Ezen változók mellett a talajlégzést a talajművelés is befolyásolta. A mérések alapján megahtároztuk a talajlégzés részarányát a veteményes időszakra (57%) és a parlag időszakra vonatkozóan (100%) a teljes ökoszisztéma légzésen belül.

A laboratóriumi kísérletek során az egyes változók talajlégzésre gyakorolt hatásait próbáltuk szétválasztani, illetve vizsgáltuk a műtrágyázás hatását, különböző dózisokra beállított kezelésekben. Az első kísérletsorozatban a legnagyobb légzésintenzitást a nem műtrágyázott mintákon mértük, valamint kimutattuk a talajnedvesség pozitív hatását. A második méréssorozat eredményei alapján a műtrágyázott minták magasabb kumulatív CO_2 kibocsátást mutattak, mint a nem műtrágyázott minták, valamint a növényi aktivitás hatása is egyértelműen kimutatható volt. Ezek mellett a legfontosabb tényező a talajnedvesség volt, magasabb talajnedvesség mellett magasabb kumulatív összegeket kaptunk, ennek a növekedésnek a mértéke a növnyi aktivitás hatását is meghaladta. A harmadik kísérletben szintén vizsgáltuk a víztartalom hatását, azt tapasztaltuk, hogy magasbb (40%) víztartalom mellett sem mutatkozott tlaljlégzést gátló hatás. Ebben a kísérletben glükóz hozzáadásával manipuláltuk a légzést, amelynek erős pozitív hatása volt, mind a növényes, mind pedig az üres (nem beültetett) talajokban. A talajlégzés stabil izotóparányát ($\delta^{13}C$) is befolyásolta a vizsgált faktorok többsége, eltérést figyeltünk meg a növénnyel beültetett és üres talajminták között, valamint a különböző talajnedvesség szintek között, míg a műtrágyázás nem befolyásolta az izotóparányt.

A gázcseremérések mellett a talajmikróbák aktivitását is vizsgáltuk, ami szintén mutatott szezonális változásokat. A BIOLOG EcoPlate módszerrel történt vizsgálatok alapján a különböző szénforrások közül a szénhidrátok váltották ki a legnagyobb metabolikus aktivitást a vizsgált talajban, míg a legalacsonyabbat az aminok/amidok.

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11. APPENDIX

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