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Separation of root and microbial respiration in wetland soil

Bachelor Thesis

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Annotation: This work serves as the grant application for project dealing with methods for separation of microbial and roots respiration in the soil.

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Abstract

Soil respiration is a group of complex and interconnected processes which run in plant roots, soil microorganisms and soil fauna. Soil fauna release only a small portion of total CO_2 production (approximately 5%), whereas plant roots and microbial respiration produce the largest portion (95%). Respiration is affected mainly by temperature and moisture. Total soil carbon balance is an important parameter because CO_2 released from soil affects global climate significantly.

It is difficult to measure root and microbial respiration separately and to calculate their portions of the total soil respiration. Therefore we suggest to evaluate the root and microbial respiration ratio in our project. We will measure soil respiration in the field regularly and evaluate seasonal pattern of soil respiration in the study site, which is wetland meadow. We will also estimate the SOM-derived CO_2 portion of total soil respiration in this field experiment. In addition, we will set up a mesocosm experiment with seedlings of *Carex acuta*. Nutrient input and water level effect on soil respiration will be studied. The effect of eutrophication will be also investigated. The results should increase our knowledge about carbon cycling through plants and microorganisms. This information will subsequently allow us to calculate total carbon balance of the whole ecosystem and to follow C fluxes.

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List of abbreviations

ADP = Adenosine diphosphate

AOP = Alternative oxidase pathway

ATP = Adenosine triphosphate

COP = Cytochrome oxidase pathway

GC = Gas chromatography

HR = Heterotrophical respiration

IRGA = Infrared gas analyzer

NAD^+ = Nicotinamide adenine dinucleotide

NADH dehydrogenase = Nicotinamide adenine dinucleotide dehydrogenase

$NADP^+$ = Nicotinamide adenine dinucleotide phosphate

NADPH oxidase = Nicotinamide adenine dinucleotide phosphate-oxidase

MAnR = Microbial anaerobic respiration

MR = Microbial respiration

MRRFS = Microbial respiration of root-free soil

OM = Organic matter

PEP = Phosphoenolpyruvate

RMR = Rhizomicrobial respiration

RR = Root respiration

SIR = Substrate induced respiration

SOM = Soil organic matter

TCA cycle = Tricarboxylic acid cycle

TSR = Total soil respiration

CONTENTS

1 Review 1
1.1 Introduction 1
1.1.1 Biochemistry of aerobic respiration process 1
1.2 Soil respiration 2
1.2.1 Microbial respiration 2
1.2.2 Plant respiration (root respiration) 3
1.2.3 Fauna respiration 3
1.2.4 Ratio of plant, microbial and fauna respiration in soil 4
1.3 Carbon dioxide emission from soil 4
1.4 Organic matters as source for microbial respiration 4
1.5 Controlling factors of plant respiration 5
1.6 Controlling factors of microbial respiration 7
1.7 Methods for soil respiration measurement 8
1.7.1 Non-isotopic methods: 8
1.7.2 Isotopic methods: 10
1.8 Conclusion 11
2 Aims of the project 12
3 Hypotheses 12
4 Approach 12
4.1 Study site 12
4.2 Methods 13
4.2.1 Field measurement 13
4.2.2 Mesocosm experiment 13
4.3 Time schedule 15
4.4 Finances 16
5 Expected research results 16
6 References 17

1 Review

1.1 Introduction

Plant and soil microbial community respiration are the processes that are intensly studied. Since carbon dioxide forms 60% of greenhouse gases, if water vapour is not considered, understanding of its fluxes becomes very important in relation to global warming. Soils generally are one of the biggest active C pools. Among them wetland soils store about one quarter of total C stored in soils in the world and so they are very important pools of C which may be released to the atmosphere if the ecosystems are disrupted (Gorgham, 1998). The C balance of ecosystems is strongly affected by their management. Recently drainage and eutrophication of wetland ecosystems are the most serious threats affecting their functioning. Both drainage and eutrophication affect soil processes directly through effect on microbial processes but also indirectly through change in plant community species composition, plant biomass production and roots exudation. The total C flux from soil is a sum of animal, plant roots and microbial respiration. Separation of microbial and root respiration is difficult but an important task which allows us to calculate and to understand C fluxes through plants and soil microbial community. This knowledge is necessary for the calculation of C balance and C fluxes through the whole ecosystem.

1.1.1 Biochemistry of aerobic respiration process

Respiration is a metabolic pathway of catabolic activity carried out by organisms. In other words respiration is a biological oxidation of organic matter (OM) which runs while oxygen works as an electron acceptor (oxidant) and a certain amount of energy is released. (2,879kJ from one glucose molecule). The reaction of glucose being used as a substrate is described by the formula: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$. When oxygen becomes limiting, other oxidants replace it and only the respiration of some microorganisms can occur (Reddy & DeLaune, 2008).

The whole aerobic process starts by organic polymers decomposition provided by hydrolysis enzymes. Subsequently, monomers are transported into the cytosol of cell where glycolysis runs (glucose transition into 2 pyruvate molecules). There are 2 molecules of ATP (adenosine triphosphate) as a form of conserved chemical energy produced from one glucose molecule during glycolysis (Reddy & DeLaune, 2008).

Pyruvate decarboxylation comes as the next step in respiration processes. Pyruvate decarboxylation leads to acetyl-CoA which is the starting substance for the TCA cycle (Trycarboxylic acid cycle). TCA cycle is also called Kreb´s Cycle or Citric Acid Cycle and

occurs in the matrix of mitochondrions (Reddy & DeLaune, 2008). Intermediate products of TCA cycle follow in order: citrate is formatted after acetyl-CoA combines with oxalacetate, subsequently isocitrate, 2-ketoglutarate, succinyl CoA, succinate and fumarate are processed. The cycle is completed through malate back into oxalacetate. CO_2 molecules are released within reactions of TCA cycle until OM is completely converted into CO_2 (Hill, 1997).

The last process of the whole respiration complex is oxidative phosphorylation occurring beside the respiratory chain in the inner membrane of mitochondrion. There are electrons released, during the above described reactions, and are transported across four protein complexes into the terminal acceptor (White, 2007), which is oxygen in aerobic conditions (Hall et al., 1982). The reaction of oxygen being reduced by electrons acceptance is described by the formula: $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$. The main goal of this proceeding is to reverse oxidation of NADH into NAD^+, which can be used in TCA cycle or other metabolic pathways as a reductant again (Reddy & DeLaune, 2008).

In total, the yield of energy coming from respiration processes is 38 ATPs and only 40% of released energy is conserved (approximately 1,140 kJ). The rest of energy is unleashed as heat (Reddy & DeLaune, 2008).

1.2 Soil respiration

Soils are pools of carbon storage and annually release enough carbon to impact global climate (Bowden et al., 1993). Soil respiration is the largest part of ecosystem respiration (Ryan & Law, 2005). Three CO_2 sources of total soil respiration are distinguished and described in this chapter: microbial respiration, root respiration and soil fauna.

1.2.1 Microbial respiration

Besides the aerobic respiration described above, microbes get energy through a few anaerobic processes as well. In general, carbon dioxide is released from the soil within a few processes running in anaerobic conditions which are: fermentation, methanogenesis, and respiration of nitrate, manganese, iron, and sulfate. *Microbial anaerobic respiration* (MAnR) differs in the electron acceptor. Oxygen as the terminal acceptor in aerobic conditions is replaced by nitrate, Mn(IV), Fe(III), sulfate, carbon dioxide or simple organic compounds in anoxic conditions. A yield of energy reached by MAnR is lower than energy profit from aerobic respiration. Compounds which don´t require initial oxygenation are preferred to become substrate in MAnR. Anaerobic conditions cause accumulation of reduced substances as methane, sulfides, volatile fatty acids, ferrous iron, manganous manganese, ammonium nitrogen, and hydrogen in the soil (Reddy & DeLaune, 2008). Another way how microbes

produce CO_2 is *fermentation,* which is an anoxic process of monomer transformation into fatty acids, alcohols, CO_2 and H_2 in soil (Glissmann & Conrad, 2000). The energy yield of fermentation is also significantly smaller (8 ATPs from one glucose molecule) in comparison to the aerobic respiration (38 ATPs from one glucose molecule) (Scandalios, 1993). *Methanogenesis* is another process of anaerobic conversion of plant materials (1% of assimilates from photosynthesis) into CO_2 and CH_4, which is provided by syntrophic associations of microbes and leads to energy yield (Thauer, 1998).

1.2.2 Plant respiration (root respiration)

In comparison to the microbial respiration (MR), using photosynthates as substrates root respiration (RR) runs as a direct release of carbon, which is fixed through photosynthesis (Cheng et al., 1993). Poorter et al. (1990) found a correlation between root respiration and unit of root dry weight in his experiment with 24 plant species. Fast growing species produce 2,7 times more CO_2 per unit of total plant dry weight than typical slow growing species. Fast growing species spent 8 -19% of fixed CO_2 in root respiration and allocate 18% of assimilated carbon into the root biomass. Slow growing species respire proportionally more of daily fixed carbon in contrast to the fast growing ones (Poorter et al., 1990). Root respiration follows seasonal patterns in which respiration decreases in winter time (from November to March) depending on the plant species (Edwards, 1991). 54-90% of annual CO_2 emissions from soil are released during the growing season which starts in the middle of May and ends in the middle of September (Bond-Lamberty et al., 2004). Root respiration varies among roots with different diameters where finest and smallest roots can respire 2,4 - 3,4 times more than bigger roots. Root respiration also declines with the depth in which the roots are placed. The measurement of sugar maple has shown the respiration of surface roots (0-10cm depths) is 40% greater than deeper roots (Pregitzer et al., 1998).

1.2.3 Fauna respiration

There are mesofauna and macrofauna considered as soil fauna in this chapter. Their main characteristic is the body size, which is bigger in comparison to soil microfauna. Mesofauna organisms are defined in size 0,1 – 2 mm, and macrofauna are animals bigger than 1 cm (Lavelle et al., 1997). The account of soil fauna in the total soil respiration is only 5% (Chapin et al., 2002). The most important function of soil fauna is mixing plant residues, making available substrate for microorganisms and stimulation of microbial activity in soil (Reichstein & Beer, 2008). Soil macrofauna increases the translocation of organic matter in soil into the mineral layer, what results in higher microbial respiration and biomass (Frouz et

al., 2006). Since the portion of CO_2 produced by soil macrofauna is so small it will not be considered furthermore in this review.

1.2.4 Ratio of plant, microbial and fauna respiration in soil

Total soil respiration is the sum of root respiration (RR), rhizomicrobial respiration (RMR), and microbial respiration of root-free soil (MRRFS). Kelting et al. (1998) measured following numbers: RR = 32%, RMR = 20%, and MRRFS = 48%. Cheng et al. (1993) found the portions for RR and RMR 40,6 and 59,4%, respectively. Wang et al. (2006) estimated contribution of RR into the TSR in the range from 38% to 76%. However MR seems to dominate in total CO_2 efflux from soil.

1.3 Carbon dioxide emission from soil

There are a few types of gas transport from the soil into the atmosphere. There will be described (i) advective flux, (ii) molecular diffusion, (iii) bulk flow, (iv) ebullition, and (v) transport through vascular tissue in this chapter. (i) *Advective flux* runs in a soil pressure gradient caused by external forces (f.e. atmosphere pumping). Viscose gas flows from the point of higher pressure into the point of lower pressure. (ii) *Molecular diffusion* is the flux which runs under isothermal and isobaric conditions, dependent on molecular weights of transported gases and temperature. (iii) *Bulk flow* consists of molecular and nonequimolar diffusion. Nonequimolar diffusion is caused by different molecular weights of components and is nonsegregative, what means gas components are not separated in contrast to molecular diffusion. Lighter gases have higher velocities than heavier gas molecules resulting in a pressure gradient (Scanlon et al., 2002). (iv) *Ebullition* is a transport way of water insoluble gases from soil through the water column into the atmosphere (Casper et al., 2000). (v) *Vascular transport through tissue* has opposite direction of oxygen respiratory consumption path. The entrance of gas into the aerenchyma tissue of plant is facilitated by diffusion gradient between soil and atmosphere. Thermo-osmosis is way of gas conductance through plants which occurs if there is temperature difference between interior and exterior (Joabsson et al, 1999).

1.4 Organic matters as source for microbial respiration

SOM consists of microbial biomass, plant and animal derived organic compounds and newly deposited litter (Kuzyakov et al., 2000). There are two fractions of SOM in the soil: resistant fraction, which consists of humic material complied of clay minerals and labile fraction, which consists of plant material and is rapidly processed by decomposition

(Schlesinger, 1997). The main sources of labile fraction compounds are: *dead roots and dead aboveground biomass (litter)* (Hernández & Hobbie, 2010), which both consist mainly of cellulose, holocellulose (cellulose and hemicellulose), lignin, and tannins (Kögel-Knabner, 2002). *Root exudates* are also a main source found in labile fraction compounds which can be divided into two groups: water-soluble compounds (for instance sugars, amino acids, organic acids, hormones and vitamines) and water-insoluble compounds (cell walls and mucilage) (Cheng et al., 1993). Meadow plants transfer 30-50% of carbon assimilates to the soil (Kuzyakov, 2001). Up to 30% of net photosynthetic production can be consumed by rhizosphere respiration (Cheng et al., 1993). The decomposition differs in its rate and need of enzymes usage by each compound. There is usually no need of extracellular enzymes for root exudates uptake by microbes unlike the plant litter containing polymers, which are hardly decomposable (Weintraub et al., 2007). Lignin:N ratio influences a decay rate of SOM. If the ratio lignin:N in litter increases, the rate of decomposition decreases (Melillo et al., 1982). The same relation runs among C:N and C:P, where with their increase the respiration rate decreases (Gnankambary et al., 2008). Microbial activity depends on plant organic matter inputs into the soil, which differs according to the plant community composition and plant productivity (Waldrop & Firestone, 2006).

1.5 Controlling factors of plant respiration

Respiration rate among plant species is variable (Lambers et al., 1991). Fast growing plant species have higher respiration in comparison to slow-growing ones due to their lower specific respiratory costs of root growth and ion uptake (Poorter et al., 1991). Bigger root biomass has also potential to enhance root respiration and subsequently higher litter offeres more substrate for microbial respiration (Pregitzer et al., 2008). On the other site high soil CO_2 concentration reduces root respiration (Qi et al., 1994).

Respiration regulation reflects the demand for energy and carbohydrate supply. There are three ways of respiration regulation: (i) partitioning electrons between cytochrome oxidase pathway (COP) and alternative oxidase pathway (AOP), (ii) regulation of glycolysis and electron transport, and (iii) two internal NADH dehydrogenases (Lambers et al., 1991). (i) COP accounts for around 90% of the respiratory carbon consumption and the remaining 10% belong to AOP which is mainly used under stress conditions (Florez-Sarasa et al., 2007). AOP doesn´t lead to adenosine triphosphate (ATP) syntesis what causes energetical efficiency of respiration decreases (Saisho et al., 2001). (ii) Glycolysis enzymes are activated by adenylates (ATP, ADP) (Lambers et al., 1991). Respiration decreases with high

ATP:ADP ratio when there is low energy demand (Loef et al., 2001). (iii) Rotenon-resistant NADH dehydrogenase is prefered in case of the high NADH/NAD ratio or low ADP availability to rotenon-sensitive NADH dehydrogenase which has the advantage because it couples with proton extrusion and subsequently creates the proton gradient to fuel ATP synthase through the membrane of mitochondrions (Lambers et al.,1991).

Environmental conditions influence root respiration and can be divided into abiotic and biotic factors. (Lambers et al., 1991).

Abiotic factors correspond to the nutrients supply and different soil conditions (Lambers et al., 1991). (i) *Nutrients supply* significantly influences RR rate. RR weakly correlates with net N mineralization (Chapin, 1980). RR decreases in case of nitrogen deficiency because energy demand on nitrogen uptake is low therefore there is no need of RR increase to gain more energy for nitrogen uptake (Lehmeier et al., 2010). Phosphate deficiency doesn´t cause change of RR rate but changes factors controlling RR rate (Wanke et al., 1998). RR can increase from a potassium deficiency causing a large energy demand on potassium uptake (Singh, Blanke, 2000). (ii) *Acidification* changes root medium where reduces H^+ release from roots while ATPase is active. RR increases immediately after pH starts decrease to adapt on a low soil pH until H^+ starts to be released again in a few hours. The main reason is to support ATPase activity in the environment where nutrients become less soluble due to lower pH. RR decreases at critical pH value (3,5 - 4 depending on species) when growth is inhibited and there is no ATP demand (Yan et al., 1992). (iii) Different species response variously on *salinity and drought*. In case salinity influences a species, root respiration is enhanced. When growth is inhibited subsequently also respiration decreases. Plant adaptations costs to saline environment are likely small in the contrary to highly salt-sensitive glycophytes (Lambers et al., 1991). (iv) RR decreases in *water stress* and the electron transport can be shifted from COP into AOP depending on species. RR becomes an important energy source in cases of water stress because photosynthesis rapidly decreases under these conditions (Ribas-Carbo et al., 2005). (v) RR rises as an exponential function of *temperature* (Smith et al., 2003) and depends on respiratory coefficient Q_{10}. Warm-acclimated and cold-acclimated species are distinguished (Lambers et al., 1991). The acclimation is considered the moment when homeostasis occurs (Atkin et al., 2000).

(vi) Low RR rate in poor *light intensity* is explained by low metabolic activity of roots (Lambers et al., 1991). But RR rate is influenced mainly by changing temperature. Higher light intensity also increases temperature and RR magnification follows (Lötscher & Gayler, 2005). (vii) Last factor influencing RR rate is *partial pressure*. The value of critical oxygen

pressure occures when oxygen in media is depleted. Such RR decrease was measured when oxygen partial pressure fell below 0,5-4,5 kPa (Armstrong et al., 2009).

Biotic factors are the second group of agents influencing RR. Symbiotic and parasitic organisms are considered as biotic factors (Lambers et al., 1991). *Rhizobium* is one example of *symbiotic organisms* which create nodules on roots and supply fixed atmospheric nitrogen. The respiration of nodules is five times higher than RR what provides enough energy for N_2 fixation. Nodules take up to 23% daily produced photosynthates from plant in return to provide nitrogen (Lambers et al.,1991). Mycorrhizal roots respire more than nonmicorrhizal ones (Snellgrove et al., 1982). The second group of biotic factors is *parasitic organisms*, which cause increase in RR by attacked plants in order to fill the higher energy demand (Haigh et al., 1991).

1.6 Controlling factors of microbial respiration

The main factors of microbial respiration are: (i) moisture, (ii) temperature, (iii) oxygen and alternative acceptors availability, (iv) OM availability, and (v) nutrients availability.

(i) *Moisture* is a major factor influencing HR. Extremely low or high moisture reduces HR through changing aeration status (Li et al., 2006). The HR dependence on moisture follows Gaussian form. The water level conditions in peatlands affect the temperature sensitivity of HR. Bacteria are more sensitive to the low moisture and higher temperature condition in comparison to fungi (Mäkiranta et al., 2009). (ii) *Temperature* sensitivity is expressed by quotient Q_{10} defined as "a factor by which CO_2 production increases for a 10°C increase in temperature" (Fierer et al., 2006). HR rises exponentially usually up to 35°C but an HR rate increase was observed up to 55° C in tropical forests (Holland et al., 2000). There rapid declination of HR rate was observed up to 20% after a few first days of increased HR in the experiment of Townsend et al. (1997). This change is explained by depletion of available substrate, which is light fraction of SOM, and switch to flux derived from resistant SOM fraction (Townsend et al., 1997). Substrate availability becomes a key determinant of HR response to temperature in this moment (Holland et al., 2000). 90% of temporal HR variation was explained by temperature variation (Minkkinen, 2007). (iii) *Oxygen availability* to the individual cell should be considered in evaluation of microbial response to the oxygen input. The *electron flux* goes mainly via AOP which alters COP under the conditions with low oxygen availability. Oxygen works as a terminal electron acceptor in respiratory chain under aerobic conditions (Alexeeva et al., 2002). Pure oxygen is toxic to the heterotrophs. Organisms are sensitive to oxygen only in early stages of their growth, but they become less

sensitive after first established growth (Gundersen, 1966). (iv) *SOM* and MR are strongly influenced by ecosystems or dominant plant species. MR declines rapidly with the soil depth in all ecosystems which is connected to the organic carbon availablity. The most of available organic carbon is located in the top layer of soil. Only about 30% of total organic carbon was observed 8 cm below the soil surface. Subsequently MR decreased rapidly (Fang & Moncrieff, 2005). The surface layer of OM is the biggest source of substrate for MR, but if this SOM is relocated into the belowground it becomes less available (Li et al., 2006). (v) The most important *nutrients* for HR are nitrogen and phosphorus if microbes are not limited by soil organic carbon. MR generally follows the pattern of microbial biomass (Tiunov & Scheu, 1999). However, microbial biomass can be reduced by nitrogen fertilization (Lee & Jose, 2003).

1.7 Methods for soil respiration measurement

The criteria for selection of the best method used to distinguish microbial and plant respiration in total CO_2 efflux from soil are: (i) The less disturbance of ecosystem the better technique it is. (ii) Ability to separate as many CO_2 sources as possible (RR, RMR, SOM-derivered CO_2,...). (iii) Universality for applications into the different ecosystems. (iv) Method should provide reproductive and reliable results. (v) The equipment, maintenance and analysis should be reachable in acceptable costs (Kuzyakov, 2006). There are two main groups of methods: non-isotopic and isotopic ones.

CO_2 released from the soil is measured in chambers using gas chromatography (GC) (Raich et al., 1990) or infrared gas analyzer (IRGA) (Bowden et al., 1993). GC is more expensive and difficult and needs more equipment, but can be used for tracing of more gases (N_2O, CH_4, CO_2) (Raich et al., 1990). IRGA can be connected into the portable chambers and each measurement takes less than 2 min. Another way of CO_2 flux measurement is passive CO_2 absorption in an alkali trap which takes a longer time (usually 24 hours). Total CO_2-C in alkali traps is determined by titration of the NaOH solution by HCl from pH 8,3 to 3,7 after precipitation with $BaCl_2$ (Jensen et al., 1996).

1.7.1 Non-isotopic methods:

Several non-isotopic methods are used: (i) root exclusion technique, (ii) trenching, (iii) shading and clipping, (iv) component integration, (v) excised roots, (vi) regression technique, and (vii) substrate induced respiration. Methods (i)-(vi) can be used for separation of RMR, and SOM-derived CO_2 (which includes MRRFS and MR) (Kuzyakov, 2006).

(i) **"Root exclusion technique"** uses removal of roots from examined soil (to the depth of 30 cm) (Hanson et al., 2000). This method may be applied both in situ and under field conditions. The disadvantage is strong disruption of soil and therefore budgets and cycling of C, N and H_2O are strongly affected (Kuzyakov, 2006). (ii) In **"trenching method"** soil is cut around to kill all roots which remain in soil and where a physical barrier is installed to keep a root growth out of the block (Li et al., 2006). The measurement of respiration should be done after most of roots are decomposed (approximately one to two years). Subsequently respiration of soil without roots is compared with unaffected control blocks (Sayer & Tanner, 2010). The disadvantage of this method is that measurement can be affected by decomposition of dead root mass remaining in soil (Bowden et al., 1993). (iii) "**Shading and clipping"** methods are based on shading of plants or clipping of plant aboveground parts in grasslands. It causes photosynthesis in leaves halts and assimilates aren´t transported into the roots anymore. Exudation of assimilates also stops (Kuzyakov & Larinova, 2005). The disadvantage is that microbial respiration of previously produced rhizodeposits are not eliminated (Kuzyakov, 2006). (iv) "**Component integration"** is technique based on a mechanical separation of rhizosphere soil, nonrizosphere soil, selected roots and washed roots from the soil sample. The removal of soil from roots is done by hands or it is washed with water (Sapronov & Kuzyakov, 2007). Big losses of fine roots is a disadvantage of this method (Larinova et al., 1988). Then all subsamples are incubated separately (Sapronov & Kuzyakov, 2007). Rooted soil incubation is compared to root-free soil to gain RR value as a difference (Larinova et al., 1988). Total CO_2 efflux is calculated as a sum of CO_2 production from separated subsamples, which are multiplicated by the weights of components (Sapronov & Kuzyakov, 2007). (v) The method **"respiration by excised roots"** works with physical removal of roots from the soil sample. In comparison to root exclusion technique, this method is executed only under the controlled laboratory conditions. This method is a short version of component integration technique but only root respiration is measured in this case (Burton & Pregitzer, 2003). Manual brushing or shaking is preferred to the root washing in this method because small rests of soil remaining on roots doesn´t significantly increase the measured root respiration (Kuzyakov & Larinova, 2005). The large disturbance followed by a strong CO_2 flush as a reaction to injuries is a disadvantage of this method (Kuzyakov, 2006). (vi) "**Regression technique"** is method which estimates linear relationship between root respiration and the biomass of roots where the CO_2 evolution from the soil is the dependent variable. The relatively low R^2 (determination coefficient) in high root biomass variation is a disadvantage (Kucera & Kirkham, 1971). The decrease of root respiration rate

during aging has to be taken in account in method evaluation (Volder et al., 2004). (vii) "**Substrate induced respiration" (SIR)** is based on the principle of glucose or other compound addition as the respiratory substrate which strongly increases MR. The measurement is provided 2-4 hours after glucose addition. The assumption glucose addition doesn´t increase RR has to be taken into account and is based on the fact, that glucose solution concentration is below the carbohydrates concentration in roots (0,5 – 5% or 5-50 mg/g) (Larinova et al., 2006). This technique can be used for separation of RR and RMR (Kuzyakov,2006).

1.7.2 Isotopic methods:

(i) **"Modelling of $^{14}CO_2$ efflux dynamics"** has two alternatives: continuous and pulse labelling with ^{14}C or ^{13}C isotopes. The labelled $^{14}CO_2$ is supplied through the air flushed into the chamber in "*continuous labelling"* method (Whipps & Lynch, 1983). This method has a few difficulties: maintenance of constant isotope ratios over long time period and stable moisture sustainment are complicated (Kuzyakov, 2006). The $^{14}CO_2$ is assimilated by plants and then $^{14}CO_2$ released from soil is measured - it is the product of both RR and MR (Kuzyakov, 2001). "*The pulse labelling"* can be used for estimation of recently assimilated C contribution into the total soil CO_2 efflux (Kuzyakov, 2006). The non-labelled (^{12}C) glucose can be added as a substrate for MR to restrict consumption of labelled exudates by rhizosphere microbes and subsequent release of $^{14}CO_2$ through MR (Cheng et al., 1993). Its calculation is simple because we know the exact amount of supplied (^{14}C) isotope (Kuzyakov et al., 2001). At first $^{14}CO_2$ is released through RR. CO_2 produced by microorganisms starts with some delay so these two components can be separated (Kuzyakov et a., 1999). (ii) "**Exudate elution method"** is an isotopic technique based on measurement of ^{14}C labelled exudates eluted from soil before microorganisms start to utilize them. Root exudates are decomposed by microorganisms due to their energy-richness and easy availability in soil, which is why measurement should be done before decomposition starts (Kuzyakov & Siniakina, 2001). Exudates are eluted by water-air mixture and subsequently collected into the flask separately from alkali CO_2 traps. Plants are grown in $^{14}CO_2$ or $^{13}CO_2$ atmosphere in order to distinguish CO_2 coming from RMR and RR (Kuzyakov & Larinova, 2005). Labelled CO_2 originates from root respiration and is blown out. This is only one method that uses physical separation of CO_2 flows, but RR can be strongly overestimated while rhizodeposition is underestimated (Kuzyakov, 2006).

1.8 Conclusion

CO_2 is produced by plants, microfauna, mesofauna, and macrofauna in soil. Both soil mesofauna and macrofauna participate in total soil respiration only by 5% (Chapin et al., 2002) and therefore are negligible if total CO_2 flux in soil is determined. CO_2 is produced through aerobic respiration, anaerobic respiration, fermentation and methanogeneses (Reddy & DeLaune, 2008). Total soil respiration is distinguished into three components: root respiration (RR), rhizomicrobial respiration (RMR), and basal respiration (microbial respiration of root free soil, MRRFS) (Kuzyakov, 2006). In average, following portions of each component respiration were found in scientific studies: 32% for RR, 20% for RMR, and 48% for MRRFS (Kelting et al., 1998).

The most important factors influencing RR are plant species, abiotic factors (temperature, moisture, nutrient supply etc.), and biotic factors (symbionts and parasites). MR is affected especially by moisture, temperature, organic substrate availability, oxygen supply, nutrients and (Lambers et al., 1991).

RR and RMR together create an interconnected and complex system, therefore their separation is very difficult. The measurement and the separation of total soil respiration are hardly executable by only one method and therefore combination of two or more methods seems to be the best solution (Kuzyakov & Larinova, 2005). Regression technique seems to be the most suitable method for separation of basal respiration (MRRFS) from total soil respiration in situ. Pulse labelling and $^{14}CO_2$ efflux dynamics modelling is probably the best method to separate RR and RMR (in mesocosm, under laboratory conditions). However, all above mentioned methods have some disadvantages and failures. There is a need for development of a new approach to exact RR and MR ratio determination.

2 Aims of the project

1) to choose or develop the best suitable method for partitioning of respiration of plant roots and soil microorganisms
2) to determine root-derived and SOM-derived CO_2 efflux and their ratio in wet meadow soil
3) to determine effect of eutrophication and water level on soil respiration

3 Hypotheses

1) According to the reviewed literature, "**regression technique"** and "**modeling of $^{14}CO_2$ efflux dynamics"** (pulse variant) are the most suitable methods for SOM and plant derived respiration.
2) Two thirds of soil respiration will originate in microorganisms, and one third in roots. However, the ratio of roots to microbial respiration will not be stable but will change with plant phenology and it will also depend on other factors like available organic substrate, temperature, moisture etc.
3) Root respiration will rise with temperature and it will follow the exponential function.
4) Soil respiration rate will decrease with the water level will increase.

4 Approach

4.1 Study site

The research will be done on wet meadow "Záblatské Louky" located in the Třeboň Basin Biosphere Reserve (TBBR), South Bohemia, Czech Republic. The study site is on peaty soils. It is subjected to several-weeks-long shallow flooding or summer drought occasionally. The altitude is 426 m above sea level. *Carex acuta* is the dominant of sedge meadow Záblatské louky.

The fertiliser NPK will be added into four experimental plots, each on the area 15 x 15m. The fertiliser will be applied in form of solution, twice a year. Respiration rate will be compared with unfertilized plots.

4.2 Methods

4.2.1 Field measurement

Regression technique will be used in the field. Regression technique is based on linear correlation of root biomass and root respiration rate which was firstly described by Kucera and Kirkham (1971). Measurement of emitted CO_2 will be done according to Wang et al. (2005), using static chambers equipped with infrared gas analyser. We will measure soil respiration rate regularly twice a month.

Twenty-four chambers will be installed into the study site at least 1 day before measurement. Twelve chambers will be placed on the fertilised plots and the other twelve will be placed on the unfertilised plots. The measurements will always be done on the same fertilised plots and compared to the same unfertilised ones (plots will be marked). Data will not be collected in the time when soil will remain frozen in 10 cm surface layer. The chamber will be cylindrical 10cm in diameter and will be placed on the ground without aboveground parts of vegetation. Temperature and moisture will be recorded continually using datalogers at 2 cm soil depth and 5 cm above the soil surface. Roots will be extracted from experimental plots (into 20 cm depth) in the end of vegetation season, oven dried at 70°C for 24 hours and weighed.

Correlation analysis will be done to determine relationship between soil CO_2 evolution and biomass of roots. The *y*-intercept of the linear regression between soil surface CO_2 efflux and root biomass will be the value of soil basal respiration (microbial respiration of soil unaffected by plant roots). The temperature dependency model of Lloyd and Taylor (1994) will be used for data analysis. Model works with two different respiration rates which are dependent on various temperatures and transfers them into the form in which they are comparable. The calculation of the net soil respiration will be done within this model.

4.2.2 Mesocosm experiment

Modelling of $^{14}CO_2$ efflux dynamics will be done under the laboratory controlled conditions. The pulse variant of measurement will be used in mesocosm experiment according to the scenario of Cheng et al. (1993). The seedlings of *Carex acuta* will be grown in the soil from study site. When the seedlings are big enough, they will be transplanted into the PVC containers (5 x 5 x 15 cm) provided by air inlet tubing at the top and air tubing at the bottom. Containers will be filled with soil from the study site. The labelling apparatus will be organised according to Cheng et al. (1993), as the Fig.1 shows.

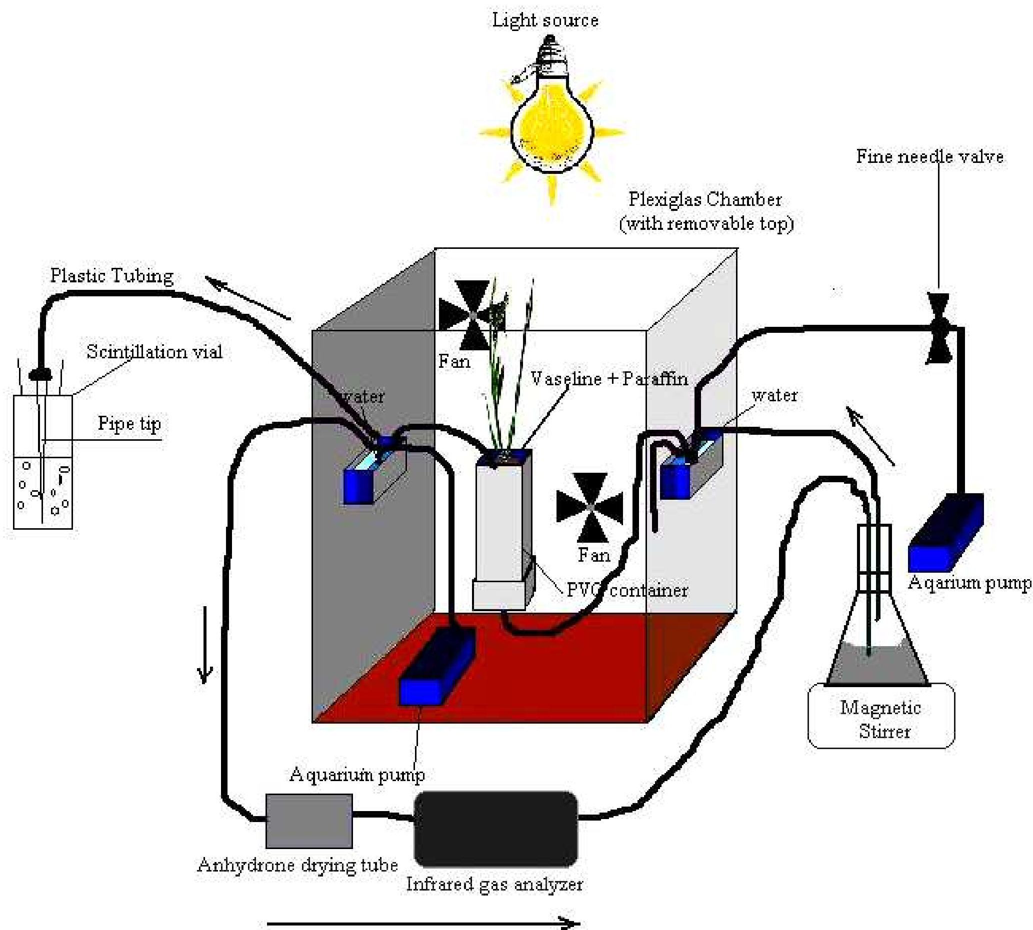

Fig.1. Equipment setting for ^{14}C pulse labelling of plant shoots respiration and tracing of below-ground $^{14}CO_2$ evolution (according to Cheng et al., 1993).

The treatment will be: (i) soil with glucose solution addition, (ii) soil with deionized water addition, (iii) soil with fertiliser addition and (iv) the variant with higher water level. The temperature of air in containers will be maintained constantly at 22°C in all variants. The measurement will be done five times in each variant of treatment.

(i – ii) Glucose (or deionized water as a control variant) will be added 1 hour before pulse labelling. Glucose will be added in order to restrict microbial consumption of labelled assimilates released by roots. It will cause preferential use of unlabelled glucose by microbes and therefore $^{14}CO_2$ flux from the soil should originate in root respiration. RMR is partially included in RR measurement, because organisms living in the rhizosphere consume root exudates and subsequently respire also labelled $^{14}CO_2$. Roots are independent on the unlabelled glucose addition.

(iii) Ten plants of *Carex acuta* will be grown in fertilised soil. The NPK solution will be added regularly during watering of experimental plants.

(iv) Water level will be maintained just on the soil surface in containers for the fourth treatment variant of mesocosm experiment.

Each container with plant will be sealed with the mixture of vaseline and paraffin in order to sealed containers will be a closed systems. The separation of soil respiration from shoots respiration will be done in this way. Air flow will be maintained at the rate of 50 cm^3 min^{-1}. Labelled CO_2 will be added in form of $NaH^{14}CO_3$ solution injected into the flask with acid. After $NaH^{14}CO_3$ injection to the flask CO_2 will be released to the inner atmosphere of the chamber. 10-min pulse labelling of experimental plants will be done.

The incubation time after labelling will be 30 minutes. After that $^{14}CO_2$ evolved from container will be trapped by continually pumping room air (50 cm^3 min^{-1} flow rate). The air will pass through the ethanolamine scintilation mixture. Scintilation mixture will be changed each 10 min. $^{14}CO_2$ released from containers will be measured 5 hours continually. Counting will be provided directly by liquid scintillation counter (Beckman LS 3801). After measurement will be done, soil will be removed from the containers. Soluble ^{14}C in the soil will be extracted in 0,5M K_2SO_4 solution. Radioactivity will be measured via liquid scintillation counting. Remaining roots and shoots will be washed in tap water and oven-dried at 70°C. All root and shoot samples will be pulverized in a ball mill and subsequently radioactivity analysis will be provided by liquid scintillation after combusting in an OX-300 Biological Oxidizer. The amounts of labelled carbon in soil, roots, shoots and air in the chamber will be summarized and will give a scheme of carbon balance.

4.3 Time schedule

	2012				**2013**				**2014**				**2015**			
Field experiment preparation																
CO_2 measurement in the field																
Data from field experiment evaluation																
Mesocosm experiment preparation																
Mesocosm experiment measurement																
Data from mesocosm experiment evaluation																
Results presentation																

4.4 Finances

	2012 (thousands CZK)	**2013** (thousands CZK)	**2014** (thousands CZK)	**2015** (thousands CZK)
Consumables	100	100	100	250
Salary expenses	100	100	100	100
Overhead	30	30	30	60
Travel expenses	10	10	10	30
Services	10	5	200	50
Total/year	250	245	440	490
Total	**1 425**			

Consumables: material for analyses – chemicals (glucose, $NaH^{14}CO_3$, K_2SO_4, vaseline and paraffin), laboratory equipment, equipment for field work, pots, seeds

Salary expenses: salary of the half-time employer and people who will help with sampling and analyses

Overhead: is set as 15% from all consumables and salary expenses

Travel expenses: traveling to the study site and sample transport, travel expenses for participation in conference

Services: expenses for company, which will make field chambers, pots and chamber costs for mesocosm experiment, equipment repairs, posters printing, conference fees

5 Expected research results

The major impact of the project will include:

- New information about the microbial and plant respiration rates in wetland soil and quantification of C fluxes through plant and soil.
- Assessment of eutrophication effect on soil respiration in wetland soil.
- Evaluation of water level effect on soil respiration in wetland soil.

The results should increase our knowledge about carbon cycling through plants and microorganisms in soil. This information will allow us to calculate total carbon balance of the whole ecosystem and to follow C fluxes (like C assimilation, exudation, respiration etc.) through ecosystem components (vegetation, microorganisms, soil). The project will also have practical implication regarding wetland management in order to maintain wetlands as carbon pool.

6 REFERENCES:

1. **Alexeeva, S., Hellingwerf, K.J., Teixeira de Mattos, M.J. 2002.** Quantitative assessment of oxygen availability: Perceived aerobiosis and its effect on flux distribution in the respiratory chain of *Escherichia coli*. Journal of Bacteriology 184(5), 1402 - 1406

2. **Armstrong, W., Webb, T., Darwent, M., Beckett, P.M. 2009.** Measuring and interpreting respiratory critical oxygen pressures in roots. Annals of Botany 103, 281-293

3. **Atkin, O.K., Edwards, E.J., Loveys, B.R. 2000.** Research review Response of root respiration in temperature and its relevance to global warming. New Phytologist 147, 141-154
4. **Bond-Lamberty, B., Wang, C., Gower, S.T. 2004.** Contribution of root respiration to soil surface CO_2 flux in a boreal black spurce chronosequence. Tree Physiology 24, 1387-1395

5. **Bowden, R.D., Nadelhoffer, K.J., Boone, R.D., Melillo, J.M., Garrison, J.B. 1993.** Contribution of aboveground litter, below-ground litter, and root-derived respiration to total soil respiration in the temperate mixed hardwood forest. Canadian Journal of Forest Research 23, 1402–1407

6. **Burton, A.J., Pregitzer, K.S. 2003.** Field measurement of root respiration indicate little to no seasonal temperature acclimation for sugar maple and red pine. Tree Physiology 23, 273-280

7. **Casper, P., Maberly, S.C., Hall, G.H., Finlay, B.J. 2000.** Fuxes of methane and carbon dioxide from a small productive lake to the atmosphere. Biogeochemistry 49, 1-19

8. **Chapin III., F.S. 1980.** The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11, 233-260

9. **Chapin III., F.S., Matson, P.A., Mooney, H.A., Chapin, M.C. 2002.** Terrestrial decomposition. In Principles of terrestrial ecosystem ecology. Springer-Verlag, New York, 436 p., 0-387-95443-0

10. **Cheng, W., Coleman, D.C., Carroll, C.R., Hoffman, C.A. 1993.** In situ measurement of root respiration and soluble C concentrations in the rhizosphere. Soil Biology and Biochemistry 25(9), 1189-1196

11. **Edwards, N.T. 1991.** Root and soil respiration responses to ozone in *Pinus taeda L.* seedlings. New Phytologist 118, 315-321

12. **Fang, C., Moncrieff, J.B. 2005.** The variation of soil microbial respiration with depth in relation to soil carbon composition. Plant and Soil 268, 243-253

13. **Fierer, N., Colman, B.P., Schimel, J.P., Jackson, R.B. 2006.** Predicting the temperature dependence of microbial respiration in soil: A continental-scale analysis. Global Biogeochemical Cycles 20, 1-10

14. **Florez-Sarasa, I.D., Bouma, T.J., Medrano, H., Azcon-Bieto, J., Ribas-Carbo, M. 2007.** Contribution of the cytochrome and alternative pathways to growth respiration and maintenance respiration in *Arabidopsis thaliana*. Physiologia Plantarum 129, 143-151

15. **Frouz, J., Elhottová, D., Kuráž, V., Šourková, M. 2006.** Effects of soil macrofauna on other soil biota and soil formation in reclaimed and unreclaimed post mining sites: Results of a field microcosm experiment. Applied Soil Ecology 33, 308-320

16. **Glissmann, K., Conrad, R. 2000.** Fermentation pattern of methanogenic degradation of rice straw in anoxic paddy soil. FEMS Microbiology Ecology 31, 117-126

17. **Gnankambary, Z., Ilstedt, U., Nyberg, G., Hien, V., Malmer, A. 2008.** Nitrogen and phosphorus limitation of soil microbial respiration in two tropical agroforestry parklands in the south-Sudanese zone of Burkina Faso: The effects of tree canopy and fertilization. Soil Biology & Biochemistry 40, 350-359

18. **Gorgham, E. 1998.** The biochemistry of northern peatlands and its possible response to global warming. In Biotic Feedbacks in the global climatic systems. Edited by: Woodwell, G.M., Mackenzie, F.T. Oxford University Press, New York. In: Adhikari, S.,

Bajracharaya, R.M., Sitaula, B.K. 2009. A review of carbon dynamics and sequestration in wetlands. Journal of Wetlands Ecology 2, 42-46

19. **Gundersen, K. 1966**. The growth and respiration of *Nitrosocystis oceanus* at different partial pressures of oxygen. Journal of General Microbiology 42, 387-396

20. **Haigh, G.R., Carver, T.L.W., Gay, A.P., Tartar, J.F. 1991.** Respiration and photosynthesis in oats exhibiting different levels of parasitoval resistence to *Erysiphe graminis* D.c. ex Merat f.sp. *avenae* Marchal. New Phtytologist 119, 129-136

21. **Hall, J. L., Flowers, T. J., Roberts, R. M. 1982.** Plant cell structure and metabolism. Longman, London, 543 p., 0-582-44119-6

22. **Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A. 2000.** Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biochemistry, 48: 115-146

23. **Hernández, D.L., Hobbie, S.E. 2010.** The effects of substrate composition, quantity, and diversity on microbial activity. Plant Soil. 35 (1-2). 397-411

24. **Hill, S.A.. 1997.** Mitochondrial metabolism. In Plant metabolism. Edited by: Dennis, D.T., Turpin, D.H., Lefebvre, D.D., Layzell, D.B., Longman, Harlow, 631 p., 0-582-25906-1

25. **Holland, E.A., Neff, J.C., Townsend, A.R., McKeown, B. 2000.** Uncertainties in the temperature sensitivity of decomposition in tropical and subtropical ecosystems: Implications for models. Global Biogeochemical Cycles 14(4), 1137-1151

26. **Jensen, L.S., Mueller, T., Tate, K.R., Ross, D.J., Magid, J., Nielsen, N.E. 1996.** Soil surface CO_2 flux as an index of soil respiration in situ: A comparison of two chamber methods. Soil Biology and Biochemistry 28 (10/11), 1297-1306

27. **Joabsson, A., Christensen, T.R., Wallén, B. 1999.** Vascular plant controls on methane emissions from northern peatforming wetlands. Tree 14, 385-388

28. **Kelting, D.L., Burger, J.A., Edwards, G.S. 1998.** Estimating root respiration, microbial respiration in the rhizosphere, and root-free soil respiration in forest soils. Soil Biology & Biochemistry 30, 961–968

29. **Kögel-Knabner, I. 2002.** The macromolecular organic composition of plant and microbial residuem as inputs to soil organic matter. Soil Biology & Biochemistry 34, 139-162

30. **Kucera, C.L., Kirkham, D.R. 1971.** Soil respiration studies in tallgrass prairie in Missouri. Ecology 52(5), 912-915

31. **Kuzyakov, Y. 2001.** Tracer studies of carbon translocation by plants from the atmosphere into the soil. Eurasian Soil Science 4(1), 28-42

32. **Kuzyakov, Y. 2006.** Review: Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biology & Biochemistry 38, 425-448

33. **Kuzyakov, Y., Kretzschmar, A., Stahr, K. 1999.** Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil. Plant and soil 213, 127-136

34. **Kuzyakov, Y., Friedel, J.K., Stahr, K. 2000.** Review of mechanisms and quantification of priming effects. Soil Biology & Biochemistry 32, 1485-1498

35. **Kuzyakov, Y., Ehrensberger, H., Stahr, K. 2001.** Carbon partitioning and below-ground translocation by *Lolium perenne*. Soil Biology & Biochemistry 33, 61-74

36. **Kuzyakov, Y., Larionova, A.A. 2005.** Root and rhizomicrobial respiration: A review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. Journal of Plant Nutrition and Soil Science 168, 503–520

37. **Kuzyakov, Y., Siniakina, S.V. 2001.** A novel metod for separating root-derived organic compounds from root respiration in non-sterilized soils. Journal of Plant Nutrition and Soil Science 164, 511-517

38. **Lambers, H., Scheurwater, I. 1991.** Respiratory patterns in roots in relation to their functioning. In Plant roots: the hidden half. Edited by Waisel, Y., Eshel, A., Kafkafi, U., Dekker, New York, pp. 323-362

39. **Larinova, A.A., Yermolayev, A.M., Blagodatsky, S.A., Rozanova, L.N., Yevdokimov, I.V., Orlinsky, D.B. 1998.** Soil respiration and carbon balance of gray forest soils as affected by land use. Biology & Fertility of Soils 27, 251-257

40. **Larinova, A.A., Sapronov, D.V., Lopez de Gerenyu, V.O., Kuznetsova, L.G., Kudeyarov, V.N. 2006.** Contribution of plant root respiration to the CO_2 emission from soil. Eurasian Soil Science 39(10), 1127-1135

41. **Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O.W., Dhillion, S. 1997.** Soil function in a changing world: the role of invertebrate ecosystem engineers. European Journal of Soil Biology 33(4), 159-193

42. **Lee, K.H., Jose, S. 2003.** Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. Forest Ecology and Management 185, 263-273

43. **Lehmeier, C.A., Lattanzi, F.A., Schäufele, R., Schnyder, H. 2010.** Nitrogen deficienty increases the residence time of respiratory carbon in respiratory substrate supply system of perennial ryegrass. Plant, Cell and Environment 33, 76-87

44. **Li, Y., Xu, M., Zou, X. 2006.** Heterotrophic soil respiration in relation to environmental factors and microbial biomass in two wet tropical forests. Plant and soil 281, 193-201

45. **Loef, I., Stitt, M., Geigenberger, P. 2001.** Increased levels of adenine nucleotides modify the interaction between starch synthesis and respiration when adenine is supplied to discs from growing potato tubers. Planta 212, 782 – 791

46. **Lötscher, M., Gayler, S. 2005.** Contribution of current photosynthates to root respiration of non-nodulated *Medicago sativa*: Effects of light and nitrogen supply. Plant Biology 7, 601-610

47. **Lloyd, J., Taylor, J.A. 1994.** On the temperature dependence of soil respiration. Functional Ecology 8, 315-323

48. **Mäkiranta, P, Laiho, R., Fritze, H., Hytönen, J., Laine, J., Minkkinen, K. 2009.** Indirect regulation of heterotrophic peat soil respiration by water level via microbial community structure and temperature sensitivity. Soil Biology & Biochemistry 41, 695-703

49. **Melillo, J.M., Aber, J.D., Muratore, J.F. 1982.** Nitrogen and lignin control of hardwood leaf liter decomposition dynamics. Ecology 63(3), 621-626

50. **Minkkinen, K., Laine, J., Shurpali, N.J., Mäkiranta, P., Alm, J., Pentlila, T. 2007.** Heterotrophic soil respiration in forestry-drained peatlands. Boreal environment research 12, 115-126

51. **Poorter, H., Remkes, C., Lambers, H. 1990.** Carbon and nitrogen economy of 24 wild species differing in relative growth rate. Plant Physiologist 94, 621-627

52. **Poorter, H., Van der Werf, A., Atkin, O.K., Lambers, H. 1991.** Respiratory energy requirements of roots vary with the potential growth rate of a plant species. Physyologia Plantarum 83, 469-475

53. **Pregitzer, K.S., Jaskowski, M.J., Burton, A.J., Lessard, V.C., Zak, D.R. 1998.** Variation in sugar maple root respiration with root diameter and soil depth. Tree Physiology 18, 665-670

54. **Pregitzer, K.S., Burton, A.J., King, J.S., Zak, D.R. 2008.** Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric CO_2 and tropospheric O_3. New Phytologist 180, 153-161

55. **Qi, J., Marschall, J.D., Mattson, K.G. 1994.** High soil carbon dioxide concentrations inhibit root respiration of Douglas fir. New Phytologist 128, 435-442

56. **Raich, J.W., Bowden, R.D., Steudler, P.A. 1990.** Comparison of two static chamber techniques for determining carbon dioxide efflux from forest soils. Soil Science Society of America Journal 54(6), 1754-1757

57. **Reddy, R.K., DeLaune, R. D. 2008.** Biogeochemistry of wetlands: Science and Applications. CRC/Taylor & Francis, Boca Raton, 774 p., 978-1-56670-678-0

58. **Reichstein, M., Beer, C. 2008.** Soil respiration across scales: The importance of a model-data integration framework for data interpretation. Jounar of Plant Nutrition and Soil Science 171, 344-354

59. **Ribas-Carbo, M., Tailor, N.L., Gilda, L., Busquets, S., Finnegan, P.M., Day, D.A., Lambers, H., Medrano, H., Berry, J.A., Flexas, J. 2005.** Effects of water stress on respiration in soybean leaves. Plant Physiology 139, 466-473

60. **Ryan, M.G., Law, B.E. 2005.** Interpreting, measuring, and modeling soil respiration. Biogeochemistry 73, 3-27

61. **Saisho, D., Nakazono, M., Tsutsumi, N., Hirai, A. 2001.** ATP synthesis inhibitors as well as respiratory inhibitors increase steady-state level of alternative oxidase mRNA in Arabidopsis thaliana. Journal of Plant Physiology 158, 241-245

62. **Sapronov, D.V., Kuzyakov, Y. 2007.** Separation of root and microbial respiration: Comparison of three methods. Euroasian Soil Science 40(7), 775-784

63. **Sayer, E.J., Tanner, E.V.J. 2010.** A new approach to trenching experiments for measuring root-rhizosphere respiration in a lowland tropical forest. Soil Biology & Biochemistry 42, 347-352

64. **Scandalios, J.G. 1993.** Oxygen stress and superoxide dismutases. Plant Physiology 101, 7-12

65. **Scanlon, B.R., Nicot, J.P., Massmann, J.W. 2002.** Soil gas movement in unsaturated systems. Soil Physics Companion. CRC Press, Boca Raton, p. 389

66. **Schlesinger, W.H. 1997.** Biochemistry: An analysis of global change. Academic press, Amsterdam: 588 p.

67. **Singh, P., Blanke, M.M. 2000.** Deficienty of potassium but not phosphorus enhances root respiration. Plant Growth Regulation 32, 77-81

68. **Smith, K.A., Ball, T., Conen, F., Dobbie, K.E., Massheder, J., Rey, A. 2003.** Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. Eouropean Journal of Soil Science 54, 779-791

69. **Snellgrove, R.C., Splittstoesser, W.E., Stribley, D.P., Tanker, P.B. 1982.** The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytologist 92, 75-87

70. **Thauer, R.K. 1998.** Biochemistry of methanogenesis: a tribute to Marjory Stephenson. Microbiology 144, 2377-2406

71. **Tiunov, A.V., Scheu, S. 1999.** Microbial respiration, biomass, biovolume and nutrient status in burrow walls of *Lambricus terrestris L.* (Lumbricidae). Soil Biology and Biochemistry 31, 2039-2048

72. **Townsed, A.R., Vitousek, P.M., Desmarais, D.J., Tharpe, A. 1997.** Soil carbon pool structure and temperature sensitivity inferred using CO_2 and $^{13}CO_2$ incubation fluxes from five Hawaiian soils. Biogeochemistry 38, 1-17

73. **Volder, A., Smart, D.R., Bloom, A.J., Eissenstat, D.M. 2004.** Rapid decline in nitrate uptake and respiration with age in fine lateral roots of grape: implication for root efficiency and competetive effectiveness. New Physiologist 165(2), 493-502

74. **Waldrop, M.P., Firestone, M.K. 2006.** Response of microbial community composition and function to soil climate change. Microbial Ecology 52, 716-724

75. **Wang, W., Ohse, K., Liu, J., Mo, W., Oikawa, T. 2005.** Contribution of root respiration to soil respiration in a C_3/C_4 mixed grassland. Journal of Biosciences 30(4), 507-514

76. **Wang, W., Guo, J-X., Feng, J., Oikawa, T. 2006.** Contribution of root respiration to total soil respiration in a Leymus chinensis (Trin.) Tzvel. grassland of northeast China. Journal of Integrative Plant Biology 48(4), 409-414

77. **Wang, W., Feng, J., Oikawa, T. 2009.** Contribution of root and microbial respiration to soil CO_2 efflux and thein environmental controls in a humid temperate grassland of Japan. Pedosphere 19(1), 31-39

78. **Wanke, M., Ciereszko, I., Podbielkowska, M., Rychter, A.M. 1998.** Response to phosphate deficiency in bean (*Phaseolus vulgaris* L.) roots. Respiratory metabolism, sugar localization and changes in ultrastructure of bean root cells. Annals of Botany 82, 809-819

79. **Weintraub, M.N., Scott-Denton, L.E., Schmidt, S.K., Monson, R.K. 2007.** The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutriet availability in a subalpine forest ecosystem. Oecologia 154, 327-338

80. **White, D. 2007.** The physiology ang biochemistry of prokaryotes. Third edition. Oxford University Press, New York, 628 p. 978-0-19-530168-7

81. **Yan, F., Schubert, S., Mentek, K. 1992.** Effect of low root medium pH on net proton release, root respiration, and root growth of corn (*Zea mays* L.) and broad bean (*Vicia faba* L.). Plant physiologist 99, 415-421