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Niina Tavi

Soil Carbon Cycling and Microbial Dynamics in Boreal Organic Soil Cultivated with a Perennial Crop

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EASTERN FINLAND

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ABSTRACT

Boreal peatlands are a huge storage of carbon (C). In pristine peatlands, waterlogged and anoxic conditions promote slow decomposition which leads to peat accumulation. Peatlands in Finland have been extensively drained for forestry, agriculture and peat extraction which changes peatlands from sinks to sources of C. Various agricultural practices such as tilling, liming and fertilization can further accelerate the loss of C. Cultivating perennial crops instead of annual crops could reduce C losses but research data on organic soils is lacking.

The research site of this thesis, Linnansuo in Eastern Finland, is a drained peatland cultivated with a perennial crop, reed canary grass (*Phalaris arundinacea*, RCG). Extensive research was conducted (years 2003 – 2011) at the site to study the greenhouse gas balance of the RCG cultivation and the factors affecting it. This thesis focuses on differentiating the soil-related CO_2 sources and identifying the importance of various microbial groups in C turnover. Additionally, new isotopic methods to study the flow and origin of C in soil were developed and tested.

The effects of RCG cultivation on the soil microbes and the role of microbial groups in soil C cycling were studied. Cultivation of RCG increased microbial biomass and activity in general. Fungi were the most active microbial group in allocation of fresh, plant-derived C. Also Gram-negative bacteria preferred fresh C, while Gram-positive bacteria had delay in their uptake of the labelled plant C indicating that they can use more processed C sources. Fungi likely play an important dual role in acidic organic soils: they are key decomposers of fresh C and simultaneously act as primary organisms in the humification process leading to formation of recalcitrant C compounds.

Another part of this thesis was the source separation of soil CO_2 flux. Within the peak growing season, respiration from the microbial decomposition of fresh C was the largest component (42%) in the soil CO_2 flux, followed by root respiration (36%) and peat decomposition (14%). Lime-derived, abiotic CO_2 flux

accounted for 8% of the soil CO_2 flux. The peat-derived CO_2 flux was approximately the same as from adjacent, uncultivated site, i.e. the cultivation does not accelerate the decomposition of the native soil organic matter. Cultivation of perennial crops on organic soils can thus be a tool to mitigate C losses from these soils.

Universal Decimal Classification: 582.542.11, 631.442.5, 631.46, 633.21

CAB Thesaurus: organic soils; peat soils; agricultural soils; perennials; Phalaris arundinacea; carbon cycle; carbon dioxide; microbial activities; respiration; soil bacteria; soil fungi

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Boreaalisen alueen suot sisältävät suuren määrän hiiltä (C). Luonnontilaisissa soissa vedenpinta on korkealla ja hapettomissa olosuhteissa orgaanisen aineksen hajoaminen on hidasta, minkä seurauksena soihin on kertynyt turvetta. Suuri osa Suomen soista on ojitettu metsätaloutta, maataloutta ja turpeen tuotantoa varten, mikä aiheuttaa soiden muuttumisen hiilinieluista hiilen lähteiksi. Viljelytoimet, kuten kyntäminen, kalkitus ja lannoitus voivat edelleen lisätä hiilen katoa turvemaista. Monivuotisten kasvien viljely yksivuotisten sijaan voisi vähentää hiilen katoa, mutta tutkimustietoa turvemailta on vasta vähän.

Tässä väitöskirjassa käytetty koealue, Linnansuo Joensuussa, on ojitettu suo, jossa viljeltiin monivuotista heinäkasvia, ruokohelpeä (*Phalaris arundinacea*). Tämä tutkimus oli osa suurempaa kokonaisuutta, jossa selvitettiin ruokohelpiviljelmän kasvihuonekaasutaseita sekä niihin vaikuttavia tekijöitä vuosien 2003–2011 aikana. Tässä väitöskirjassa keskityttiin maaperän hiilidioksidin lähteiden erittelyyn sekä maaperän mikrobien rooliin hiilen kierrossa. Hiilen lähdettä ja alkuperää selvittävien isotooppimenetelmien kehittäminen ja testaus olivat myös merkittävä osa tätä väitöskirjatyötä.

Tutkimuksessa määritettiin ruokohelven viljelyn vaikutuksia maaperän mikrobeihin sekä mikrobiryhmien roolia hiilen kierrossa. Ruokohelven viljely lisäsi mikrobibiomassan määrää ja mikrobien aktiivisuutta. Ruokohelvestä peräisin oleva tuore hiili sitoutui ensimmäisenä sienibiomassaan. Myös Gram-negatiiviset bakteerit hyödynsivät tuoretta hiiltä, kun taas Gram-positiivisissa bakteereissa leimatun hiilen määrä lisääntyi vasta myöhemmin osoittaen, että ne voivat käyttää pitemmälle muokattuja hiilen lähteitä. Sienten rooli happamissa turvemaissa on erityisen tärkeä. Ne ovat toisaalta tärkeimpiä tuoreen hiilen hajottajia ja toisaalta niillä on tärkeä osa humifikaatioprosessissa, joka tuottaa maahan stabiileja hiiliyhdisteitä.

Toinen osa tutkimusta oli maaperän CO_2-lähteiden erittely. Kasvukauden aikana suurin CO_2-lähde (42 %) oli tuore, kasviperäinen hiili. Seuraavaksi tärkein oli juurirespiraatio (36 %) ja kolmanneksi tärkein turpeen hajotus (14 %). Maan tuottamasta hiilidioksidista 8 % oli peräisin kalkista. Tutkimuksessa todettiin myös, että ruokohelven viljely ei lisännyt maan orgaanisen aineksen hajotusta. Monivuotisten kasvien viljely turvemailla voi siis hillitä näiden maiden hiilivarantojen häviämistä.

Universal Decimal Classification: 582.542.11, 631.442.5, 631.46, 633.21

Yleinen suomalainen asiasanasto: eloperäiset maat; turvemaat, maatalousmaa, monivuotiset kasvit; ruokohelpi; hiilitase; hiilidioksidi; hengitys; mikrobit; bakteerit; sienet

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final boost to finish this thesis. Finally I want to thank my husband Mika for his unconditional love and encouragement.

This thesis is dedicated to the memory of my parents, Elsa and Pertti Pekkarinen.

Tampere, July 2014

Niina Tavi

LIST OF ABBREVIATIONS

FE	Fumigation- extraction
OM	Organic matter
PLFA	Phospholipid fatty acid
RCG	Reed canary grass (*Phalaris arundinacea*)
RR	Autotrophic respiration
SIR	Substrate induced respiration
SMR	Heterotrophic respiration
SOM	Soil organic matter
VPDB	Vienna Pee Dee Belemnite

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to in the text by the Roman numerals.

I Tavi NM, Keinänen-Toivola MM, Koponen HT, Huttunen JT, Kekki TK, Biasi C and Martikainen PJ. 2010. Impact of *Phalaris arundinacea* cultivation on microbial community of a cutover peatland. *Boreal Environment Research* 15: 437-445.

II Tavi NM, Martikainen PJ, Lokko K, Kontro M, Wild B, Richter A and Biasi C. 2013. Linking microbial community structure and allocation of plant-derived carbon in an organic soil using $^{13}CO_2$ pulse-chase labeling combined with ^{13}C-PLFA profiling. *Soil Biology and Biochemistry* 58: 207-215.

III Biasi C, Tavi NM, Jokinen S, Shurpali N, Hämäläinen K, Jungner H, Oinonen M and Martikainen PJ. 2011. Differentiating sources of CO_2 from organic soil under bioenergy crop cultivation: A field-based approach using ^{14}C. *Soil Biology and Biochemistry* 43: 2406-2409.

IV Biasi C, Pitkämäki AS, Tavi NM, Koponen HT and Martikainen PJ. 2012. An isotope approach based on ^{13}C pulse-chase labeling vs. the root trenching method to separate heterotrophic and autotrophic respiration in cultivated peatlands. *Boreal Environment Research* 17: 184-192.

V Biasi C, Lind SE, Pekkarinen NM, Huttunen JT, Shurpali NJ, Hyvönen NP, Repo ME and Martikainen PJ. 2008. Direct experimental evidence for the contribution of lime to CO_2 release from managed peat soil. *Soil Biology and Biochemistry* 40: 2660-2669.

AUTHOR'S CONTRIBUTION

II Niina Tavi contributed to the design of the study and had main responsibility of the data collection and processing. She wrote the first version of the article together with Christina Biasi and Pertti Martikainen after which the other co-authors contributed to the writing process.

III Niina Tavi contributed to the design of the study, field work and laboratory work. She had the main responsibility of data processing. Niina Tavi wrote the first version of the article together with Christina Biasi and Pertti Martikainen after which the other authors contributed to the writing process.

IV Niina Tavi contributed to the acquisition of the data and interpretation of results. Christina Biasi had the main responsibility of data processing and wrote the first version of the manuscript after which Niina Tavi and the other co-authors contributed to the writing process.

V Niina Tavi contributed to the design of the study and field work. Christina Biasi had the main responsibility of data processing and wrote the first version of the manuscript after which Niina Tavi and the other co-authors contributed to the writing process.

VI Niina Tavi (née Pekkarinen) contributed to experimental set-up, field work and laboratory work. Christina Biasi had the main responsibility of data processing and wrote the first version of the manuscript after which Niina Tavi contributed to the writing process with the other co-authors.

Contents

1 General Introduction

1.1 BOREAL PEATLANDS

Northern peatlands are estimated to contain 125-621 Gt of carbon (C) (Yu, 2012). This huge storage of C has accumulated after the last glacial period because the decomposition of organic matter is slow in the waterlogged, anoxic conditions of cool and humid climate. There is variation in the overall annual C balance of different peatland types but on average, natural northern peatlands act as a sink of C (32.3 g C m^{-2} yr^{-1}, Yu, 2012). On the other hand, natural peatlands produce methane (CH_4), which is a potent greenhouse gas. Another important greenhouse gas, nitrous oxide (N_2O) has only minor importance in natural peatlands.

Peatlands cover large areas of northern landscapes. For example 32%, 23%, 22% and 17% of the land area in Finland, Sweden, Estonia and Canada, respectively, are comprised of peatlands (Vasander et al., 2003, Poulin et al., 2004). While some parts of these peatlands are undisturbed, large portions have been intensively used for various economical purposes. For example, peatlands have been extensively drained for forestry, agriculture and peat extraction (energy production and horticulture). In Finland out of the original peatland area (10.4 Mha), 55% have been drained for forestry (5.7 Mha), 7% for agriculture (0.7 Mha), and 0.5% for peat extraction (57 000 ha) (Vasander et al., 2003). Drainage of peatlands creates oxic conditions to a larger part of the peat column and thus accelerates the decomposition of organic matter and changes the overall C balance and greenhouse gas dynamics in general (Maljanen et al., 2010).

1.2 ORGANIC AGRICULTURAL SOILS

The most important management practice of peatlands which results in significant C losses is agriculture. In addition to drainage, tilling, fertilization, liming and other agricultural management practices further increase the loss of C from organic soils. Tilling improves aeration and breaks down soil aggregates which induce an increase in microbial activity and soil organic matter (SOM) decomposition (Paustian et al., 2000). Fertilization provides nutrients not only to plants, but also to soil microbes thus increasing microbial growth (Majumder & Kuzuakov, 2010). As mentioned earlier, N_2O fluxes from natural peatlands are negligible but agricultural peatlands are generally important sources of N_2O (Maljanen et al, 2010). Peat is naturally acidic, which can hinder plant growth and therefore liming is a common agricultural management practice on organic boreal soils. The higher pH, induced by liming, favours microbial activities (Rangel-Castro et al, 2004). As a result of higher C content in organic soils the risks for C loss and atmospheric carbon dioxide (CO_2) loading from organic soil are higher than from mineral soils if soil management increases microbial activities (Heikkinen et al., 2013).

Carbon balance of cultivation systems depends on agricultural practices applied. For example, annual and perennial crops require different management practices. In contrast to perennial crops, annual crops require regular tilling and large amounts of fertilizer which lead to high CO_2 fluxes (Maljanen et al. 2010, Six et al, 2006). The possible benefits associated to the cultivation of perennial crops have received little attention in boreal organic soils.

1.2.1 Cultivation of reed canary grass on peat extraction areas

Peat extraction is controversial topic in Finland and other countries utilizing peat for energy production. It is a local source of energy and can reduce the demand for oil and coal. Peat extraction sites are sources of CO_2, also after they are abandoned (Mäkiranta et al., 2007). After-use options which reduce C losses

from abandoned peat extraction sites have thus been explored. One option suitable for Finnish climatic conditions is the cultivation of reed canary grass (RCG, *Phalaris arundinacea*), a perennial crop that can be used for bioenergy production. RCG is a tall (150-300 cm) grass which grows well on organic soils (Lewandowski et al., 2003). It tolerates both drought and high water table and has an exceptional efficiency to utilize water in its growth (Shurpali et al., 2013). As an endogenous plant in the Nordic regions, it is well adjusted to low temperatures and short growing periods. The crop is harvested in spring. The delayed harvest improves combustion qualities (drier crop, lower concentration of N and K). In recent years the C balance of RCG has been intensively studied in Finland. The results suggest that even on organic soil the system can act as a sink for C especially during the wet years (Shurpali et al. 2009).

1.3 SOIL CARBON DIOXIDE FLUX

1.3.1 Soils as a source of carbon dioxide

Carbon dioxide (CO_2) is the most important greenhouse gas. The CO_2 concentration in the atmosphere is rising, which is one of the main reasons for global climate change (IPCC, 1997). Annually global soil respiration releases 50-75 Pg of C (Raich and Schlesinger, 1992). Due to the magnitude of soil-derived CO_2 flux, the understanding of the processes and the factors affecting them is crucial. The CO_2 flux from the soil can be separated into six sources, 1) root respiration, 2) rhizomicrobial respiration, 3) decomposition of fresh organic matter (OM), 4) decomposition of SOM, 5) CO_2 stemming from priming effect and 6) weathering of soil carbonates and/or abiotic CO_2 flux (Subke et al., 2006). A more general division of biological soil respiration is frequently used: autotrophic respiration (RR) includes root and rhizomicrobial respiration and heterotrophic respiration (SMR) includes decomposition of both fresh and native OM in addition to priming effect.

1.3.2 Biotic carbon dioxide flux and controlling factors

Separating the sources of respiration is important as they have different implications for the C balance of the whole ecosystem. Root respiration returns the recently assimilated C back to the atmosphere and the increases in RR mainly reflect increases in photosynthetic activity and primary production. On the contrary, increase in SMR means that native C is lost from the soil. Since largest stocks of C are found in the soil, there is also a great risk for large CO_2 losses and thus feedbacks to the climate system (Davidson & Janssens, 2006). In order to relate the CO_2 emissions to quantity and decomposability of SOM, more knowledge on the magnitude and regulation of SMR are needed. Temperature is the most important controller of respiration and the temperature dependence among terrestrial ecosystems is similar (Yvon-Durocher et al., 2012). Other controlling factors are moisture, availability of substrates and nutrients, and substrate quality (Raich and Schlesinger, 1992). The old and fresh C pools can have different decomposition dynamics. Fresh C consists of labile C compounds which are easily degraded by the soil microbial community. The old C consists of more stable components. The decomposition of recalcitrant C pools can accelerate if fresh C is provided e.g. in the form of plant litter and root exudates (priming effect, Kuzyakov et al, 2000, Fontaine et al, 2007). Land-use changes can thus produce large and unpredictable changes in the decomposition of soil C and better understanding on this subject is crucial.

1.3.3 Abiotic carbon dioxide flux from soils

Soil abiotic CO_2 flux (not derived from respiration of soil microbes or fauna) is the CO_2 that is produced by chemical processes. Natural abiotic flux of CO_2 can have importance in calceraeous soils (Ramnarine et al., 2012). In acidic soils the abiotic CO_2 flux needs to be considered when lime is applied. Lime is generally applied to increase pH in order to improve crop yield on acidic organic soil. It is added as calcitic limestone ($CaCO_3$) or as dolomitic limestone [($CaMg(CO_3)_2$)]. When lime

dissolves in water, CO_2 is chemically released (Butler, 1982). The standard methodology of Intergovernmental Panel of Climate Change (IPCC, 1997) to estimate the release of CO_2 from lime is based on the assumption that all of the C in lime is emitted as CO_2. However, direct measurements are lacking and needed especially when determining the respiration rates of acidic soils, since the lime derived CO_2 should not be included to the overall biological respiration estimate.

1.4 SOIL MICROBIAL COMMUNITY AND CARBON CYCLING

1.4.1 Role of microbial groups in soil carbon cycling

The decomposition of C in the soil is carried out by bacteria, fungi and soil fauna. It is generally accepted that various microbial groups in soil exhibit different roles in the turnover of C. Gram-negative bacteria are considered to be associated with the turnover of labile, plant-derived C (Kramer & Gleixner 2006, Denef et al., 2007). Gram-positive bacteria are often reported to be able to use more recalcitrant C sources and utilize C derived from dead fungal or root biomass (Denef et al., 2007). Fungi have an important role since their exoenzymatic capabilities allow them to decompose macromolecules (Rabinovich et al, 2004) but fungi are also able to compete for the fresh, easily available C (Treonis et al., 2004, Denef et al., 2007). The microbial community in the soil is dynamic and can react to changes in the environmental conditions. Changes in the community structure can even affect soil C sequestration as e.g. fungal-dominated soil microbial communities are considered to be more efficient to sequester C than those dominated by bacteria (Six et al, 2006). The chemical composition of fungal biomass is less susceptible to degradation than bacterial biomass (Six et al., 2006). Soil conditions supporting the growth of fungi may thus favour C sequestration (Bailey et al. 2002). However, there is generally still lack of knowledge on the role of specific microbial groups in soil C stabilization and destabilization processes.

1.4.2 Microbes in agricultural organic soils

The studies focusing on CO_2 fluxes of agricultural organic soils are abundant but the microbial community and its mediation of these fluxes has received less attention. All of the agricultural management practices and plant cultivation as such affect the microbial community and, in turn, the functioning of the ecosystem. Drainage would be expected to increase the abundance of fungi, when a larger part of the soil column is oxic. This effect has been observed on drained fens but not on bogs (Jaatinen et al., 2007). Fertilization (nitrogen) favours bacteria in expense of fungi (Innes et al., 2004, Bardgett et al, 2005, Paterson et al., 2007). Increases in microbial activity (Fuentes et al., 2006, Rangel-Castro et al., 2004) and microbial diversity (Rangel-Castro et al., 2005) as a result of liming have been observed. Substrate quality is considered to be an important controlling factor of microbial activity. Native OM in peatlands is a poor substrate for microbes (Waddington et al., 2001, Andersen et al., 2006). However, increased input of fresh C from plants (litter, roots, root exudates) as a result of enhanced net primary productivity due to cultivation could activate the microbial community and modify its structure. For example, the amount of Gram-negative bacteria can increase since they thrive well in rhizosphere where fresh C availability is high (Butler et al., 2003, Denef et al, 2009). The effects of plant cultivation and agricultural management practices are diverse and sometimes controversial, favouring different microbial groups. The overall effect of agricultural practices, including drainage, on the structure and functioning of peatland microbial community is not yet well understood.

1.5 ISOTOPE APPLICATIONS IN STUDIES OF SOIL CARBON CYCLING

1.5.1 Stable isotopes of carbon

Carbon (C) has two naturally occurring stable isotopes; ^{12}C (98.93%) and ^{13}C (1.07%). The differences in the natural $^{13}C/^{12}C$ ratios are small (e.g. in the third decimal) but they have important implications for C turnover studies. To make the differences more clearly visible, the delta (δ) notation is generally used to report C isotope data. The $\delta^{13}C$ values are calculated by relating the abundance values to an international standard (Vienna Pee Dee Belemnite, VPDB) according to the following equation:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{VPDB}} - 1\right) \times 1000 \qquad \text{Equation 1}$$

where R_{sample} is the abundance ratio ($^{13}C/^{12}C$) of a sample and R_{VPDB} is the abundance ratio of VPDB (0.0111802).

Most of the natural processes fractionate isotopes, which means that in a chemical reaction a larger proportion of the lighter isotope (^{12}C) is utilized leaving the source enriched with the heavier isotope (^{13}C). For example, photosynthesis in C3 plants fractionates C isotopes resulting to approximately 20‰ difference between the atmospheric CO_2 (-8 ‰) and plant C (-27 ‰ for C3 plants). Respiration, on the other hand, fractionates only to a minor extent, i.e. the $\delta^{13}C$ value of the respired CO_2 is (approximately) the same as the $\delta^{13}C$ of the source (Fry, 2006). Thus, the $\delta^{13}C$ of respiration can be used to separate contributing sources if these carry sufficiently different isotope signals.

Stable isotopes of C can be used in ecological studies either by utilizing the natural differences in $\delta^{13}C$ values (natural abundance) or by adding a substrate with a known, distinct isotopic signature (tracer studies). The benefits of natural abundance studies are that they cause no disturbance to the ecosystem and reflect natural processes. For separating fluxes, they require a clear and consistent difference in the $\delta^{13}C$ values,

which often cannot be obtained (e.g. for separating plant- and soil-derived respiration). Since the carbon isotope signal of abiotic C is distinct from the one of biotic C (Fry, 2006), both sources can be identified and separated in theory. Tracer studies have many advantages since the label can be followed in various ecosystem components which give valuable information on C flow and turnover rates. They can also be used to partition flux components and calculate source contributions in various ecosystem pools. In pulse-chase labelling experiments plants take up enriched $^{13}CO_2$ in photosynthesis and the fate of fresh C can be followed both in plants and in soil. This method has been employed both in laboratory (Paterson et al., 2008, Butler et al., 2003) and field conditions (Ostle et al., 2000, Denef et al., 2007, Kaštovská and Šantrůčková, 2007, Reinsch & Ambus, 2013) to study various grassland types and it has also been widely applied to label trees (Epron et al., 2012).

1.5.2 Radiocarbon (^{14}C)

Radiocarbon (^{14}C) is the only radioactive C isotope found in the nature. It has a half-life time of 5700 years. Therefore dead, deposited organic material is depleted of ^{14}C with time. The age of C containing material can be thus determined by ^{14}C analyses (i.e. radiocarbon dating). Nuclear tests performed from 1950 to 1963 produced a peak in ^{14}C levels. This "bomb carbon" peak can be used to separate SOM-derived and plant-derived C (Hahn et al, 2006, Gaudinski et al, 2000). Peatlands provide a special opportunity to utilize ^{14}C analyses since the lower layers of peat are thousands of years old. In post-extracted peatlands the upper layers of peat have been removed and the remaining peat is very old and has thus a very different ^{14}C signature than the plants which have a modern ^{14}C signature. As respiration does not substantially fractionate isotopes, the age difference can be used to separate SOM- and plant-derived respiration in cultivated cut-away peatlands.

1.6 MATERIALS AND METHODS

1.6.1 Study site

The research site of this study, peatland complex called Linnansuo, is situated in Eastern Finland (62˚30′N, 30˚30′E; 110 m a.s.l.). Mean annual temperature is 2.1 ˚C and precipitation is 669 mm (Drebs, 2002). This originally ombrotrophic Sphagnum fuscum pine bog (Tolonen, 1967) was drained for peat extraction in 1976. In 2001, 15 ha of the abandoned peat extraction site was cultivated with a perennial crop, reed canary grass (*Phalaris arundinacea*). Reed canary grass was cultivated for bioenergy purposes. Since the beginning of cultivation, the RCG site has been annually fertilized (350 kg ha^{-1}, N:P:K=17:4:13). Liming (7800 kg ha^{-1}) with fine-crushed dolomitic limestone [$CaMg(CO_3)_2$] was done in the first year of cultivation and repeated every four years to improve yield. The site has no previous cultivation history which offers a unique possibility to elucidate the changes in soil microbial processes induced by drainage, agricultural practice and plant cultivation. The overall C balance and greenhouse gas fluxes of the site have been studied intensively (Shurpali et al., 2008, Shurpali et al., 2009, Shurpali et al., 2010, Shurpali et al, 2013, Hyvönen et al,, 2009, Hyvönen et al., 2013).

1.6.2 Methods

The methods used in this study are listed on Table 1. Respiration measurements in the field and laboratory were combined with ^{13}C and ^{14}C analyses to separate components of the soil CO_2 flux at both natural abundance and enrichment levels. The isotopic method for separating autotrophic and heterotrophic respiration was compared with the root trenching method. The role of microbes in the soil C flow was also a focus point of this thesis. The impacts of land-use change on soil microbial biomass C (SIR), soil microbial activity (laboratory respiration measurements) as well as the composition of the soil microbial community (PLFA analyses) were studied. By

combining the PLFA analyses with the ^{13}C pulse-chase labelling study, the flow of fresh, plant-derived C was followed in the microbial community (^{13}C-PLFA analyses) in limed and unlimed soils. The effects of liming on the soil microbial biomass (FE method) and respiration (both field and laboratory measurements) were also determined.

***Table 1**. Field and laboratory methods used in this thesis Detailed descriptions of the methods are provided in the articles.*

Method	**Articles**
Field methods	
$^{13}CO_2$ pulse-chase labelling	III,V
Partitioning sources of respiration (^{13}C)	V
^{14}C dating of respired CO_2 and flux partitioning	IV
Field respiration measurements	V,VI
Root trenching	V
Laboratory methods	
Laboratory respiration measurements	II,V,VI
Substrate induced respiration (SIR)	II
Fumigation-extraction (FE)	VI
PLFA analyses	II,III
^{13}C-PLFA analyses	III
^{13}C analyses of soil, roots, root lipids and root sugars	III

1.7 AIMS OF THE STUDY

The overall aim of this study was to increase knowledge on soil C dynamics, microbial functioning and processes behind the fate of C when perennial crop is cultivated on organic soil. In addition, soil respiratory fluxes were investigated by applying various techniques to separate different flux components. The more specific aims were:

-To elucidate the changes in soil respiration, soil microbial biomass and soil microbial community induced by cultivation of a perennial crop on an organic soil (Articles II and VI)

-To determine the role of microbial groups in processing of fresh, plant-derived C in non-limed and limed acidic organic soils (Article III)

-To partition the following sources of CO_2 flux from soil:
1) CO_2 derived from old native SOM (peat) and new plants/ plant-residues (Article IV)
2) Autotrophic and heterotrophic respiration (Article V)
3) Abiotic and biotic CO_2 fluxes (Article VI)

-To discuss the findings within the view of climate change and soil C sequestration potential of perennial crop cultivation on organic soil

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2 Impact of Phalaris arundinacea cultivation on microbial community of a cutover peatland

3 Linking microbial community structure and allocation of plant-derived carbon in an organic soil using $^{13}CO_2$ pulse-chase labeling combined with ^{13}C-PLFA profiling

4 Differentiating sources of CO_2 from organic soil under bioenergy crop cultivation: A field-based approach using ^{14}C

5 An isotope approach based on ^{13}C pulse-chase labelling vs. the root trenching method to separate heterotrophic and autotrophic respiration in cultivated peatlands

6 Direct experimental evidence for the contribution of lime to CO_2 release from managed peat soil

7 General Discussion

7.1 CHANGES IN SOIL RESPIRATION, SOIL MICROBIAL BIOMASS AND SOIL MICROBIAL COMMUNITY AS A RESULT OF REED CANARY GRASS CULTIVATION ON AN ORGANIC SOIL

The magnitude of soil respiration of the RCG cultivation was measured in the laboratory (Article II) and in the field (Articles IV and V). A direct comparison between cultivated and uncultivated sites was largely based on soil incubation experiments (Article II), thus the discussion on the effects of cultivation on soil respiration focuses to some extent on laboratory conditions. Generally, the laboratory experiments showed that the microbial activity (respiration) increased as a result of RCG cultivation, especially in the uppermost (0-5 cm) soil layer (Article II). Root exudates and dead plant material contain C compounds that are easily degradable and thus promote microbial activity. In addition, some of the respiration in laboratory incubation experiments can be derived from roots remaining in the soil despite of careful sieving, since fine roots are difficult to separate from peat. The magnitude of CO_2 emissions from cultivated peat in situ ranged between 432 and 504 mg CO_2 m^{-2} h^{-1} in peak growing season of 2006 (Articles IV and V). These values were in the range found for agricultural organic soils from the boreal region (e.g. 325-407 mg CO_2 m^{-2} h^{-1} and 345-382 mg CO_2 m^{-2} h^{-1} for barley and grass fields, respectively, Maljanen et al., 2001, Maljanen et al., 2004). Soil respiration from cultivated peat is comprised of CO_2 from root respiration and decomposition of peat and fresh, plant-derived C. Based on bulk CO_2 analysis alone we cannot separate various sources and discuss effects of cultivation on decomposition of native SOM (so-called priming effect; Kuzyakov et al., 2000). However, microbial activity is overall increased with cultivation which may translate into higher soil C turnover; but increased

soil C losses may be compensated by C input into the soil in the form of plant-derived C. I will discuss later (Chapters 7.3 and 7.5) the possible effects of RCG cultivation on soil C balance based on CO_2 fluxes from various pools.

In addition to microbial activity, microbial biomass increased in the cultivated soil compared to the non-cultivated soil (Article 2). This is most likely also a result of fresh C input in the form of easily degradable plant material which promotes microbial growth (Thomson et al, 2013). There were specific changes in microbial community structure. Gram-negative bacteria were relatively the most abundant in the uppermost soil profile of the RCG site since they are closely connected with the plant derived C (Kramer & Gleixner, 2006, Lu et al. 2004, Butler et al., 2003, Denef et al., 2009).On the contrary, in the uppermost soil profile of the uncultivated peat (bare peat) PLFA biomarkers of Gram-positive bacteria were relatively the most abundant which can be explained by their ability to utilize recalcitrant C sources (Denef et al., 2007, Balasooriya et al, 2008). The cultivation practices (liming, tilling) could potentially inhibit the growth of fungi (Innes et al, 2004, Bardgett et al. 2005, Paterson et al., 2007). However, the positive effects of plant cultivation (fresh C provided by plants) seem to have exceeded the possible negative effects since the abundance of fungi was higher in the cultivated site than in the bare peat site. In addition, RCG is a perennial plant which shows occasionally mycorrhization (A. Kasurinen, personal communication).

When discussing effects of cultivation of a perennial crop on organic soils, effects of management practices have to be also considered. As previously mentioned, in addition to fertilization, acidic peat soils are limed regularly at a relatively high rate with unpredictable effects on soil C cycling. In the RCG cultivation, liming induced no statistically significant changes in the amount of microbial biomass (FE method, Article 6) and total PLFAs. There were also no remarkable changes in microbial biomarker structure (Article 3). However, the manipulation experiment where the effects of liming were studied was of relatively short duration, and it may take more

time for effects to become evident. The ^{13}C pulse-chase labeling experiment (Article 3) enabled to follow the flow of fresh C in soil microbial groups in more detail. Two trends were observed in the allocation of fresh C when comparing the limed and unlimed soils. Firstly, the allocation of fresh C appeared to be higher in the limed soils. Secondly, the label peak was observed earlier in the limed soils (day 1 after labeling) than in the unlimed soils (day 3 after labeling). In earlier studies liming has induced an increase in microbial activity and subsequently faster microbial C turnover (Rangel-Castro et al, 2004). In our study the results were not consistent and statistically significant in the whole data pool as there were large differences between the replicates, but the observed trends from the pulse-chase labeling experiment are in agreement with the earlier findings.

7.2 THE FLOW OF CARBON FROM PLANTS TO SOIL MICROBIAL COMMUNITY AND ROLE OF VARIOUS MICROBIAL GROUPS IN TURNOVER OF FRESH CARBON

The flow of C from plants to soil microbes was followed over a period of 464 days after labelling RCG by $^{13}CO_2$ in July 2006 (Article 3). The highest $\Delta\delta^{13}C$ values in PLFAs were measured 1-3 days after labelling, thus, fresh photosynthesates are relatively quickly transported from aboveground tissues into belowground compartments. Among the microbial groups, the label allocation was highest in fungal biomarkers throughout the sampling period, even though fungal biomarkers comprised only a minor part of the microbial community (12-17% of microbial PLFAs). However, the label allocation in fungal biomarkers was lower one year after labeling (July 2007) than immediately after labeling. The importance of fungi in turnover of fresh, plant-derived C has been observed also in other soil ecosystems (Treonis et al., 2004, Denef et al, 2007, Denef et al. 2009; De Deyn et al., 2011). As mentioned above, the biomass of fungi also increased as a result of RCG cultivation, supporting the assumption that fungi play a dominant role in processing

new photosynthesate C. On the other hand, when comparing $\Delta\delta^{13}C$ values of the microbial PLFAs one year after labeling, fungal biomarkers still have the highest values. This indicates that fungi have a role also in decomposition of more recalcitrant C. Fungi produce enzymes that enable the decomposition of more complex C compounds (Rabinovich et al., 2004, Blagodotskaya and Kuzyakov, 2008). Immediately after labeling, Gram-negative biomarkers had the second highest $\Delta\delta^{13}C$ values after fungal biomarkers indicating that these microbes also utilize preferentially fresh C derived from root exudates which has been observed also in other soil ecosystems (Denef et al. 2007, Reinsch et al., 2014). On the contrary, in the Gram-positive PLFA biomarkers there was a trend of increase in the label allocation with time indicating the ability of Gram-positive bacteria to utilize dead fungal or root biomass, as well as other more complex C compounds, as substrates (Denef et al., 2007, Balasooriya et al, 2008). In general, increase in relative abundance in microbial groups was found with concomitant increase in label allocation, suggesting that functional changes are reflected by structural changes in microbial community in this ecosystem. Microbial community structure can have implications for the soil C balance. It has been suggested that more C can be incorporated in fungal biomass than in bacterial biomass and fungal biomass can be less susceptible to degradation (Six et al., 2006). Furthermore, fungi might promote the formation of microaggregates in the soil thus increasing soil C sequestration (Six et al., 2006).

The role of microbial groups in utilizing fresh, plant-derived C was also studied by estimating the contribution of root exudates (root sugars) in microbial PLFAs (Article 3). This was done by a two-pool mixing model (Fry, 2006) using the $\delta^{13}C$ values of root sugars, soil and PLFAs within the first week after labeling. In a laboratory experiment using artificial exudates the microbial biomass mainly consisted of exudate-derived C (Marx et al., 2007). In our study the relative amount of fresh C in the PLFAs was overall relatively low (max. 6.5% in fungi), most likely because the system has not yet been in isotopic

equilibrium. But even if we consider that these estimates are too conservative, these values seem low in regard of general understanding of microbes as utilizers of root exudates (Marx et al., 2007) and they suggest that root exudates are not fueling microbial growth to a large extent in these systems, but rather serve as energy sources. The differences between microbial groups – with fungi incorporating 2 – 3 times the amount of root exudates than bacteria – remain the same even though the actual values might be too low. This approach highlighted thus again the importance of fungi in turn-over of root-derived C. This method requires further development but it offers new interesting possibilities to explore the flow of C from plants to soil microbes in situ.

7.3 COMPONENTS OF THE SOIL CARBON DIOXIDE FLUX

Soil respiration is derived from many sources (see Chapter 1.3.1). Separation of these sources is important since they have different implications for the soil C balance but it is difficult due to methodological constraints. Since boreal organic soils contain about 30% of global soil C content (Gorham, 1991), especially rates of soil-derived CO_2 losses as a result of land use change are important to know within the context of climate change. Within this thesis, the contribution of SMR and RR to overall soil CO_2 emissions was determined by using two methods, an isotopic method based on ^{13}C pulse-chase labelling and root trenching method (Article V). Isotopic approaches are considered to be the most reliable method to separate SMR and RR (Kuzyakov, 2006). The root trenching method is also widely used (e.g. Bond-Lamberty et al., 2004, Ngao et al., 2007). The advantages with the root trenching method are that it requires no special equipment and is thus applicable also in remote research sites. According to the isotopic method, SMR accounted for 50% of the soil respiration in peak season. In earlier experiments with Finnish organic soils plant roots have contributed by 35-45% to the total soil respiration (Silvola et al. 1996). However, with the

root trenching method, a higher value (71%) was obtained here. The methodological aspects which may affect the results are discussed in Chapter 7.2.

The separation of CO_2 derived from native soil (peat) and plant-derived C sources (SOM derived from plants and autotrophic respiration) was obtained here based on the large age difference between old native peat and fresh, plant-derived C enabling their separation by ^{14}C analyses (Article IV). On average, 70% of the respired CO_2 was from new, plant-derived C. The age of the respired C increased significantly towards the autumn, i.e. the contribution of the peat derived CO_2 increased. Similar trend has been found also in other ecosystems (Garnett and Hardie 2009, Hirsch et al., 2002). The reasons for this phenomenon are not fully clear. It has been suggested that as the temperature of the surface soil decreases in autumn, a higher proportion of the respiration is derived from lower (and older) peat layers where the decrease in temperature is less (Hirsch et al., 2002). It is also plausible that in the autumn as the growing season ends and plants wither, less fresh, plant-derived C in the form of root exudates is available for the soil microbes and relatively more CO_2 is derived from peat C. RCG is staying in the field over winter leading to accumulation of plant litter material in snow. This material is then decomposed in the spring after the snow-melt increasing the contribution of plant derived CO_2 in respiration. These results show that factors other than temperature affect the magnitude and sources of respiration over the season which is important when modeling soil CO_2 release.

Abiotic CO_2 fluxes are often neglected when soil CO_2 flux is measured. However, the abiotic CO_2 release may have importance in calcareous soils (Ramnarine et al., 2012) and in acidic soils, like peat soils, after the application of lime. There the role of lime-derived CO_2 should be considered because it can distort respiration measurements. Within this thesis, the contribution of lime-derived CO_2 was determined by utilizing the difference between ^{13}C content in lime and SOM (Article VI). Lime has naturally high $\delta^{13}C$ values (2.1‰, Article VI) while the

typical $\delta^{13}C$ value of SOM derived from C3 plants is -27‰ (Kuzyakov, 2006). Field measurements showed that a relatively small, but significant portion of the total annual CO_2 flux can be lime-derived (4.6%) in the year of application. In the year following the application, lime-derived emissions were negligible. IPCC 2006 Guidelines for National Greenhouse Gas Inventories (IPCC, 2006) suggests that all lime carbonates are released in the form of CO_2. Based on the results here this might be an overestimation at least in peat soils. In the peat studied here a total of 15% of the C in applied lime was released as CO_2, i.e. 85% remained in the soil or was dissolved in the soil water. It has to be noted that in the laboratory incubations up to 70% of the CO_2 was lime-derived. This was probably induced by the water addition in the laboratory (soil moisture was adjusted to 60% WHC). The higher soil water content could have favored lime dissolution leading to higher lime-derived CO_2 emissions than from drier soil *in situ*. So, in the field conditions the amount of precipitation could regulate the lime derived CO_2 emissions. We may have missed such hot-events since the field measurements were done once per month. Overall, the role of lime-derived CO_2 cannot be neglected in respiration measurements on acidic lime-treated peat soils to avoid overestimation of biological CO_2 emissions.

A summary on the contribution of various sources to soil CO_2 flux in RCG cultivation, estimated by using the data from Articles IV, V and VI, is presented in Figure 1. This approximation shows how the CO_2 is derived from different sources within the peak growing season (June - August) 2006. Autotrophic respiration and CO_2 derived from plant-derived SOM (36% and 42% on average, respectively) are the most important sources of CO_2 in this site. Five years after cultivation with RCG, CO_2 from peat contributes on average 14% to total respiration. The smallest amount of CO_2 stems from lime with 8% at max., immediately after liming.

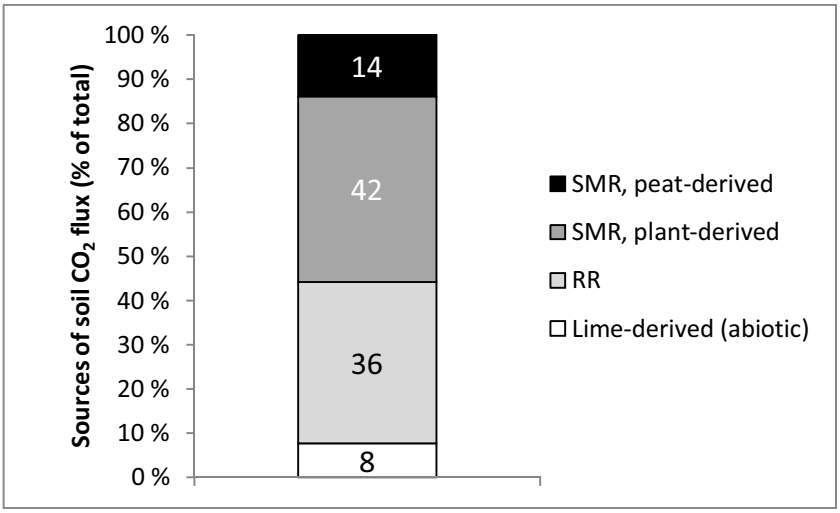

***Figure 1**. Sources of soil CO_2 flux in RCG cultivation on an organic soil. The data from June, July and August 2006 were used to determine the relative share of each component of the soil CO_2 flux. The figure is based on the data used in Articles IV, V and VI and represents an average value found for the different months in peak season and for the different methods used (e.g. isotopic labelling and root trenching approach).*

7.4 SEPARATING COMPONENTS OF SOIL RESPIRATION: METHODOLOGICAL CONSIDERATIONS

There was a discrepancy between the results obtained by root trenching and isotopic method in the relative proportion of RR and SMR as mentioned above. In case of trenching, total root exclusion is difficult to obtain and the remaining roots might continue to decompose causing an overestimation in the SMR (71% of total respiration). With the isotopic method which measures SMR in presence of roots the inaccuracy associated to the roots is avoided. As expected, the isotopic method gave thus lower value (50%) for the SMR. The divergence of the results between the two methods may, however, in part also be explained by the fact that the root associated microorganisms (e.g. mycorrhizal fungi) were contributing to RR in the isotope

partitioning approach while they are associated to SMR in the trenching experiment. Different methods thus separate different sources to some extent, and defining the sources of consideration is important in partitioning experiments. A huge advantage with the isotopic method is that it can be applied in situ without disturbing the system and therefore further testing and development should be done with this method.

The "bomb C peak", i.e. the ^{14}C peak derived from nuclear testing during 1950-1963 can be used to separate plant- and soil-derived CO_2 (Hahn et al., 2006, Gaudinski et al., 2000). Vegetation change from C3 to C4 plants (or vice versa) enables also the separation of plant- and soil-derived C (Blagodatskaya et al., 2011, Kuzyakov, 2006). These photosynthetic pathways fractionate isotopes differently producing distinct $\delta^{13}C$ signatures; -13‰ for C4 plants and -27‰ for C3 plants (Kuzyakov, 2006). All these methods have been widely used in various ecosystems, and offer possibilities for reliably partitioning SMR and RR in soil respiration. However, differences in isotope signal between the sources are often relatively small causing uncertainties in the outcome of the isotope mixing model. To avoid this, a novel approach for separating plant- and soil-derived CO_2 was introduced in Article IV. This method is based on ^{14}C analyses and can be applied on peat soils, where the native peat is thousands of years old (e.g. after peat extraction as here). Due to the age difference of thousands of years between plant- and soil-derived C, these two sources have clearly distinguishable ^{14}C signals which improves accuracy of the analyses. Cultivated cut-away peatlands are thus ideal model systems to study fate of peat after land-use change. Peat soils are thick deposits of C which has been accumulated over thousands of years. However, it can be rapidly lost by e.g. land-use changes (Trumbore 2009). New field methods that provide understanding on the processes affecting the C cycling of these soils are therefore necessary.

7.5 IMPLICATIONS OF FINDINGS ON SOIL CARBON BALANCE, SOIL CARBON SEQUESTRATION POTENTIAL AND TOTAL CARBON SINK/SOURCE STRENGTH OF THE ECOSYSTEM

The research site Linnansuo has been intensively studied to determine the overall greenhouse gas balance of perennial crop cultivation in an organic boreal soil. By using micrometeorological and chamber methods, it has been shown that the site acts overall as a sink of C, especially during wet years (Shurpali et al., 2010). This is a very positive result and a rare situation, as generally organic agricultural soils lose substantial amounts of C (Maljanen et al., 2010). Usually, the fresh C from plants increases soil microbial activity, which may accelerate also the decomposition of the native SOM (priming effect, Kuzyakov et al., 2000) which produces CO_2 to the atmosphere. The crucial question is how much of the native soil C is lost and whether these losses can be compensated by net primary productivity and plant input. In this last chapter, I attempt to link the findings on soil C cycling and dynamics of this thesis with the overall C balance of the RCG cultivation.

The soil C balance is the net difference between C inputs from plant roots and litter and C outputs in form of soil microbial respiration and C leaching (the last component is low in RCG cultivations and can be neglected here; Hyvönen et al., 2013). Root growth of RCG in organic soil is high with net belowground productivity averaging about 225 g C m^{-2} yr^{-1} (Shurpali et al., 2008, Shurpali et al., 2010). If hourly root growth rates are roughly calculated from this value (assuming 4 months of growing season and constant production rates) we arrive at a root C input rate of 75 mg C m^{-2} h^{-1}. If further litter input is added (~40 mg C m^{-2} h^{-1}; C. Biasi, personal communication) the total C input equals to ~115 mg C m^{-2} h^{-1}. This is a conservative estimate for the peak growing season since root productivity is higher then (Shurpali et al., 2008). Soil respiration rate in peak season on the other hand is in the range of ~130 mg C m^{-2} h^{-1} (peak respiration rates expressed as mg C instead of mg CO_2 here; see above). With 56% stemming from SMR (Figure 1), soil

C losses equal to about 75 mg C m^{-2} h^{-1} which is less than the assumed rate of soil C gains. Despite all the uncertainties in the calculations for C losses the respiration rates could be lower or at least in the same order of magnitude as total C input (including root growth and litter input). In a second exercise, peat-derived respiration rates can be estimated by multiplying the relative proportion of peat respiration (14%) with absolute soil respiration rates (see above). This calculation yields a peat respiration rate of about 70 mg CO_2 m^{-2} h^{-1} for growing seasons, values which are in the lower range of peat respiration rates reported for similar period in the adjacent uncultivated peatlands (Shurpali et al., 2008). The results of this study show that the peat-derived respiration in the RCG site was similar, if not lower than in the unplanted site. This means that the plant cultivation likely did not induce an increase in peat decomposition (priming effect), one of the likely reasons for high CO_2 losses from organic agricultural soils. Even though further studies are needed to confirm that, it is possible that organic soils cultivated with RCG do not loose huge amounts of soil C, instead, they may be in balance or even act as soil C sinks, which favors overall C acquisition of the RCG cultivation. Possible reasons are large root C inputs of perennial plants with huge root stocks and lack of priming effect. This is further supported by the relative increase of fungi and their overall importance in soil C cycling in the system, since fungi are often connected to soil C sequestration. Studies which are currently underway will further elaborate on soil C balance in perennial cropping systems of organic soils.

The results from the studies here on microbial dynamics, soil respiration and CO_2 sources support the C balance studies in situ which have shown that with perennial crops C loss could be prevented even from cultivated organic soils.

7.6 SUMMARY AND CONCLUSIONS

This thesis provided new information about the sources of soil CO_2 flux and the role of microbial communities in soil C cycling in a cultivated organic boreal soil. The work also contributed to the development of methods in the field of isotopic research.

The main findings were:
- As expected, the cultivation of RCG induced an increase in microbial biomass and activity compared with the unplanted site. Respiration was mostly derived from new plant material and roots.
- Fungi have the most important role in the immediate turnover of fresh, plant-derived C and likely also in soil C sequestration.
- In addition to fungi, Gram-negative bacteria also incorporated rapidly large amounts of fresh C.
- Gram-positive bacteria, on the other hand, had higher label allocation one year after $^{13}CO_2$ pulse-chase labeling than immediately after it indicating that they use more processed sources of C.
- The use of label in root sugars as a proxy for root exudates following the $^{13}CO_2$ pulse-chase labeling experiment provides new opportunities to study the flow of C from roots to soil microbial community *in situ*.
- Liming induced a trend of faster allocation of fresh C in microbes but the results were inconclusive.
- Lime-derived CO_2 was of minor importance in the field measurements of soil CO_2 flux but laboratory incubations showed that e.g. rain events can induce substantial emissions of lime-derived CO_2.
- ^{14}C analyses proved to be a promising tool to separate CO_2 derived from fresh and native OM in cultivated cutaway peatland soil – these plant-soil systems thereby act as ideal model site to study fate of native C after cultivation/re-vegetation of organic soils.
- Root trenching method and an isotopic method based on ^{13}C labelling were used to separate SMR and RR. This comparison

of methods highlights the potential of the non-invasive isotopic method.

- Priming effect was not observed as the flux of peat-derived CO_2 in the cultivated was not higher than in the non-cultivated site.
- Agricultural organic boreal soils are generally losing soil C but cultivation of perennial crops could decrease this loss. This is likely connected with overall C sequestration of perennial cropping system on organic soil. This cultivation system would thus be an option to mitigate negative climatic effects of cultivated organic soils.

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Niina Tavi

Soil Carbon Cycling and Microbial Dynamics in Boreal Organic Soil Cultivated with a Perennial Crop

Boreal peatlands are a huge storage of carbon. When drained and cultivated, they become large sources of atmospheric carbon dioxide. Within this thesis, the sources of soil carbon dioxide flux on an organic boreal soil cultivated with a perennial crop, reed canary grass (*Phalaris arundinacea*) were determined. The results show that cultivation of perennial crops instead of annual crops could mitigate the carbon losses. The study also elucidated the role of various soil microbial groups in the turnover of plant derived fresh carbon.

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