The contribution of various carbon sources to soil organic carbon: agronomical and environmental implications

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Preface

This master thesis completes my two year Master of Science degree in Agroecology. The study of carbon dynamics associated with agricultural soil deals with the very process that provide the energy needed to sustain all life depending on the functions of agroecosystems, including humans. I find the ecosystem approach to such a process most interesting and I hope that the findings of this study will be of value for the future management of the carbon resources involved in agroecosystems. In order to enable a holistic approach, often not accomplished by a narrow experiment and the reported findings, the thesis contains a literature review, followed by an experiment report.

Especially I salute my supervisor, Professor Marina Azzaroli Bleken for being at service during the writing process, and for designing the experiment of which this thesis partly is based. Further I thank Toril Trædal and Øyvind Peder Vartdal for being at service during the field work, Trygve Fredriksen for helping me both during the field work and in the laboratory, Hanna Marika Silvennoinen for conducting the isotope ratio analyses, my co-supervisor Professor Tor Arvid Breland and Peter Dörsch for expert advisory and Astrid Haavik for commenting on my script and solving computer issues.

The incubation experiment is a part of the ongoing “Soilborne greenhouse gas project” at the Institute of Plant and Environmental Sciences.

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Abstract

This thesis contains a literature review, where the ecology of soil organic carbon (SOC) in agricultural soils is addressed in a wide perspective. In addition it reports the findings of a laboratory incubation experiment. Several earlier studies suggest that aboveground plant parts (shoots), either incorporated into soils or left on the soil surface, are decomposed at a faster rate than their belowground counterparts (roots and roots derived components). To test whether such a relationship also exists in mineral soils in southeast Norway, oat (*Avena sativa* L.) and a green manure mixture containing perennial ryegrass (*Lolium perenne* L.), timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.) and red clover (*Trifolium pratense* L.) was pulse labeled in the field with $^{13}CO_2$ throughout the growing season 2009. Shoots and soils were then used to establish six different incubation treatments (unlabeled control soil, unlabeled soil + ^{13}C enriched oat shoots, unlabeled soil + ^{13}C enriched grass shoots, unlabeled soil + ^{13}C enriched clover shoots, soil containing ^{13}C labeled green manure roots and soil containing ^{13}C labeled oat roots) was incubated at 15 °C and the respired CO_2 was collected using lye (NaOH) as CO_2 traps. Two approaches were used to quantify the relative amount of respired shoots. The respired CO_2 from the control soil was subtracted from treatments amended with ^{13}C enriched shoots, to provide the apparent amount of CO_2 derived from shoots. In addition the $^{12}C/^{13}C$ signature of the respired CO_2 together with the $^{12}C/^{13}C$ signature of the soil and organic matter in question was used to quantify the relative contribution of the various carbon sources to the total CO_2 pool, using a two source mixing model. Using the latter approach, it was found that after 104 days of incubation: 29.9 ± 1.29 %, 33.8 ± 2.67 % and 26.2 ± 1.49 % (mean values ± standard deviation) of the original carbon in shoots had been respired from oat shoots, grass shoots and clover shoots, respectively. The same approach applied to root respiration provided lower values: 7.6 ± 0.12 % and 10.2 ± 0.33 % of the original carbon contained in green manure roots and oat roots, respectively had been respired. The differences in degradability of the two substrates are believed to be partly caused by structural differences (inherent properties), and partly by differences in the ability to become physically protected. As roots and root exudates interact with the soil structure in a more profound way than do shoots incorporated into the soil, they have a better chance of becoming an integrated part of soil aggregates and thus gain protection from the degrading actions of microorganisms.

Sammendrag

Masteroppgaven omfatter både en litteraturgjennomgang, der økologien til organisk karbon i jordbruksjord blir satt i en vid sammenheng, samt en del som omhandler et inkubasjonsforsøk og de funn som er gjort. Funn fra flere tidligere studier indikerer at overjordiske plantedeler, enten de er blandet inn i jord eller plassert på jordoverflaten, har en høyere nedbrytningsrate enn underjordiske plantedeler (røtter og rotderivater). For å teste om dette også stemmer for mineraljord i Sørøst-Norge, ble havre (*Avena sativa* L.) samt en grønngjødselbanding bestående av flerårig raigras (*Lolium perenne* L.), timotei (*Phleum pratense* L.), engsvingel (*Festuca pratensis* L.) og rødkløver (*Trifolium pratense* L.), eksponert for $^{13}CO_2$ gjentatte ganger i løpet av vekstsesongen 2009. Seks behandlinger med jord (kontrolljord, jord + ^{13}C anriket havrehalm, jord + ^{13}C anriket gress-skudd, jord + kløverskudd, jord med ^{13}C anrikede havrerøtter og jord med ^{13}C anrikede grønngjødselrøtter) ble inkubert ved 15 °C og respirert CO_2 ble akkumulert i lut (NaOH). Den relative andelen av skudd som var respirert ble estimert på to like måter. CO_2 i kontrollen ble trukket fra CO_2 respirert i behandlingene tilsatt skudd. I tillegg ble en blandingsmodell der $^{12}C/^{13}C$ signaturen til oppsamlet CO_2 sammen med $^{12}C/^{13}C$ signaturen til tilhørende jord og plantedeler brukt, til å kvantifisere det relative bidraget ulike typer substrat hadde til den samlede $^{12}C/^{13}C$ signaturen til respirert CO_2. Ved bruk av sistnevnte metode ble det estimert at etter 104 dager hadde 29,9 ± 1,29 %, 33,8 ± 2,67 % og 26,2 ± 1,49 % (middel ± standardavvik) av opprinnelig karbon blitt respirert, fra henholdsvis havreskudd, gress-skudd og kløverskudd. Ved å bruke samme prosedyre, ble det funnet at bare: 7,6 ± 0,12 % og 10,2 ± 0,33 % av opprinnelig karbon i henholdsvis havrerøtter og grønngjødselrøtter var respirert. Forskjellen i nedbrytningsrate er antatt delvis å være forårsaket av substratforskjeller (iboende forskjeller) og delvis av forskjeller i evnen til å bli fysisk beskyttet mot nedbrytning. Fordi røtter og roteksudater blir dannet i tett nærhet til mineralpartikler vil disse i større grad kunne bli innlemmet i aggregater, og således bli skjermet fra nedbrytningsorganismer, enn skudd som tilføres jorden.

List of content

Introduction

Soil organic matter (SOM) is continuously generated and degraded through a complex series of events. Whether the SOM content in soils is changing in either direction or remains stable depends on the balance between the SOM building mechanisms and the rate of SOM mineralization (Janzen 2006). It is difficult to distinguish between the properties of SOM and soil organic carbon (SOC) because they are inherently connected. In this paper SOC will be used when it is possible to handle carbon more or less specifically. When a broader perspective is applied, SOC is implied within the concept of SOM.

It has been estimated that agricultural soil prior to cultivation contained some 222 Pg C. At this date, the very same soil contains 156 Pg C and the CO_2 generated during depletion of SOC has contributed some 20 % of the anthropogenic atmospheric CO_2 emissions. By the means of agricultural manipulation of the processes leading to SOM depletion it is possible to shift the situation, transforming agricultural soil to a sink rather than a source of CO_2 (Paustian et al. 1997).

In addition to merely being a constituent of the soil body, SOM facilitates an array of agronomic beneficial properties such as: increased cation exchange capacity (CEC) (Brady & Weil 2004), enhanced soil structure (Golchin et al. 1994 a; Hillel 2004; Six et al. 2000), increased micro nutrient availability (Havlin et al. 2005), lowered albedo (Brady & Weil 2004), increased pH buffering capacity (Magdoff & Bartlett 1985), positive effect on erosion (Bresson et al. 2001) and the release of nutrients and energy upon degradation of SOM itself (Janzen 2006).

The decay rate of SOM is in general considered to follow first order kinetics, which implies that the decay rate increases proportionally with an increase in available substrate (Six et al. 2002). From this relationship it follows that with a given regular input of organic matter, a dynamic equilibrium, where the carbon added to the soil is equal to the mineralized carbon, will be established thus underlining the dynamic nature of SOC. The longevity of organic matter added to the soil is termed the turnover time and it may range from a few years (Oades 1988) to as long as 10,000 years (Nguyen et al. 2009).

The turnover time of soil organic carbon (SOC) is determined by factors such as substrate quality (e.g. C/N ratio) (Brady & Weil 2004; Morvan & Nicolardot 2009), temperature and water content (Karhu et al. 2010; Smith et al. 2003), pH (Foereid et al. 2006), oxygen status (e.g. water logging) (Gao et al. 2009; Grønlund et al. 2008), spatial distribution (Breland 1994), and chemical and physical protection (Gale et al. 2000a; Golchin et al. 1994 a; Puget & Drinkwater 2001; Six et al. 2002) which in turn is affected by extent of soil disturbance (e.g. tillage) (Six et al. 2000).

By fractionating soil it has been revealed that SOM is tightly connected to the soil structure and that a large portion of the SOM content is enclosed within microaggregates (Golchin et al. 1994 a; Six et al. 2000; Six et al. 2002) or chemically associated with the clay fraction (Oades 1988). By using plant material enriched with carbon isotopes (^{13}C and ^{14}C) it has been shown that roots contribute to a larger extent to the stable SOM pool than do shoots when added to the same soil (Buyanovsky & Wagner 1987; Gale & Cambardella 2000; Puget & Drinkwater 2001; Rasse et al. 2005). It has thus been hypothesized that shoots amended on the soil surface or incorporated into the soil body are more prone to rapid mineralization than roots.

The aim of this thesis is to (i) review the dynamic nature of SOC and how different agricultural regimes interact with this dynamic process (ii) test, by conducting a short term laboratory incubation with ^{13}C enriched roots and shoots of oats (*Avena sativa* L.) and green manure, if there are any differences in mineralization rate between shoots and roots that are incorporated into a loamy soil.

Literature review

Carbon dynamics in agricultural soil

In ecology, assimilation of carbon-containing compounds through photosynthesis is termed primary production. Gross primary production (GPP) is the total amount of harvested energy, whereas net primary production (NPP) corrects for plant respiration. The overall changes of carbon (C) storage (Δ storage) in an ecosystem equals C inputs minus C outputs (Krebs 2001).

Agricultural soil is constantly replenished with organic matter from a wide range of sources. Crop residues, compost, green manure and animal manure is added on top of the soil or incorporated into the soil through tillage, faunal activity or by natural physical processes. Roots growing within the soil are constantly producing organic exudates and when they die, they become a source of SOC. The organic matter (OM) added to the soil follows a succession from labile and easily decomposable plant material to stabile and recalcitrant humus (Magdoff & Weil 2004). In a geological time perspective, some of the OM added to the soil will be preserved and transformed into fossil fuel. The NPP associated with agricultural soil is high, but so is the turnover of SOC. Whether total SOC increases or is depleted, or in which state carbon compounds occur in the soil matrix depends on many factors, some of which will be presented in this section.

Classification of SOM (SOC)

Organic matter (carbon) can be divided into a hierarchy based on origin, stage of degradation, density, chemical properties and recalcitrance. Oades (1988) suggests that the following terminology should be applied to distinguish between different fractions:

- ***Organic matter*** **(OM):** natural C-containing organic materials living or dead, but excluding charcoal.
- ***Phytomass*****:** materials of plant origin usually living, but it also includes standing plants which are dead, e.g. trees.
- ***Microbial biomass*****:** the living population of soil microorganisms.
- ***Macroorganic matter*****:** organic fragments in soils from any source which are > 250 μm.
- ***Light fraction*****:** organic fragments obtained from soils by flotation on heavy liquids of densities 1.6 – 2.0 Mg m^{-3}

- ***Humus*:** material remaining in soils after removal of macroorganic matter.

The light fraction may exist either as free particulate organic matter (POM) or as occluded POM. The amount of free POM is quantified by physical fractioning, a process where a soil sample is suspended in a high density liquid (e.g. sodium polytungstate of 1.6 g cm^{-3}) and centrifuged, whereby POM floats on the surface. The occluded POM may be quantified by exposing the aggregates, in which the POM is situated, to ultrasonic sound waves which crack the aggregates open, thereby liberating the POM, which in turn may be quantified by physical fractioning (Golchin et al. 1994 b).

Another important category is litter, which by Buyanovsky & Wagner (1987) is defined as plant residues from the previous year. In this paper, litter will include all dead organic matter of plant origin that enters the soil. Dissolved organic carbon (DOC) is water-soluble carbon compounds in dissolved state. DOC only constitutes a small fraction of the total carbon in the soil, but may, due to its dissolved state, be prone to leaching (Zimmermann et al. 2007).

Variables affecting mineralization of SOC

It has been stated that soil microbial biomass is "the eye of the needle through which all the natural organic material that enters the soil must pass" (Jenkinson 1978), reported by (Vanveen & Kuikman 1990). Heterotrophic decomposers in soil can exist within a wide range of environmental conditions. Even so, there are still some boundaries that restrict the biological activity of the soil dwelling organisms. Within these boundaries there exists a gradient ranging from habitable → suboptimal → optimal living conditions. Thus, the factors governing the mineralization rate of SOC are indeed complex and difficult to articulate.

Temperature, water content, oxygen status, pH and distribution of SOC

Temperature and precipitation is closely associated with SOC turnover. On a global scale mineralization of SOC has a positive correlation with rising temperature and negative correlation with increasing precipitation. This is illustrated by the fact that the amount of SOC is positively correlated with increasing latitudes from about 30° (Smith & Smith 2006). However, in tropical rainforest, the amount of sequestered SOC is marginal (varies) even though vast quantities of water is deposited as precipitation (Oades 1988).

Thus, there is no universal correlation between temperature, precipitation and sequestration of SOC. Many studies reviewed by Smith et al. (2003) have found that the respiration rate of OM is multiplied by a certain factor for every 10 °C rise in temperature and the phenomenon is referred to as the Q_{10} factor. The Q_{10} factor is not consistent between different studies and Q_{10} values ranging from 2.5 to 4 have been reported. The relationship between temperature and soil water content (moisture) is also reviewed by Smith et al. (2003) and they conclude that temperature is the limiting factor for SOM mineralization as long as moisture levels are optimal and that moisture becomes the limiting factor under dry conditions.

The oxygen content of the soil is of utmost importance when it comes to mineralization rate and it is tightly connected to the water content of the soil (Gao et al. 2009; Grønlund et al. 2008) which in turn is partly governed by the texture and structure of the soil (Hillel 2004). In a long term study of the effect of drainage of marches for agricultural purposes in Norway, it has been shown that the aeration associated with drainage and tillage has lead to massive mineralization of SOM manifested as field subsidence. It has been estimated that the annual CO_2 emissions associated with cultivated peat soil in Norway is some 2 million tones $year^{-1}$ (Grønlund et al. 2008). Given that cultivated peat soils worldwide harbor large quantities of SOC (Biasi et al. 2008) that becomes prone to mineralization upon drainage, cultivation of such soils has an obvious impact on the atmospheric CO_2 level.

Low pH is generally regarded as a retardant for decomposing organisms (Oades 1988). By labeling grasslands with ^{13}C-carbon it has been shown that liming with $CaCO_3$ is positively correlated with mineralization of SOM (Foereid et al. 2006). However, Oades (1988) reports that liming with $CaCO_3$ at first increased the mineralization of SOM, but that the total mineralization in the limed soil over time was less than in the control. The overall retarding effect of liming on mineralization is attributed to the stabilizing effect associated with the added Ca^{2+} originating from the carbonate (Oades 1988).
The spatial distribution of litter influences the mineralization rate. It has been shown that a heterogeneous distribution of litter is more prone to rapid mineralization than litter evenly incorporated into the soil (Breland 1994).

C/N relationship and nutrient status

In heterotrophic organisms, energy released from oxidizing carbon containing compounds is used to fuel biochemical reactions, while nitrogen containing compounds are used as structural compounds within the cells of the decomposers. For each gram of nitrogen in the substrate roughly 24 g of carbon is needed for oxidative, structural and other purposes. Hence, if the substrate has a C/N ratio roughly above 25, the decomposition is retarded unless mineral N can be assimilated from the soil reserves (N immobilization). If the C/N ratio is below 25 the excess nitrogen will be released to the soil matrix through mineralization (Brady & Weil 2004). The same principle is applicable to all nutrients, both as an intrinsic property of the organic substrate and inorganic nutrients in the soil matrix, but since nitrogen is regarded as the limiting factor the C/N ratio is preferred.

Priming effect

Priming effect was defined by Bingeman et al. (1953) as "the increase in soil organic matter mineralization following the input of fresh C residue" (Nottingham et al. 2009). Some studies have found evidence of a positive priming effect (i.e. enhanced mineralization of SOM) (Fontaine et al. 2004; Nottingham et al. 2009). Nottingham et al. (2009) traced the additional CO_2 evolved after amendment of fresh OM to be of old SOM origin and not as a result of increased microbial turnover (apparent priming effect). Other studies have found that addition of litter may have no priming effect (Martens et al. 2009) or cause a negative priming effect (i.e. relieve SOM from mineralization) (Potthast et al. 2010; Torbert et al. 2000).

Alternative oxidative pathways and their outcomes

During anoxic conditions, often associated with water logging or soil compaction, microorganisms are unable to utilize oxygen as the ultimate electron acceptor. In order to keep up the oxidative biochemical processes, the microorganisms suffering from anoxia are forced to utilize alternative electron acceptors. Examples of alternative electron acceptors are different reactive nitrogen compounds, which are reduced in the following order: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. The precursor for NO_3^- is ammonium (NH_4^+) which, under aerobic conditions, is oxidized by bacteria to NO_3^- in the two step process known as nitrification (Hillel 2004). In many cases the reduction sequence of NO_3^- is incomplete, leaving N_2O prone to escape the soil and enter the atmosphere and contribute to the green house effect. Another property of anoxic conditions in soil is production of

methane (CH_4) by methanogenic archaea and bacteria (Conrad 1999). Production of CH_4 is not initiated until all available electron acceptors are exhausted, and a prolonged period of water logging is needed before CH_4 is emitted from the soil. Examples of such conditions are marshes and paddy rice fields. In addition to being a contributor of CH_4, soils and especially aerated soils, may act as a sink for CH_4 through the oxidative actions of certain bacteria. Since production of NO_3^- is an oxidative event, and because utterly anoxic conditions often lead to total reduction of NO_3^- to N_2, prolonged water logging (i.e. marshes and paddy rice fields) does not lead to substantial N_2O emissions. However, soils that are subjected to fluctuations in water table or are frequently flooded can potentially lead to immense N_2O emissions (Smith et al. 2003).

Stabilization of SOC

Three basic mechanisms for stabilization of SOC are recognized by Six et al. (2002) namely chemical stabilization, physical protection and biochemical stabilization. In addition the stabilization of SOM due to anoxic conditions is an important factor for the preservation of large quantities of carbon worldwide, and will therefore also be reviewed.

Chemical stabilization of SOC

Particles in the clay fraction have, due to isomorphic substitution of the core atom in the structure, a permanent negative charge. In addition to the permanent negative charge, the edges of the same particle may have a pH dependent charge where the negative charge is positively correlated with increasing pH. Humus particles have both positive and negative sites within the particle, but always exert a net negative charge. As with the edges of clay particles, the charge of the humus fraction is also pH dependent. Because the clay fraction and humus share many of the same properties they are jointly labeled the colloid fraction (Brady & Weil 2004). The electrical charge of mineral particles and humus may result in creation of organo-mineral complexes where divalent and polyvalent cations function as bridging units, connecting the negatively charged clay and humus particles (Oades 1988). The chemical stabilization capacity of soils is according to these principles limited to the physiochemical properties of the soil, leading to the hypothesized prediction of the existence of a saturation level of SOC (Chung et al. 2010; Kimetu et al. 2009). Organic molecules may also bind to clay particles by hydrogen bonds, anion properties of humus, van der Waals forces or precipitate on mineral particles and thus function as a cementing agent in microaggregate formation (Hillel 2004).

Physical protection of SOC

Soil organic carbon that would otherwise be suitable as substrate for microbial degradation, can, by physically excluding microorganisms or by altering the conditions unfavorable, be protected from further decomposition. Six et al. (2002) suggests that SOC may be physically protected through three different mechanisms; 1) the compartmentalization of substrate and microbial biomass 2) the reduced diffusion of oxygen into macro- and (especially) microaggregates and 3) by the compartmentalization of microorganisms and their predators. The formation of physical conditions facilitating such properties may be either as a function of the forces mentioned for chemical stabilization or by sticky substances that glue particles together into aggregates. Such substances are often root exudates (mucilage) or secondary metabolites of plant, bacterial, faunal or fungal origin (Golchin et al. 1994 a; Oades 1984). In addition to the entrapment within aggregates, pores may also exert physical protection for SOC (Oades 1988). Due to the simple fact that roots grow in close contact with soil particles and that they constantly shed exudates of various chemical composition has led to the hypothesized prediction that roots are more easily physically protected from decomposition than shoots incorporated into soil, and studies suggests that this hypothesis holds true (e.g. Puget & Drinkwater 2001; Buyanovsky & Wagner 1987). Because both physical protection and chemical stabilization is enhanced by increasing proportions of the silt and clay fraction (Vanveen & Kuikman 1990), it is expected that soils with high content of these fractions are able to hold a larger stock of occluded carbon as compared to sandy soils.

Biochemical stabilization of SOC

Organic carbon entering the soil will immediately be exposed to the action of degrading organisms. Since carbon dynamics is all about realizing the energy stored within the organic compounds, it is logical that the suitability as a substrate for oxidation is reduced as the amount of energy decreases. SOM varies in chemical composition and the suitability as a substrate for degradation is equally diverse. By incubation of various ^{14}C labeled compounds it has been shown that those compounds prone to oxidation (glucose, amino acids, etc.) were mineralized at a higher rate than more recalcitrant compounds like lignin (Haider & Martin 1975). In addition it has been shown that easily hydrolysable compounds like cellulose and proteins may be protected from degradation through associations with lignin (Oades 1988). As the SOM descends down the hierarchy of

Gibbs free energy, the suitability for further degradation is reduced. The result is that a small fraction of the added organic matter is retained in the soil as recalcitrant humus that is non-hydrolysable by enzymes in the decomposer organisms' arsenal (Magdoff & Weil 2004). In soils that are depleted of N, a high C/N ratio could be regarded as a sort of temporary chemical stabilization, as long as external nitrogen is unavailable.

Anoxic stabilization of SOM

Even though water is a prerequisite for life, an excess of water in soil may lead to conditions that restrain the living conditions for the decomposing organisms and hence retard the mineralization of SOC. Whether water enhances or retards mineralization depends on the oxygen status of the soil. If the infiltration capacity of the soil is poor and the precipitation is high, or if there is a threshold that hinders excess water from draining, water logging may occur. During such an event anoxic conditions will soon limit the mineralization rate, and hence the turnover of SOC. Even though the productivity under such conditions is reduced, the overall effect on the soil ecosystem is a net accumulation of SOC. In cold and water logged locations, massive amounts of SOC may build up and form peat (Brady & Weil 2004). Even though water logging hardly ever occurs in European agriculture, water logging is an integrated part of the field management in paddy rice fields. In addition, much of the world's terrestrial sequestered carbon is to be found in marshes, and therefore play a crucial role in the global carbon dynamics. As mentioned above, anoxic condition may lead to alternative oxidative pathways, and this may have implications for the climate.

Carbon dynamic models

Mineralization of SOC is in general considered to follow first order kinetics, which implies that the decay rate is proportional with the amount of substrate (Six et al. 2002). The decay rate for a substrate subjected to a certain environment (temperature, moisture etc.) is given by a specific decay rate constant. The environmental conditions are termed decay rate modifiers, and variations in the environmental conditions may profoundly modify the decay rate constant (Tor Arvid Breland pers. comm). The decay rate of change may be expressed as: $dC_{soil}/dt = -kC_{soil}(t)$, where C_{soil} is the amount of C in the soil and k is the decay rate constant. The amount of substrate (C) at any time (t) may be given as: $C_t = C_0e^{-kt}$ (La Scala et al. 2008). It is important to emphasize that this is the theoretical amount of one type of substrate (pool) at any given time and that SOC consists of several

qualitative distinct pools of substrate. Due to this fact, SOC decay models are dynamic by definition. This calls for a systemic approach where the decay rate of all the SOC pools are considered jointly. Several attempts have been made to articulate such models in order to predict the dynamic behavior of SOC and the RothC-, CENTURY- and DAISY models are examples of such models (Petersen 2007).

The longevity of SOC is either expressed as turnover time or half life ($T_{1/2}$). The turnover time is regarded as the average longevity of litter added to the soil and is calculated by dividing the SOC content of the soil with the annual input. The $T_{1/2}$ of SOC is calculated by dividing 0.693 by the decay rate constant. The average turnover time for SOC globally is considered to be 30-40 year (Oades 1988). The decay rate, and hence the SOC turnover and the equilibrium threshold is as mentioned largely dependent on temperature and water logging. These factors are largely responsible for the up to 2000 years turnover time for peats and for the positive correlation between increasing latitude and the amount of sequestered SOC in the soil, with turnover times exceeding 100 years in the arctic tundra (Oades 1988). The first order kinetics involved in OM degradation makes it practically impossible to deplete the SOC content completely as long as there is some plant production, but is it equally difficult to sequester large amounts of SOC without constant additions of substantial amounts of organic material, unless the decay rate constant is low. Because of different decay rates at different locations and different degradability of different types of litter, there is no absolute level of annual litter supply needed to sustain a certain level of SOC at equilibrium (Buyanovsky & Wagner 1987).

Vold et al. (1999) suggested an easily comprehensible conceptual model, similar to the DAISY model, that considers the decay of litter and thus the biochemical stabilization of SOC. In order to fulfill the assumption of a constant decay rate, the litter is conceptually divided into two pools, namely readily decomposable litter (sugars, amino acids, starch, cellulose, etc.) and slowly decomposable litter (hemicelluloses, lignin etc.). As the SOC is decomposed, most of the carbon is mineralized to CO_2, while some of the carbon is retained in the microorganisms. The proportion of the original C that is retained is termed the microbial growth yield efficiency and usually lies between 0.2 and 0.5 (Tor Arvid Breland pers. comm). Some of the microorganisms will enter the humus pool when they die and some will reenter the microbial biomass. In addition the soil fauna plays a role in that they prey on the biomass (Ferris et al. 1998), and carbon from faunal feces may yet

again become part of the microbial biomass or enter the humus pool. In the end, the initial carbon from the litter is either stabilized as humus or mineralized to CO_2.

The SOM flow chart created by Vold et al. (1999) gives an excellent illustration of the fate of litter added to the soil system in terms of biological decay. However it does not consider the dynamic interaction between organic carbon and the mineral fraction of the soil. Merging the models of Vold et al. (1999) and Six et al. (2002) into one joint conceptual model enables a total evaluation of organic matter with emphasis on carbon flow (Figure 1).

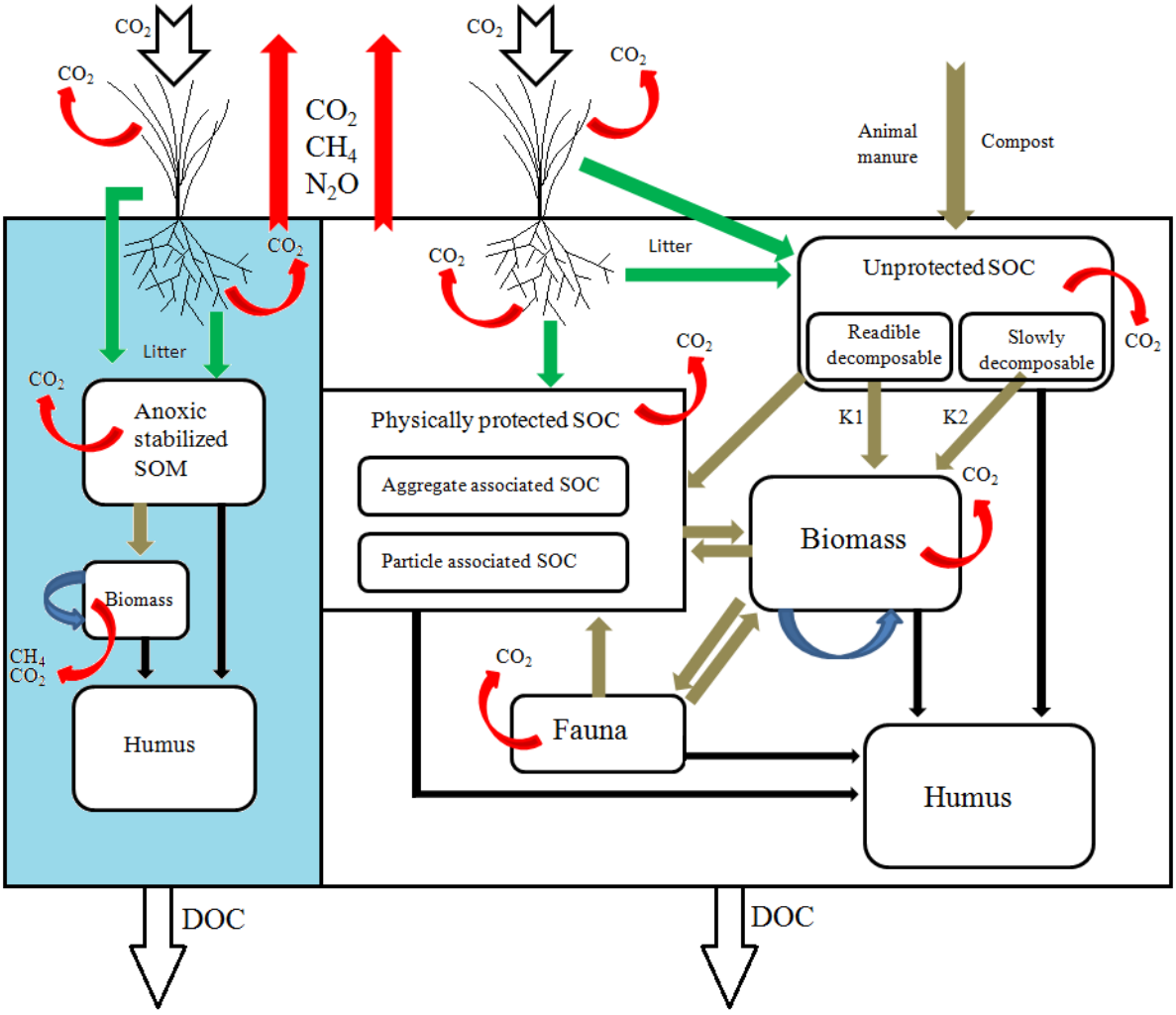

Figure 1. Soil organic carbon flow model. The left part (Blue) represents water logged soil whereas the right part represents aerated mineral soil. Green arrows represent litter, brown arrows represent organic intermediates, blue arrows represent recycling, black arrows represents humus formation and red arrows represents respiration.

Agronomic implications of SOM

Evolution of terminology and perception of SOM

The term humus was in the roman age used more or less synonymously with soil. Since then the term has frequently been associated with what we today classify as mould or organic matter. Before it in the mid eighteen-hundreds became clear that the substrate for primary production is water, soluble minerals and CO_2, it was believed that the juices from SOM was the source of plant biomass, and SOM was thus regarded as a precious resource. It was common agronomical procedure to add a certain share of the organic matter back to the soil as animal or human manure, compost or green manure. Once it became clear that the substrate for photosynthesis is the above mentioned factors, the attention given to SOM fainted as the mineralistic era of agronomy began. During the last couple of decades SOM has once again gained some attention as it has been shown that SOM facilitates many beneficial soil properties that both may increase production and have implications for the long term sustainability of agriculture. Today SOM is recognized as a resource both from an agronomical point of view and as a potential CO_2 sink. The term humus is no longer synonymous with OM, but has a far more specific meaning (Manlay et al. 2007).

Humus as part of the nutrient exchange complex

As mentioned, the clay and the humus fraction in soil exert colloid properties, and this has vast agronomical implications. The negatively charged colloids play a vital role in the cation exchange capacity (CEC), and are thus important for the nutrient status in the soil matrix. Since the humus colloids both have positive and negative charges, humus also influences the anion exchange capacity (AEC) of the soil matrix. The CEC of humus is positively correlated with rising pH, and in high pH soils the weight by weight CEC of humus exceeds the CEC of clay (Brady & Weil 2004). In addition to playing a part in the nutrient exchange complex, the SOM is a substantial source of nutrients that are released as available for plants when degraded (Janzen 2006).

Soil structure and the involvement of SOM

The soil structure is governed by many factors, and the soil structure is important for the overall production capacity of the soil ecosystem (Hillel 2004). Soil structure is important for the stabilization of SOM and this has important implications for carbon sequestration.

Whereas an environmentalist is interested in how soil structure is involved in stabilization of carbon, the agronomist takes interest in how stabilization of SOM affects soil structure. The soil structure has implications for: soil density, water conducting properties (and thus water holding capacity and infiltration rate), soil aeration, root penetration and nutrient availability (Hillel 2004). The soil structure is made up from soil aggregates which may conceptually be divided into microaggregates (< 30 μm) and macroaggregates (> 30 μm), consisting of clusters of microaggregates (Brady & Weil 2004).

Soil aggregate dynamics

Six et al. (2000) proposed a conceptual model for the formation and turnover of soil aggregates. They suggest that macroaggregates are formed as POM becomes enclosed within mineral particles by mechanisms mentioned above. As microbial activity modifies the organic core of the macroaggregate, new and smaller (micro) aggregates are formed within the macroaggregates. If the macroaggregates are destroyed, free microaggregates are released, and these might again merge to form new macroaggregates. The findings of Gale et al. (2000a) strongly support that such a relationship exists. The turnover time for carbon contained within aggregates has been estimated to be 412 years and 140 years for micro- and macroaggregates, respectively (Six et al. 2002).

The ecological performance of the structure depends on the stability of macroaggregates. Gale et al. (2000b) found that the stability of macroaggregates constantly increased 180 days after addition of litter, before a decline was observed. It was also found that roots contributed to a larger extent than shoots, to the genesis of stable macroaggregates (Gale et al. 2000b). This indicates that a constant replenishment of litter is a prerequisite for the maintenance of a functional soil structure.

Other physiochemical properties of SOM

Micro nutrients often have a low solubility in the soil solution, and may thus potentially become a limiting factor for plant growth. Organic compounds in the soil matrix may combine with micro minerals and form organo-mineral complexes called chelates, which increases the solubility of the micro nutrient in question. The chelated minerals may in turn be utilized by plants through interactions with the chelates (Havlin et al. 2005).

Because of its complex structure, humus has the ability to bind toxic compounds originating from pesticides applied in agriculture or from other sources. However, humus

may also act as a source for toxic compounds (Magdoff & Weil 2004). Facilitated by the black color of humus, the association of humus with mineral soil lowers the total albedo of the soil, and this has implications for the temperature dynamics of the soil (Brady & Weil 2004). Compared with the mineral fraction, SOM has a low density, and the composite effect of mixing mineral soil and SOM is a reduced soil density (Havlin et al. 2005). The pH buffering capacity of soils is enhanced by SOM (Magdoff & Bartlett 1985).

Erosion

Phosphorous (P) binds tightly to clay particles (Havlin et al. 2005). Erosion causes loss of soil particles and associated nutrients like phosphorous, and this has implications both from an agronomical and an environmental point of view. The farmer looses valuable soil and the environment suffers from eutrophication of waterways. Due to the positive effect of SOM on the soil structure and other soil properties, soils prone to erosion may benefit from a high level of SOM. A good soil structure throughout the soil profile increases the infiltration rate. A soil surface with stable aggregates reduces the amount of aggregate destruction upon exposure of rain drops and thus reduces the amount of free particles that are prone to become carried away by runoff water. The same effect may be attributed to surface mulch of plant residues (Havlin et al. 2005). Bresson et al. (2001) found that a combination of good infiltration rate and stable surface aggregates due to added municipal waste had profound positive effect on erosion in unstable soils that were initially low in SOM.

Organic fertilizers in agriculture

In organic agriculture, application of easy soluble plant nutrients is prohibited. The soil fertility of organic agriculture therefore depends on proper soil management and on utilizing knowledge about ecological processes in the soil.

Green manure is defined as “plant material incorporated with the soil while green, or soon after maturity, for improving the soil” (Brady & Weil 2004). Although the green manure does not have to be “incorporated with the soil” the definition pretty much covers the application. Leguminous plants live in mutual symbiosis with nitrogen fixating bacteria, where the plant houses the bacteria in anoxic conditions in nodules in the roots. The plant provides the bacteria with photosynthetic assimilated carbon compounds, whereas the

bacteria use the energy to assimilate atmospheric N (Havlin et al. 2005). The overall process provides the soil with reactive nitrogen which in turn fuels the whole food web.

In order for the leguminous nitrogen to become available for growing crops, the added green manure has to be mineralized trough a complex series of events. Even though the C/N relationship is favorable for mineralization, the challenge in application of green manure is to ensure that the mineralization happens at the time when it is needed by the growing crop (Sanchez et al. 2001). If the green manure is mineralized in absence of a growing crop, nitrogen will be prone to leaching as nitrate (NO_3^-). It has been demonstrated that some mineralization may take place also under low temperature (Andersen & Jensen 2001) and it may therefore be questioned whether application of green manure is positive in terms of resource management and pollution of the environment.

Animal manure has passed through the digestive tract of animals and both the structure and chemical properties is different from that of the feed (Persson & Kirchmann 1994). Some of the nitrogen that is absorbed and integrated into the animals is excreted as urea in urine. Urea is then converted to ammonium (NH_4^+) by the action of the enzyme urease, produced by bacteria (ammonification), some of which will volatilize as ammonia (NH_3) during storage and application. The ammonium may then be converted to nitrate (NO_3^-) by yet other bacteria through the process of nitrification (Havlin et al. 2005). Thus, at the time of application on the field, animal manure also contains mineralized plant available nitrogen and can therefore be considered to be a more predictable source of nitrogen than green manure.

Compost is already partly degraded when it is applied to the field. The C/N ratio of the compost depends on the substrate, and the mineralization rate is thus difficult to predict (Hadas et al. 1996). The nitrogen use efficiency (NUE) related to the application of animal manure is somewhat lower than for inorganic fertilizers (Olesen et al. 2009) and the NUE at the farm level is lowered as the application rate of animal manure increases (Bleken et al. 2005).

Effect of agronomic practices on SOM dynamics

Effects of tillage

The effect of different plowing depths is well documented in a long term trial from 1940 to 1990 on a loamy soil in Ås, Norway, reported by Børresen & Njøs (1994). The different plowing depths applied were 12, 18 and 24 cm. The shallow plowing depth (12 cm) had significantly higher amount of SOM in the upper 12 cm compared to the deep plowing depth (24 cm). However, the total SOM throughout the 0 - 40 cm profile was not significantly different between the different treatments.

In a long term trial started in 1976 on a clay soil at Tune in Norway, a comparison between plowing with a moldboard plough to 25 cm depth and reduced tillage by a rotary cultivator to 10 cm depth was made. The crops grown were cereals and the soil was hence tilled annually. The reduced tillage resulted in a buildup of SOM in the top 5 cm and a small increase was observed in the 10 - 20 cm profile compared with plowing (Børresen & Njøs 1993).

D'Haene et al. (2009) found that reduced tillage over a 20 year period increased the percentage of SOM in the upper soil profile, but did not change the total carbon stock. Much of the SOM in the top layer of reduced tillage soils was found to be free or partly physically protected POM that is prone to mineralization. In fact, during a laboratory incubation trial the mineralization rate of the soil from the reduced tillage treatment was 1.5 to 3 times that of the soil from conventional tillage. In a similar study Kader et al. (2010) compared OM content in different soil fractions between conventional tillage and reduced tillage in Belgium. They also found that the total SOM content increased slightly under the reduced tillage regime, but that most of the observed increase in SOM was associated with the POM fraction, leading them to conclude that the potential SOM gain by the increased level of unprotected POM could be offset by the risk of a higher mineralization if the soil was to be disturbed.

In a long term trial started in 1962, moldboard plowing, reduced tillage and no tillage was applied in a monoculture with maize (*Zea mays*) on a silt-loam in Ohio. In 2004 a comparison of different soil properties between the different tillage regimes were made. The no-till treatment had nearly twice as much SOM as compared to moldboard plowing,

but no significant difference were found between moldboard plowing and reduced tillage. The SOM content was significantly higher in the 0 to 15 cm profile in the no tillage treatment compared to the other treatments, but no difference was found in the 15 to 30 cm profile between the different treatments. Based on natural abundance $\delta^{13}C$ values it was found that maize derived SOM vas confined to the upper 15 cm in the no till treatment, whereas maize derived SOM was distributed throughout the 0 to 30 cm profile in the tilled soil. It was also found that the mineralization of both new and old SOM were greater in the tillage treatments compared with the no tillage treatment. The soil temperature in the upper 5 cm were found to be higher in both tillage treatments compared with the untilled soil, and the temperature increment is attributed to the insolating effect of the crop residue surface mulch in the no till treatment. The lower mineralization rate in the no tillage treatment is by the authors concluded to be related to both the higher temperature in the tilled soil and to the poorer incorporation of litter in the no tillage treatment, leading to less interaction between the soil and litter and thus retarded mineralization (Ussiri & Lal 2009). In a short term experiment based on measurement of CO_2 fluxes from arable soil with and without tillage it has on the contrary been shown that the CO_2 flux was greater with the no till regime. The increased mineralization is attributed to the increased moisture content associated with the no tillage treatment (Hendrix et al. 1988).

A strong correlation between soil temperature and soil respiration in a barley field has been shown (Rochette et al. 1992), and some of the decreased mineralization observed by Ussiri & Lal (2009) in the no tillage treatment may be attributed to the lower soil temperature recorded in the no tillage soil. Ussiri & Lal (2009) argues that the reduced interaction between litter and soil (i.e. soil contact) has a retarding effect on decomposition of litter. However, this is not in line with the findings of Breland (1994), who concluded that a heterogeneous distribution of litter enhances mineralization of litter, compared with evenly distributed litter as accomplished by some types of tillage. Heterogeneous distribution in soil are not direct analogous with heterogeneous distribution on the soil surface and Buyanovsky and Wagner (1987) found that the mineralization rate increased when straw was incorporated into the soil as compared to surface mulch. However, the effect was attributed to limiting moisture conditions on the soil surface and not to limited contact with soil. Thus, it might be expected that

mineralization of litter applied as a surface mulch might be as high as for litter incorporated in soil as long as sufficient moisture is available.

Six et al. (2000) provides a framework for aggregate turnover as presented earlier. Following their model, microaggregates are created around cores of POM within macroaggregates. The microaggregates hence needs time to develop, and any disturbance will accordingly abort the formation of microaggregates. Based on their data they predict that the macroaggregate turnover is twice as fast with conventional tillage as compared to no tillage (Six et al. 2000). Based on the dynamic process of microaggregate genesis it could be postulated that tillage, or any soil disturbance, will lead to exposure of unprotected POM, thus leaving it prone to mineralization.

Little attention is given by Ussiri & Lal (2009) to physical protection of SOM, even though the physical protection may have profound effect on the observed increase in SOM content under no-till (Six et al. 2000). In a laboratory incubation trial where decomposition routes of ^{14}C labeled roots and shoots under simulated no-till conditions were compared, it was found that after one year, roots had contributed significantly more both to the POM fraction and to the silt/clay associated fraction than did shoots (Gale & Cambardella 2000). Both D'Haene et al. (2009) and Kader et al. (2010) found an increase of POM in the upper soil profile under reduced tillage and the results of Gale & Cambardella (2000) may indicate that the bulk of this may be of root origin.

So why is it than, is the differences between full depth tillage (i.e. plowing) and reduced tillage so small? Two possible, not mutually exclusive, factors may explain the minor difference between different tillage regimes. Ussiri & Lal (2009) argues that any tillage, regardless of depth and extent, will expose the soil surface and thereby increase the temperature with enhanced mineralization as a result. The other possible explanation is that shallow tillage disrupts the important upper soil profile in which the vulnerable microaggregates with cores of root derived SOM are developing within macroaggregates and thus retards the physical protecting of SOM.

Independent of the cause of the observed differences between SOM under no tillage compared to various tillage regimes, it may be claimed that tillage in terms of carbon sequestration is an "all or nothing phenomenon", that is, tillage of any extent has equally

impact on the total SOC content. However, asides from the limited effect of reduced tillage on SOM buildup, the many other soil properties facilitated by reduced tillage, as pointed out by Børresen & Njøs (1993), and the positive effect of a high SOM content in the upper soil profile on erosion (Bresson et al. 2001), should also be considered when evaluating reduced tillage.

As reported by D´Haene et al. (2009), incubated soil from reduced tillage was decomposed 1.3 to 3 times faster compared to soil from conventionally tilled soil. The same effect may also be expected to be true for soil from a long term no tillage regime. Long term sequestration of SOC by the means of reduced- or no-tillage may therefore be rather hazardous because of the risk of a "backfire" effect in the form of increased emissions of CO_2 upon converting back to conventional tillage in the future.

Effects of cropping regimes

Continuously arable cropping implies that the crops grown are annuals or biannual. The crops grown under such cropping regimes cannot afford to allocate their limited resources to develop a wide ranging root network. However, perennials rely on an extensive root network for their prolonged survival, and thus allocate much of their resources to root development. In a review of the root/shoot ratio in the worlds biomes it has been found that the average root/shoot ratio of crops are 0.15 whereas temperate grasslands have a root/shoot ratio of 1.4 (Jackson et al. 1996). Prairie grass may serve as an extreme example having a root/shoot ratio of 13 (Oades 1988). It should thus be expected both from the observed larger contribution of roots to the SOC pool and the fact that cultivation of perennial grasses reduces the need for tillage, that continuous grass cultivation or the inclusion of perennial grass in the crop rotation have a positive effect on SOC content.

In a long term study, (Glover et al. 2010) found that fields in USA with continuous (harvested) grass cultivation had significantly higher levels of SOM compared to fields were winter wheat (*Triticum aestivum*) had been grown for some 75 years. The same results was found to be true in Canada, were fields with perennial grasses and legumes (forage) were found to have significantly larger SOM content than fields under arable cropping with cereals (Carter 1998). In Sweden, a comparison between a 6 year arable

cropping rotation including one year with black fallow and two different 6 year crop rotation including two years of forage, the latter two treatments were found to increase the SOC compared to the first treatment. The treatments including perennials were also found to increase the grain production in the overall crop rotation (Persson et al. 2008). Black fallow has a profound negative effect on SOM level (Mikhailova et al. 2000), and this may have favored the crop rotation including forage.

Crop residues may either be transported away from the field or be returned to the field. If shoots are important in contributing to SOC maintenance then returning the crop residues on the soil should lead to a higher SOC content compared to where the crop residues have been removed. In a long term experiment it has been shown that the removal of spring wheat straw only had a slight effect on the SOC content as compared with where the crop residues were returned to the field (Campbell et al. 1991). Further it has been shown that long term removal of grass did not alter the SOM content as compared with natural grassland that had not been harvested (Mikhailova et al. 2000).

In a long term experiment in Sweden (35 years) it has been demonstrated that amendment of straw and green manure (grass) had a small positive effect on SOC (Persson & Kirchmann 1994). In two long term experiments at Ås and Øsaker, lasting 30- and 20 years respectively, it was found that about 7 % of the added carbon in straw was retained in SOC (Uhlen 1991).

Lal and Kimble (1997) emphasizes the importance of production on SOC level. It should therefore be expected that an increase in the amount of litter added to the soil should be positively correlated with the SOC content. However, several long term studies where different N fertilizing levels have been compared and where the crop residues have been left on in the field, indicates that increases in N application (i.e. increases in production of litter) does not (Bertora et al. 2009; Lopez-Bellido et al. 2010), or only slightly (Børresen & Njøs 1994; Campbell et al. 1991; Clapp et al. 2000; Jenkinson 1990) affect the SOC content. However, Uhlen (1991) found variable, yet positive effect of nitrogen fertilizer application on SOC.

To this date the best documentation of the effect of fertilization on SOM level is that of the long term experiments at Rothamsted reported by Jenkinson (1990). At the continuous

wheat cultivation field (Broadbalk) where straw was returned to the field, the application of mineral fertilizer only had a slight impact on the SOM level after 140 years of cultivation as compared with the unfertilized fields (they both remained fairly constant over the whole period). This experiment also documents that it is possible to persist continuous arable cropping over a long period without depleting the SOM in the soil and in addition harvest ever increasing yield. It has been shown that as the nitrogen fertilization level rises, the root/shoot ratio diminishes (i.e. most of the additional growth is allocated to the shoots) (Li et al. 2009). This may be one explanation for the minor correlation between production and SOC content and may be taken as a strong indication towards that shoots play a limited role in SOC genesis.

The positive effect of perennial grasses on SOM content is also documented in another long term experiment at Rothamsted. At one of the experimental plots (Agdell) a part of the plot was converted to permanent grassland in 1958 whereas the rest was kept under continuous arable cropping. In 1970 the SOM content of the grassland and the arable soil was 2.4 percent and 1.5 percent respectively (Leigh & Johnston 1994).

Given that roots are so important in contributing to the SOM content of soils, it can be expected that an extensive and deep rooting system has an enhancing effect on carbon sequestration. It has been shown by Carter and Gregorich (2010) that deep rooted tall fescue (*Lolium aarundinaceum*) increased the SOC content by 23 percent after 7 years on a sandy soil in Canada.

Effect of animal manure application

Given the partly decomposed (i.e. more recalcitrant) nature of animal excreta as opposed to the forage from which it derived, it would be expected that amending the field with animal manure would have an enhancing effect on SOM level. This has been confirmed by many studies (Bertora et al. 2009; Jenkinson 1990; Moller 2009; Paustian et al. 1997; Persson & Kirchmann 1994). Magdoff & Weil (2004) reports that the effect of manure application on SOM level is highest the first 10 years of application, and that the effect is also expected to be greatest in soils that are initially low in SOM content. However, from the long term continuous wheat experiment at Rothamsted (Broadbalk) it has been shown that after 140 years of applying 35 Mg manure ha^{-1} $year^{-1}$ the SOM content tripled, and

the effect of manure application was almost equal over the whole period (Jenkinson 1990). Uhlen (1991) found that 17 % of the carbon in farm manure was retained in SOC after 30 years, at Ås, Norway. This is more than twice the amount found for straw. Persson & Kirchmann (1994) found the same relationship in Sweden.

From an incubation experiment with animal manure subjected to different types of treatments (pig slurry (PS), cattle slurry (CS), farmyard cattle manure (FYM) and composted farmyard cattle manure (CFYM)) where fractioned into six different fractions, it was found that the composition of the natural detergent fiber (NDF) was an important factor in determining the carbon and nitrogen mineralization dynamics of the different types of manure. The overall C/N ratio was similar for the PS and CFYM (around 13), but were somewhat higher in the CS and FYM (19.7 and 28.9 respectively). However, the C/N ratio of the NDF fraction were considerably lower in the CFYM (15.3) compared with FYM, CS and PS (36.7, 56.6 and 55.2 respectively). The lower C/N ratio of the CFYM is attributed to the mineralization of cellulose and hemi-cellulose (e.g. the easily degradable fractions of NDF) that had occurred during the composting process leaving the NDF highly enriched with recalcitrant lignin. The recalcitrant nature of the CFYM was manifested in a considerable lower total mineralization of carbon measured as respired CO_2. FYM had, due to the bedding materials, a high C/N ratio, and thus resulted in a net immobilization of nitrogen (Morvan & Nicolardot 2009).

Carbon sequestration in agricultural soils

The CO_2 concentration in the atmosphere has been constantly increasing since the onset of the industrial revolution and to this date an increase in temperature of 0.8 °C has been attributed to the 77 ppm increase in atmospheric CO_2 concentration in the 21th century (Bala et al. 2005). Of the increase in atmospheric CO_2 concentration, 20 percent has been estimated to originate from soils (Paustian et al. 1997). It has been estimated that the total organic carbon stock held within soils are somewhat in the order of 2400 Pg C, being the second largest C pool after oceans (39000 Pg), leaving the atmosphere as the third largest C pool (750 Pg C). This includes all organic carbon in soils to a depth of 200 cm excluding carbon held in litter and charcoal. Of this carbon some 1500 Pg C is held in the upper 100 cm and 700 Pg C is held in the upper 30 cm (Batjes 1996). In addition vegetation, accounts for about 600 Pg C. The estimated amount of carbon held within agricultural soils prior to cultivation is about 222 Pg C. It has further been estimated that

at this date soil under cultivation contains some 168 Pg C, and the loss of about 54 Pg C is attributed to the cultivation history of the soils. Of this loss, 43 Pg C is expected to have originated from upland soils, leaving only 11 Pg C of cultivated wet-land origin (Paustian et al. 1997).

Paustian et al. (1997) defines carbon sequestration as "any persistent net increase in organic C storage". An increase in the SOC content of soils is unlikely to be permanent unless the input of OM is kept at a high level, and this must be, as also commented by Paustian et al. (1997), taken into account when carbon sequestration is considered. Given the exponential nature of SOC mineralization, it should be expected that the potential rate of carbon loss from agricultural soils today is lower than at a pre-cultivated situation. This also implies that today the potential for increasing the SOC levels of agricultural soil without massive inputs of novel OM is great. Paustian et al. (1997) argues that the native condition (i.e. prior to cultivation) serves as a upper limit for the carbon holding capacity of agricultural soils, from which it follows that the 54 Pg lost upon cultivation is the theoretical potential for carbon sequestration. However, as pointed out by Six et al. (2001) some cultivation regimes results in larger SOC levels compared to the native state, and they states, that SOC level prior to cultivation do not have to be the best way to define the potential upper level for carbon sequestration. Given that the potential of carbon sequestration in agricultural soils are some 54 Pg carbon, its capacity to act as a major sink for the ever rising atmospheric CO_2 levels seems rather slim, and this calls for a novel approach.

Biochar: a potentially major CO_2 sink or a technocratic illusion?

Natural black carbon is C-rich organic material derived from incomplete combustion of vegetation, and it may persist in soils up to 10,000 years (Nguyen et al. 2009). By pyrolysis (i.e. anaerobic combustion) of organic materials, energy in the form of gas and fuel is extracted, leaving biochar as a waste product (Gaskin et al. 2008). As an example pyrolysis of rapeseed cake have been shown to generate: 27.4 % biochar, 59 % bio-oil, and 12.8 % gas (Ozcimen & Karaosmanoglu 2004). However, all sorts of organic materials can be transformed into biochar e.g. paper mill waste (Van Zwieten et al. 2010). Some of the claimed effect of amending soils with biochar is reduced acidity, increased CEC and enhanced nitrogen retention (Gaskin et al. 2008). It has also been shown that biochar has beneficial effects on mycorrhizal interactions (Warnock et al. 2007). In a

study of the microbiological impacts of biochar it was found that biochar boosted the mineralization of native SOC (i.e. priming effect), as compared to soils without biochar, but that the carbon added by the biochar by far offsets this loss (Steinbeiss et al. 2009).

In theory, the gain of both fuel and of high quality soil amender, that at the same time sequesters carbon seems like the ultimate solution for managing crop residues. Though biochar may be promising in terms of carbon sequestration and soil properties, the research in this area is still in the infant stage and more research is needed before the agricultural soils of the world are heavily amended with this, so far, little known material.

SOC dynamics under elevated CO_2 conditions

There is an increasing awareness of the possible impact an elevated atmospheric CO_2 level may have on the performance of agroecosystems (Martens et al. 2009). In a review on the possible effects of an elevated atmospheric CO_2 level, higher yields (espessially for C_3 plant species) and higher C/N ratio are amongst the expected outcomes and it is concluded that a rise in atmospheric CO_2 concentration may result in higher retention of carbon in soil and this is partly explained by immobilization of nitrogen due to higher C/N ratio (Torbert et al. 2000). In a field experiment crops were grown under ambient (360 ppm) CO_2 level and under free air carbon dioxide enrichment (FACE) at 550 ppm CO_2. Plants were pulse labeled with ^{14}C carbon and the fate of the assimilated carbon was investigated. No difference in yields was found, but the mineralization rate under the FACE treatment was slower than under ambient CO_2. Most of the sequestered carbon (80 %) was found to be associated with the clay fraction (Martens et al. 2009), indicating that physical protection could have been involved.

The possible elevated temperature associated with increased atmospheric CO_2 concentration may also affect SOM dynamics. Given the effect of temperature on respiration rate, a more rapid mineralization is expected if the temperature rises. Some evidence suggests that the temperature sensitivity of old (more or less recalcitrant) SOM is higher than for litter, and the findings are attributed to the higher activation energy needed for oxidation of recalcitrant material (Karhu et al. 2010). Thus it appears that elevated temperature may increase the mineralization of old SOM more than what can be expected from a Q_{10} factor approach. It has also been suggested that temperature perturbations may result in higher mineralization than what can be predicted by the mean

temperature (Smith et al. 2003). If climate change results in periods with extreme upper temperatures this may affect the SOM dynamic.

On the one hand, increased atmospheric CO_2 levels might promote carbon sequestration, on the other hand there are factors that equalize the equation and the total outcome is difficult to predict. A higher C/N ratio might promote carbon sequestration, however, one thing is certain, a high C/N ratio reflects a dilution of nutrients and thus food and feed of less quality, and care, in terms of fertilizing, must be taken to avoid this.

Overall evaluation of the agricultural carbon cycle

In order to understand how agriculture interacts with the SOM dynamics and hence the atmosphere, and thus influence the global climate, it is of utmost importance to consider the whole carbon dynamic process involved using a systemic approach, or in other words be holistic. By using such an approach, a conceptual model may be synthesized (Figure 2). The model does not illustrate any particular agricultural regime, but rather represents various possible routes for photosynthetic assimilated carbon. It must be emphasized that the field compartment of the model does not have to be continuous, that is, the carbon produced at one location does not have to be returned to the same site. The animal manure that is not fermented in a bio-reactor may or may not be composted before application on the field.

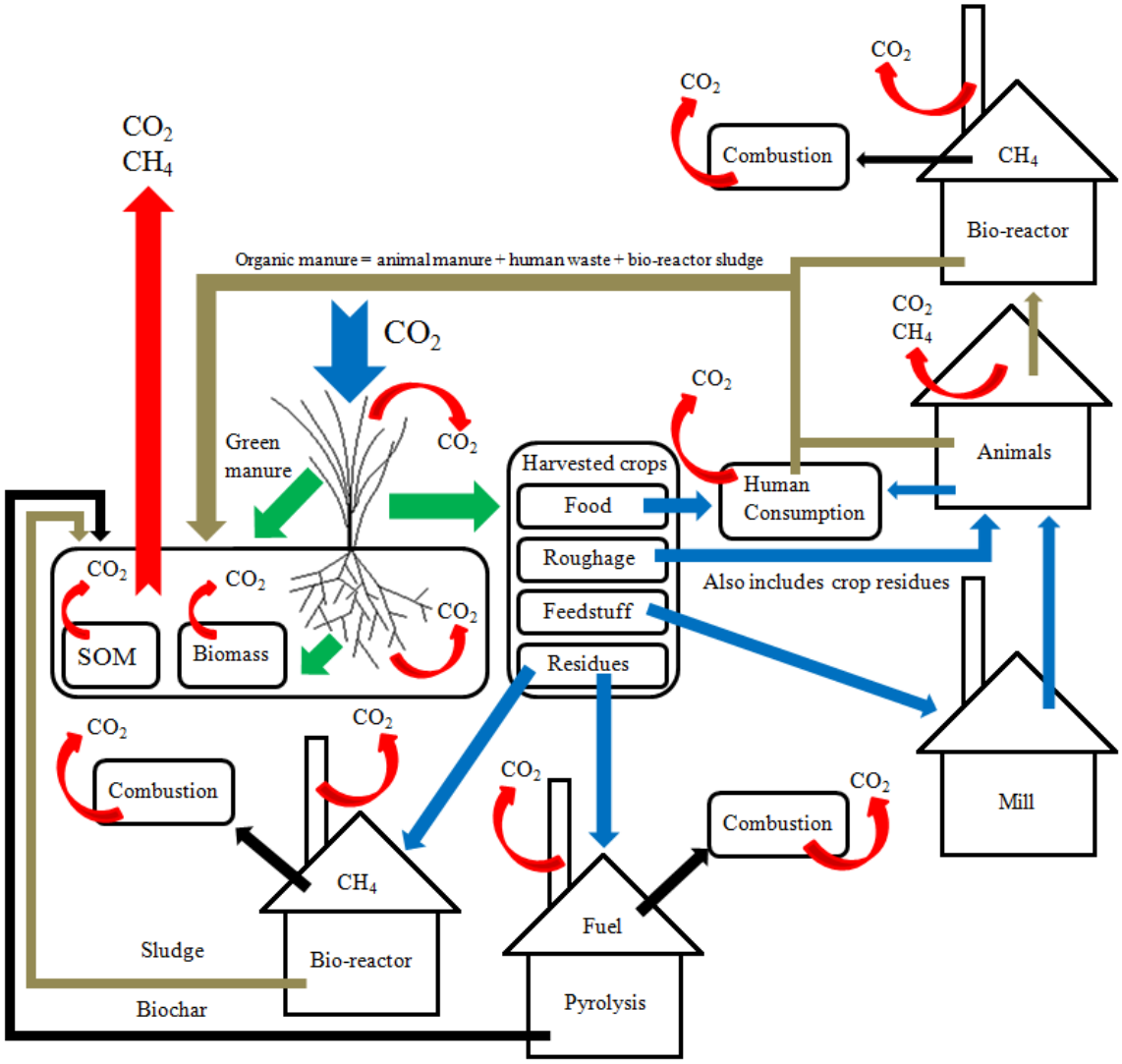

Figure 2. Conceptual model illustrating the fate of photosynthetic assimilated carbon in a agroecosystem. The red arrows represent green house gas emissions.

The model suggests 6 major managing strategies of the crops grown in the field: 1) green manure (GM); 2) fermentation of green manure in a bio-reactor, with production of methane; 3) pyrolysis of the crop residues with the production of fuel and the use of biochar (BC) as a soil amender; 4) feeding animals with forage, crop residues and concentrate feed with the subsequent application of manure as slurry, farm yard manure (FYM) or composted FYM on the field; 5) fermentation of manure in a bio-reactor, with production of methane for energy before application on the field; 6) direct human consumption of food and the subsequent use of human waste as a soil amender.

From a strict agronomical perspective, it is not a goal to increase the SOM content to an infinite high level. A critical level of 3 % SOM should be avoided in order to sustain the agronomical benefits associated with SOM. However, as SOM content exceeds 6 % the additional beneficial effects facilitated by SOM diminish (Riley & Bakkegård 2006). Thus, it is contradicting goals between the farmers, who want to benefit from a sufficient SOM level, and the environmentalist who wishes to maximize the level of SOC.

The nitrogen cycle is tightly connected to the carbon cycle. Different agricultural regimes have different impact on the nitrogen dynamics. In order to accomplish an overall evaluation of the joint impact of different agronomic practices on the carbon and nitrogen dynamics both these factors must be taken into account. Møller (2009) evaluated the effect of different manure strategies on both the humus balance (i.e. the change of humus content) and the nitrogen use efficiency (NUE). Two different farming systems were evaluated, one including animals and one stockless system. In the stockless system the manure was applied either as green manure, fermented crop residues or fermented cop residues with off field additions. The animal manure was applied as either FYM, slurry, digested slurry (digested refers to fermentation in a bio-reactor), digested slurry + crop residues and digested slurry + crop residues + off field additions. The NUE was calculated to be higher for the stockless system compared to the animal husbandry system. The fermentation of crop residues in the stockless system reduced the NUE and this negative effect was further increased when off-field additions were added to the system and fermented. The NUE was similar between FYM and slurry, however the nitrogen lost as ammonia (NH_3) was lowest with FYM application. Fermented slurry did not alter the NUE compared to slurry, but fermented slurry + crop residues increased the NUE slightly. As with the stockless system, the NUE decreased when additional crop residues were added in the fermented slurry + crop residues. The calculated biological nitrogen fixation (BNF) was lower where the green manure was left as mulch compared to where it was harvested. Based on the amounts of returned organic carbon, it was calculated that digested slurry + crop residues + external inputs resulted in the highest humus balance. The lowest gain in humus balance was calculated for fermented crop residues. Both slurry and digested slurry scored high on humus balance, with a slight lower value for FYM. All the stockless systems had substantially lower humus balance values compared to the animal manure systems. However, after 3 years with the different treatments no change in either total nitrogen content or total SOC content could be

measured (Møler 2009). It seems like stockless farming systems offers the best solution in terms of NUE, while farming systems that includes animals gives the highest SOC content, thus underlining that no farming system is perfect.

Bertora et al. (2009) compared the effect of FYM and liquid slurry on SOM content. The FYM regime used maize residues as bedding whereas the maize residues in the slurry regime were left on the ground. It was calculated that after 20 years of application the FYM and the slurry application (where all the manure originated from same field to which it was applied) would result in a 20 Mg ha^{-1} and 16 Mg ha^{-1} increase in carbon content, respectively.

The use of human waste as fertilizer is yet another way for carbon to reenter the field and Bresson et al (2001) found a positive effect on SOM level by amending human waste to the field. In many cases application of human waste to fields is difficult. In addition there are some hazards e.g. heavy metals and pathogens associated with human waste. However, these obstacles may be overcome by proper treatment of the waste. One promising way to handle human waste could be to ferment human waste together with harvested green manure or animal manure and thus both create energy and a high quality soil amender.

Because reactive nitrogen is labile, the management of nitrogen is a question of controlling the processes that leads to loss of nitrogen (i.e. leaching, volatilization). Least control is gained by applying green manure. Some control is gained by storing the nitrogen as animal manure but the losses associated with application may be substantial. Fermentation of green manure and crop residues is yet another way to control the nitrogen, and in addition it serves as a source of energy. This way of managing organic manure may be promising given that the farming system is a stockless one. In terms of controlling the nitrogen flow within the farming system, application of mineral fertilizer is probably most effective.

One of the implicit goals of agriculture is after my opinion to render as much of the photosynthetic assimilated carbon as possible suitable to meet the nutrient demands of humans without compromising the sustainability of agriculture. The ultimate agricultural regime would thus, in a short perspective, be to grow crops that can be consumed directly

by humans e.g. cereals. However, such a cropping regime relies totally on the application of artificially fixated nitrogen, that comes at a high energy cost and the sustainability of this strategy might be disputed. One way of adding nitrogen to such a system could be to exploit the BNF facilitated by leguminous crops applied as green manure. This approach implies that a substantial part of the crops grown in a rotation is applied directly on the field and is neither used for animal feed nor human consumption, and may therefore be considered to be unproductive in terms of energy. I would therefore stress that in terms of energy management, it is better to unleash the energy stored within the green manure within a producing animal, instead of letting the energy go to waste in the field. However, this point of view can be challenged, because the same energy might also become useful if the green manure is fermented.

Application of animal manure is effective in terms of carbon sequestering. However, because one unit of methane is equal to 23 units of CO_2 (Smith et al. 2003), the methane emissions associated with ruminant animals may offset this positive effect. The methane emissions are not only negative in terms of global climate, but in addition they represent energy loss for the farmer. Therefore it is not certain whether the inclusion of animals in the farming system offers the best overall solution in terms of greenhouse gas emissions.

The SOM content situation in Norway

In the nineteen fifties- and sixties a canalization policy was launched by the agricultural authorities in Norway. Through economic incentives, animal husbandry was confined to the marginal agricultural areas (i.e. mountains, valleys etc.) and the arable cropping soon became concentrated on the more productive soils (e.g. southeast Norway) (Puschmann et al. 2004). The spatial displacement of animal production and arable cropping is negative in two ways. Animal feed is displaced and concentrated in the animal production region and potentially leads to environmental problems related to the surplus of animal manure, and it can lead to depletion of SOM in the arable cropping region.

Riley & Bakkegård (2006) found that the SOM content in southeast Norway have declined since the onset of measurements in 1952 until today, and the causes of the decline are related to changes in cultivation practice and possible to the steadily increase in average temperature. Bleken et al. (2005) found that problems of nitrogen pollution are worsened by increased external inputs to the animal farming system. This calls for an

integration of arable cropping and animal husbandry. However, this is difficult given the current canalization policy. This underlines the complex nature of agroecosystems as being governed by socioeconomic relationships, and a quick fix for the decreasing SOM in south eastern Norway does not seem to be realistic. An ad hoc solution to this issue might be to ship animal manure across the segregated regions. Though the channelization policy might seem suboptimal in terms of organic matter distribution, the geography of Norway in some ways makes segregation of regions ecologically sound. This is because many of the regions of Norway are unsuited for arable cropping and relies upon ruminant animals to utilize the grass that is grown. Animals in all regions may, largely dependent of the quantities of imported feedstuffs, lead to overproduction of animal products. A situation were animal husbandry is confined to regions unsuitable for arable cropping and the introduction of fermentation of green manure and crop residues in the arable regions may therefore be a adequate solution to the issue.

Review conclusion

Based on this review it may be concluded that from an agronomical perspective, the optimal managing system includes (i) a six- to eight year crop rotation, including two- to three years of ley including leguminous plants like clover, harvested for forage and managed with as little tillage as possible (ii) a crop rotation that provides crop residues suitable as bedding material (iii) application of animal manure as FYM, CFYM or fermented manure with the subsequent use of methane as a energy source.

Because of the negative impact of methane emissions and the loss of nitrogen as volatilized NH_3 related to animal husbandry, the best suited strategy in terms of optimizing the carbon sequestration, reducing the emissions of other greenhouse gasses and utilize the energy stored within the crops, a regime that includes the following components is recommended: (i) a six- to eight year crop rotation, including two- to three years of green manure, that is managed with as little tillage as possible (ii) application of fermented green manure and fermented crop residues with the subsequent use of methane as a energy source (iii) no new cultivation of wetlands (iiii) amending soil with biochar.

Returning crop residues to the field might have a positive effect on SOM. However, given the resource that crop residues can be when used for other purposes, compared with the minor effect it has on SOM content, I would recommend that the crop residues should be utilized for purposes like; animal feed, bedding material, bio-energy by burning, pyrolysis (energy + biochar), or fermentation (energy + manure). In soils prone to erosion, returning crop residues to the soil should be considered.

Method

Field description

Cultivation and labeling of plants was conducted at the field Østre Voll (59º 40′N, 10º 47′E – 75 meters above sea level) at Voll farm located in Ås in Akershus County, Norway. The soil is described by Bakken et al. (2006) as naturally poorly drained clay/silty clay loam. According to soil samples taken in 2009 the soil contains 2.1 % total C (1.74% organic C). The average annual precipitation (1961-1990 normal values) is 785 mm. The average number of days with temperatures above 5 ºC (i.e. the growing season) is 186 (Bakken et al. 2006). During the growing season 2009 (May-August) 403 mm precipitation and 14.25 ºC as mean temperature was registered at Ås meteorological station (Hansen & Grimenes 2009).

Until 1993, the field was used for conventional cultivation of various arable crops (e.g. potatoes and cereals). Application of animal manure was not part of the cultivation regime. In 1993 the field was converted to organic cultivation and was annually amended with about 25 tons animal manure ha^{-1} (Sveistrup et al. 1997) however the soil has not been amended with animal manure the last few years. In 2008 a field trial was established (BYGGRO) with improved management of green manure for cereals in stockless organic agriculture as the overall objective. In May 2008 the entire field was sown with barley (*Hordeum vulgare* L.) and on the bulk of the plots the barley was undersawn with a green manure mix consisting of: 35 % perennial ryegrass (*Lolium perenne* L., cv Napoleon), 35 % meadow fescue (*Festuca pratensis* L., cv Fure) 10 % timothy (*Phleum pratense* L., cv Grinstad), and 20 % red clover (*Trifolium pratense* L., cv Nordia). White clover (*Trifolium repens* L.) from the soil seed bank contributed to the green manure. After tilling with a rotary cultivator, the plots that were not undersawn with green manure the previous year were sawn with oat (*Avena sativa* L., cv Gere) on 5 May 2009.

For the labeling with ^{13}C of green manure and oat, 8 microplots (Mp) were established, of which 4 were on each category of plant cover respectively. In addition 12 microplots were established on oat plots and these were to serve as control in terms of $\delta^{13}C$ ‰ values and provide soil for the incubation trial.

The microplots were divided from the surrounding field by an aluminum frame that was forced 15 cm into the soil. The microplots used for labeling with ^{13}C and the unlabeled microplots measured 91 x 91 cm and 91.5 x 91.5 cm respectively.

Field labeling with $^{13}CO_2$

Field management

Due to the management history of the field, it was presumed that the soil was depleted in readily plant available nitrogen. Therefore, nitrogen, was applied as ammonium-nitrate (NH_4NO_3) in water solution on the microplots where oats were grown (Appendix 1).
In June the recorded precipitation was only 28 mm (Hansen & Grimenes 2009). In order to reduce drought, the whole BYGGRO trial field was irrigated twice. In addition, prior to each labeling at the end of June, each microplot was supplied with 5 liters of water Mp^{-1} (6 l m^{-2}). The 4 liters of ammonium-nitrate solution also contributed to the water supply and the microplots containing oat were thus not supplied with additional water on occasions when fertilizing was concurrent with the labeling dates.

The microplots containing green manure were harvested three times. The first harvest was conducted at 3 June. The intended interval between the respective harvests was at a total temperature sum of 650 °C with 0 °C as basis temperature. The actual temperature sum between harvest 1 and 2, and between harvest 2 and 3 were respectively, 624 °C and 637 °C day-degree above 0 °C. The stubble height was 6 cm at the two first and 0 cm at the third harvest. The harvested crop was botanized into: grass, clover and herbs before drying at 60 °C (Appendix 2). The oats was harvested by cutting to the soil surface level at 14 August. The crop was dried at 60 °C and the ^{13}C labeled crops were threshed. Grain spillage was collected and weighed (Appendix 3).

Labeling with $^{13}CO_2$

The labeling chambers measured 97cm x 97 cm x 100 cm, and consisted of plexiglas attached to a aluminum skeleton. The frames dividing the microplots from the surrounding field were equipped with a pit, which when filled with water sealed off the labeling chamber, thus creating a closed atmosphere. Four labeling chambers were used at the same time in random order. A bracket for attachment of an Erlenmeyer flask was fitted to the ceiling inside the chamber and a tube (fitted with a valve) penetrating the

sealing enabled external excess to add acid to the Erlenmeyer flask. The chamber was fitted with a 12 V electric fan to facilitate adequate air circulation. The CO_2 concentration was measured with an absolute non-dispersive infrared (NDIR) CO_2 gas analyzer (LI-820) connected to a laptop computer. The temperature was monitored by inserting a thermometer inside the chamber. However, due to inadequacy of the method the temperature was merely monitored sporadically.

The relevant amount of $Na_2{}^{13}CO_3$ (99 atom-percent ^{13}C) were weight in, and placed in the Erlenmeyer flask. By adding appropriate amounts of hydrochloric acid (HCl), $^{13}CO_2$ was dissolved by the equation: $Na_2\ {}^{13}CO_3 + 2HCl \rightarrow 2Na^+ + 2Cl^- +{}^{13}\ CO_2 + H_2O$. The partial pressure (pp.) CO_2 at one fixed microplot was continuously monitored and the data were logged on the computer (Figure 3 and 4). When acid was added the pp. CO_2 immediately increased. When the pp. CO_2 stabilized at a low level more CO_2 was added by the means of human expired air. This was done both to relieve the plants from stress and to ensure that as much as possible of the added $^{13}CO_2$ was assimilated by the plants. To reveal potential variation amongst the chambers in terms of pp. CO_2, each chamber were pulse monitored at least once during each labeling. The chambers were removed when the pp. CO_2 stabilized at the lowest achievable level.

Figure 1. Labeling of green manure

Figure 2. Labeling of oats

At some occasions the temperature inside the chambers exceeded 40 °C (maximum recorded 41.7 °C). Under such conditions there was a lag before the pp. CO_2 began to decrease. A strong correlation between standing biomass and CO_2 assimilation were observed for both oats and green manure. The CO_2 assimilation rate decreased as the oats matured. An ideal CO_2 response curve during labeling is shown as Figure 5, though the

curve typically deviated somewhat from this. In total green manure and oats were labeled 12 and 10 times respectively during the course of the entire growing season (Table 1).

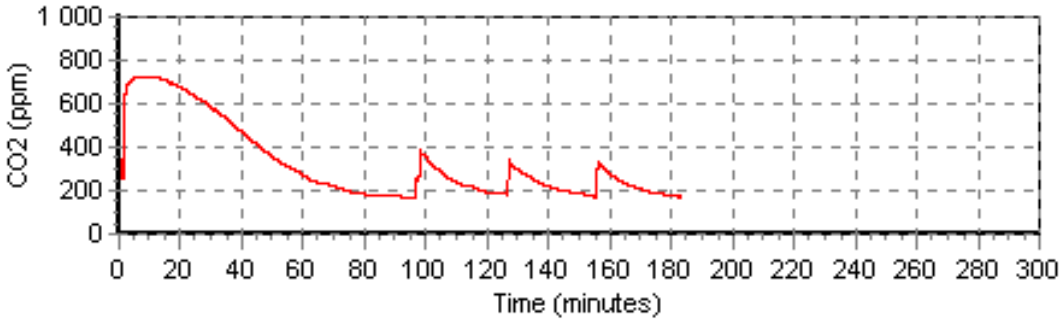

Figure 5. Pp. CO_2 as function of time. Recorded in labeling chamber 1 (Mp 1) during labeling with $^{13}CO_2$ 23 June 2009.

Table 1. Quantity added $Na_2{}^{13}CO_3$ (g Mp^{-1}), $^{13}CO_2$ (l Mp^{-1}) and 4 M HCl (ml Mp^{-1}) duration and observations made during the labeling events. The temperature (°C) was monitored at a fixed site in the shadow. The final ppm CO_2 was the recorded just before the chambers were removed. Mp 1-4 = green manure and Mp 5-8 = oat.

Mp	Date	°C	$Na_2{}^{13}CO_3$ g Mp^{-1}	CO_2 l Mp^{-1}	4 M HCl ml Mp^{-1}	Duration h	Weather	Final ppm CO_2
1-4	20.05.09	15	6.6	1.5	37.5	5.3	Sunny/rain	120
1-4	25.05.09	19.5	11	2.5	62.5	2.3	Sunny	109
1-4	29.05.09	19.5	13.2	3	75	1.6	Sunny	86
1-4	02.06.09	18	11	2.5	62.5	1.8	Sunny	87
1-4	17.06.09	19	4.4	1	25	2.8	Sunny	217
1-4	23.06.09	21	8.8	2	50	3.5	Sunny	171
1-4	01.07.09	30	13.2	3	75	3.2	Sunny	180
1-4	08.07.09	21	15.4	3.5	87.5	3.6	Sunny	122
1-4	13.07.09	19.5	15.4	3.5	87.5	3.6	Sunny/rain	200
1-4	28.07.09	18	6.6	1.5	37.5	5.1	Sunny/rain	450
1-4	05.08.09	20	11	2.5	62.5	2.0	Sunny	80
1-4	12.08.09	19	15.4	3.5	87.5	3.0	Sunny	108
			Σ 132	**Σ 30**	**Σ 750**	**Σ 37.6**		
5-8	03.06.09	12.2	4.4	1	25	5.6	Sunny/cloudy	180
5-8	09.06.09	16	6.6	1.5	37.5	5.0	Sunny/cloudy	130
5-8	16.06.09	15	8.8	2	50	2.6	Sunny	144
5-8	22.06.09	20	8.8	2	50	2.3	Sunny	110
5-8	29.06.09	27	13.2	3	75	3.2	Sunny	125
5-8	07.07.09	16	15.4	3.5	87.5	5.0	Overcast	150
5-8	13.07.09	18	11	2.5	75	5.0	Overcast/rain	>2000
5-8	14.07.09	18	8.8	2	62.5	2.1	Sunny	118
5-8	28.07.09	20	11	2.5	75	3.0	Sunny	200
5-8	05.08.09	20	6.6	1.5	37.5	3.8	Sunny	285
			Σ 94.6	**Σ 21.5**	**Σ 575**	**Σ 37.4**		

Preparation for *in situ* incubation

The purpose of the labeling of oats and green manure was to provide ^{13}C enriched substrate for both *in situ* incubation and *ex situ* incubation in laboratory. On the 25 August 2009 the 17 cm top soil from all microplots was removed, plant material was added (for some of the treatments), mixed (tilled) and returned to the respective microplots. Soil bulk samples containing 2 kg soil were taken from each microplot during the process, before adding plant material. Data from the *in situ* incubation will not be available before (earliest) autumn 2010 and will thus not be included in this thesis.

Ex situ incubation

Preparation for *ex situ* incubation

Soil collected during preparation for *in situ* incubation was raw sieved with a 2 mm mesh size sieve and water content as percent of dry weight was determined by drying a subsample at 60 °C . The soil was first stored for two days at 2 °C and than frozen.

Glass bottles (Duran, 1160 ml volume) were used as incubation chambers (IC). The bottles were fitted with a screw top, penetrated by two tubes. The tubes (one ranging the entire depth of the IC and one ending just below the top) were fitted with a valve which constituted the connection with the exterior. All the bottles were vacuum tested.

Equal amounts of ^{13}C enriched grass, clover and oats straw (chopping length 5 - 10 mm), the first two from Mp 1 to 4 and the latter from Mp 5 to 8, were mixed respectively and then divided into portions weighing 1.5 g. The grass used was from the fist harvest, and the clover was from the second harvest.

Soil from different microplots was mixed, creating three different types of substrate for six treatments each having four replicates. Portions of raw soil (equal to 400 g dry weight) were weight in, and in some of the treatments 1.5 g dry weight ^{13}C labeled plant material was incorporated. Soil was then gradually added to the incubation bottles and compacted to 1.1 g cm^{-3}. In the midst of the process of filling the incubation chambers with soil, a 50 ml plastic cup (to be filled with NaOH) was placed in the center of the IC and surrounded with soil (Figure 6 and 7). In order to avoid that inadequate amounts of nitrogen were to become a limiting factor for mineralization, the soil were amended with 0.1 M KNO_3 in appropriate amounts adjusted for the different treatments. Grass and straw was wetted with a 0.1 M KNO_3 prior to the incorporation in the soil. The KNO_3 was

amended as water solution and added to the soil surface in quantities that fulfilled a water content of 40 weight percent dry weight (Table 2). The incubation chambers were weight and placed in an incubation cabinet at 15°C.

Table 2. Type of treatment, origin of the soil used for the different treatments, soil water content as percent of dry weight, added raw soil (g IC^{-1}), amount of added ^{13}C enriched plant material (g IC^{-1}), amount of added 0.1 M KNO_3 (ml IC^{-1}) and amount of added solute (ml IC^{-1}). IC 13 to 16 was only supplied with pure water. GM = green manure.

IC nr	Treatment	Soil from Mp nr	SWC %	Soil g IC^{-1}	Shoots g IC^{-1}	0,1 M KNO_3 ml IC^{-1}	Solute ml IC^{-1}
1 - 4	Control soil	9-20	24.6	499	–	3	17
5 - 8	^{13}C oat shoots	9-20	24.6	499	1.5	1 + 9	18
9 - 12	^{13}C grass shoots	9 -20	24.6	499	1.5	1 + 3	18
13 - 16	^{13}C clover shoots	9-20	24.6	499	1.5	–	17
17	Control	–	–	–	–	–	–
18 - 21	^{13}C roots GM	1-4	23.5	494	–	1.5	22
22 - 25	^{13}C roots oat	5-8	24.6	499	–	3	17

Figure 6. Incubation chamber

Figure 7. Incubation chamber control

CO_2 harvest within the incubation chambers

Prior to the placement in the incubator cabinet, gas samples were taken and 10 ml 2 M NaOH (lye) was inserted into the plastic cup inside each of the incubation chambers. The lye served as a CO_2 trap by a two step equation: 1) $CO_2 + NaOH \rightarrow NaCO_3^-$ 2) $NaCO_3^- + NaOH \rightarrow Na_2CO_3$. By trapping CO_2 in lye, three purposes are fulfilled: (i) by constantly

trapping the access CO_2 the pp. CO_2 is kept from reaching toxic levels and (ii) from exchange with carbonate in the soil (iii) and the CO_2 may be easily taken out of the system (i.e. the incubation chamber) and the amount of trapped CO_2 gives a quantitative measure of the CO_2 mineralized between different sampling dates.

The CO_2 enriched lye (10 ml) were replaced at intervals that were in accordance with the mineralization rate within the incubation chambers and added to the bottles containing 13 ml 2 M sulphuric acid (H_2SO_4). The Na_2CO_3 was then dissolved and CO_2 was released by the equation: $Na_2CO_3 + H_2SO_4 \rightarrow Na_2SO_4 + H_2O + CO_2$. The bottle containing sulphuric acid, had been previously washed with helium (cycles of alternating evacuation and helium filling), and evacuated to 35 mbar.

Sampling of the incubation chambers

Before the lye was extracted from the incubation chambers, 15 ml gas were sampled with a syringe and added to a 10 ml evacuated vial. The gas samples were used to monitor the concentration of both CO_2 and O_2 and were used as a guideline for adding oxygen. Lye was extracted from the incubation chambers with a syringe connected to the three way valve and added to the bottles containing sulphuric acid (Figure 8 and 9). Five ml degasified distilled water was flushed trough the system and added in the same bottle.

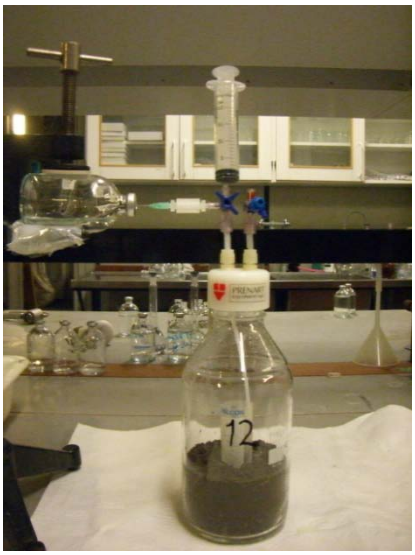

Figure 8. Extraction of lye

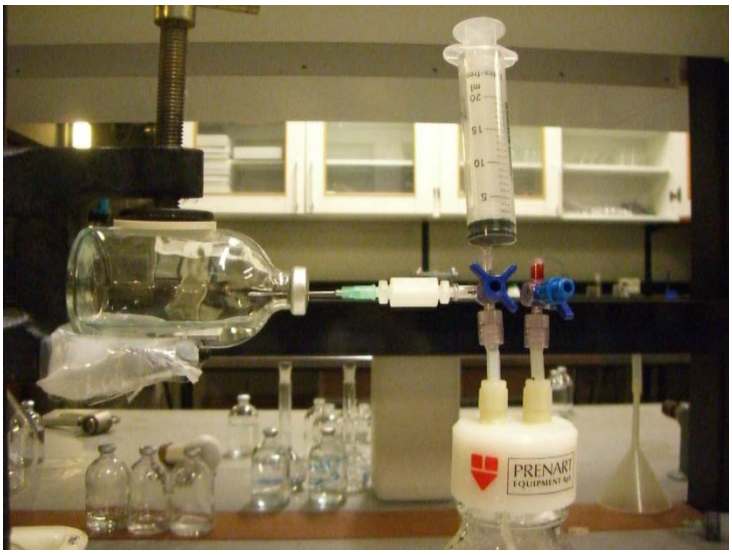

Figure 9. Extraction of lye detailed

The degasified water was made by adding distilled water to capped 119.5 ml bottles which was then subjected to a series of evacuating and helium washing under magnetic stirring followed by a prolonged period of evacuating. The purpose of the degasifying process was to reduce the quantity of dissolved CO_2 within the water. After the lye had been extracted, 10 ml new "fresh" lye, made from degasified water, was added to the incubation chambers. In all lye was changed 10 times during 104 days of incubation. Pure oxygen was added to the incubation bottles at most sampling dates. The quantities added were determent by the gas analysis from the preceding sampling.

During lye extraction on 27 November 2009 it was discovered that several of the three way valves fitted to the tubes on the incubation chamber were leaking and these were changed immediately. On 12 December 2009 even more valves were leaking and it became clear that the plastic material, of which the valves consisted, could not stand lye. On 5 December 2009 all the valves were replaced with silicon tubes fitted with a clamp.

Analyses

CO_2 concentration in the sampling bottles

Due to the different levels of dissolved CO_2 in the lye, there were correspondingly large variations in the pressure in the sampling bottles, which was below ambient in most of them. The pressure within these bottles was adjusted to 1100 mbar by addition of helium. A few bottles had a significant overpressure (above 2000 mbar), and the content of these was divided in two portions, each having around 1100 mbar pressure. Some sampling bottles had a pressure between 1100 mbar and 1500 mbar and these were not modified.
After adjusting the pressure, the CO_2 concentration in the sampling bottles was measured using gas chromatography (Agilent technologies 7890A GC system).

Isotope ratio analysis of CO_2

Only 3 out of 4 replicates were analyzed in the root treatments and only 2 out of 4 were analyzed in the shoot treatments. Subsamples from the sampling bottles, was diluted to reach approximately 5 ppm as the final concentration. A 1 ml subsample was collected from the sampling bottles and injected into a second bottle (120 ml) that had been thoroughly washed with helium and evacuated in sequences, and finally filled with helium to 2000 mbar. The pressure was then reduced to slightly above ambient and a new 1 ml subsample was taken from the second bottle and injected into a third bottle. The

final concentration of 5 ppm CO_2 was accomplished by injecting helium into the third bottle before reducing the pressure to slightly above ambient. The diluted gas samples were analyzed for $^{13}C/^{12}C$ isotope ratio using isotope ratio mass spectrometer (IRMS: Thermo Finnigan).

Carbon content and isotope ratio of soil and plant samples

Soil from the different microplots was wet sieved with a 2 mm mesh size sieve, dried at 60 °C and ground in a mortar. Plant material from all microplots (grass, straw and clover) was dried at 60 °C, finely cut, ground to 2 mm cutting length and then ball milled for 5 min at 20 Hz. Soil samples from microplots 9-11, 12-14, 15-17 and 18-20 respectively was mixed and analyzed jointly. The plant material was analyzed for total carbon content and total nitrogen content using LECO CHN-1000 analyzer. The same equipment was used to analyze soil for the total carbon content and organic carbon content. The $^{12}C/^{13}C$ isotope ratio was determined using isotope ratio mass spectrometer (IRMS: Thermo Finnigan). Soil (5 mg) and plant (1 mg) material were packed in aluminum before it was analyzed.

Given the repeated labeling of plants during the whole growing season, roots were assumed to have the same $\delta^{13}C$ value as the shoots. In order to establish a joint signature for the plant material, shoots from all three harvests of green manure was analyzed, and the mean atom percent value was designated as the green manure root isotope ratio.

Inorganic carbon isotope ratio

Samples of each of the substrates for soil analyzes were placed in sealed, evacuated 10 ml viales and 2 M sulpuric acid (H_2SO_4) was added. The carbonate bound C was thus dissolved to form CO_2 gas which was diluted to 5 ppm and analyzed for ^{13}C isotope ratio using the same procedure as for the other gas samples.

Calculations

The headspace of each sampling bottle was calculated by subtracting the volume of the added liquid (determined by weighing with and without liquid content, divided by the density of the combined liquid) from the total volume of 119.5 ml. The molar volume for the actual temperature was calculated by using the formula for molar volume: $V_m=RT/P$

where: V_m = molar volume, R = gas constant (8.31), T = temperature (Kelvin), P = pressure (Kpa). The amount of CO_2 given as mol sampling bottle^{-1} was calculated by dividing the volume occupied by CO_2 (concentration x headspace) by the molar volume of the gas at the pressure and temperature measured.

The $\delta^{13}C$ values were calculated as ‰ deviation from a standard given by the equation: $\delta^{13}C‰ = (R_{sample}/R_{reference}-1) \times 1000$.

The $\delta^{13}C$ values were converted to ^{13}C atom % by using the formula: $(\delta^{13}C + 1000)/((\delta^{13}C + 1000 + (1000/0.18))) \times 100$.

The total quantity of CO_2 from mineralized added shoots was calculated using two different approaches: (i) the total CO_2 mineralized from the reference soil (without shoot amendment) was subtracted from the total CO_2 mineralized from the soil that was amended with 1.5 gram shoots, and the difference was designated the apparent quantity of mineralized carbon from shoots (ii) the relative carbon contribution originating from shoots of the total CO_2 pool collected during the incubation, was calculated using a two source mixing model: $(f_x\ ^{13}C \text{ atom } \% * C_x) + (f_y\ ^{13}C \text{ atom } \% * C_y)$, where $f_x + f_y = 1$.

This assumes that isotope discrimination during mineralization is negligible and similar for both litter and SOC. The same approach was used both to quantify the total amount of roots IC^{-1}, and to quantify the share of root derived carbon in the respired CO_2. The percentage of the original (incubation start) carbon in shoots and roots that was mineralized during the course of the incubation period was estimated by dividing the carbon in CO_2 derived from shoot and root by the original quantity of carbon in shoots and roots.

Statistical analyzes

Differences between obtained delta values and differences between different treatments were analyzed using analyses of variance (ANOVA) and two sample t-test. The cumulative respiration curves and the mean daily respiration curves were fitted with the regression line that best explained the data.

Results

$\delta^{13}C$ and ^{13}C atom % values

The $\delta^{13}C$ ‰ values obtained for unlabeled shoots (mean value for: grass, clover and oat) collected at the BYGGRO trial field was -30.61 ± 1.12 $\delta^{13}C$ ‰ (mean value ± standard deviation). The $\delta^{13}C$ ‰ obtained from soil containing labeled green manure roots (Mp 1-4 n = 15) and from soil containing labeled oat roots (Mp 5-8 n = 15) were both significantly higher (T = 16.39 P = 0.00 DF = 14 and T = 15.02 P = 0.00 DF = 16 respectively) than the $\delta^{13}C$ ‰ of soil containing unlabeled roots (Mp 9-20 n = 19). There was no significant difference in $\delta^{13}C$ ‰ between the two soils containing labeled roots (T 2.26 P = 0.109 DF = 3). The $\delta^{13}C$ ‰ of shoots from different harvests of green manure differed significantly for both clover (ANOVA, F = 80.19 P = 0.00 DF = 11) and for grass (ANOVA, F = 7.76 P = 0.016 DF = 23) and this resulted in a high standard deviation for the estimated $\delta^{13}C$ ‰ of green manure roots. Only one sample of CO_2 derived from inorganic carbon (carbonate-C) in soil containing natural abundance ^{13}C roots and ^{13}C enriched roots respectively was analyzed (Table 3). Though some variation was observed in the beginning of the incubation, it may seem as the ^{13}C atom % in the respired CO_2 decreased with time, especially for roots (Figure 10, 11, 12, 13 and 14).

Table 3. $\delta^{13}C$ ‰ values and atom % ^{13}C of various substrates (mean values ± st.dev)

Substrate	$\delta^{13}C$ ‰ value	^{13}C atom %
Unabeled soil	-25.36 ± 1.64	1.14 ± 0.0019
Soil Mp 1-4	-12.014 ± 3.18	1.15 ± 0.0037
Soil Mp 5-8	-13.53 ± 3.08	1.15 ± 0.0036
Unlabeled oat	-30.98 ± 4.6	1.13 ± 0.0053
Unlabeled clover	-29.35 ± 0.02	1.13 ± 0.000023
Unlabeled grass	-31.50 ± 0.0023	1.13 ± 0.000026
Shoots clover	1927.87 ± 157.026	3.34 ± 0.17
Shoots grass	1101.68 ± 44.72	2.42 ± 0.05
Shoots oat	1312.64 ± 109.35	2.66 ± 0.12
Roots GM	1315.32 ± 331.72	2.67 ± 0.37
Carbonate	-12.24 ± 2.18	1.15 ± 0.0025

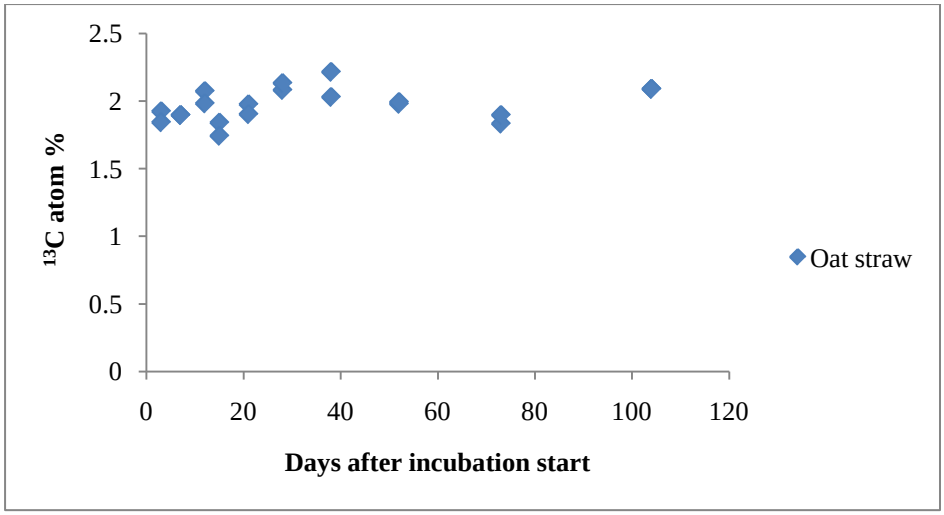

Figure 10. ^{13}C atom % in respired CO_2 from soil supplied with ^{13}C labeled oat straw.

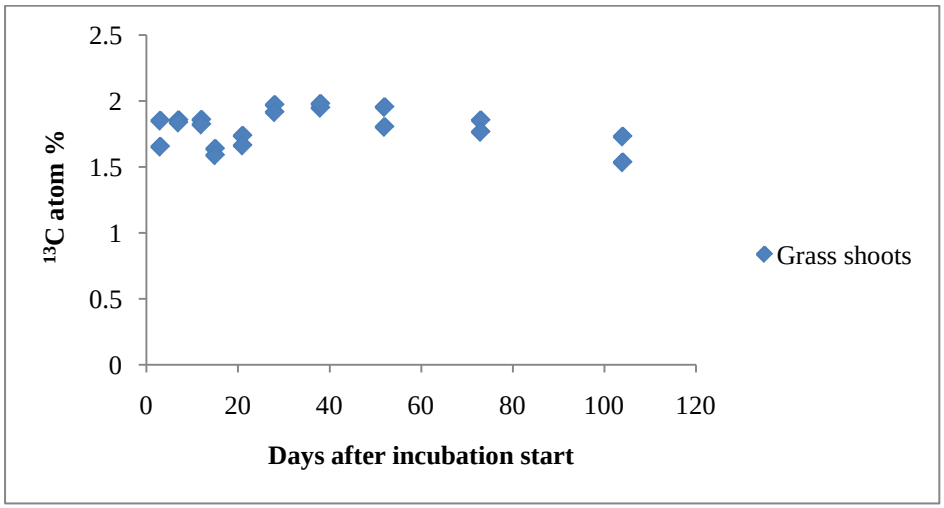

Figure 11. ^{13}C atom % in respired CO_2 from soil supplied with ^{13}C labeled grass shoots.

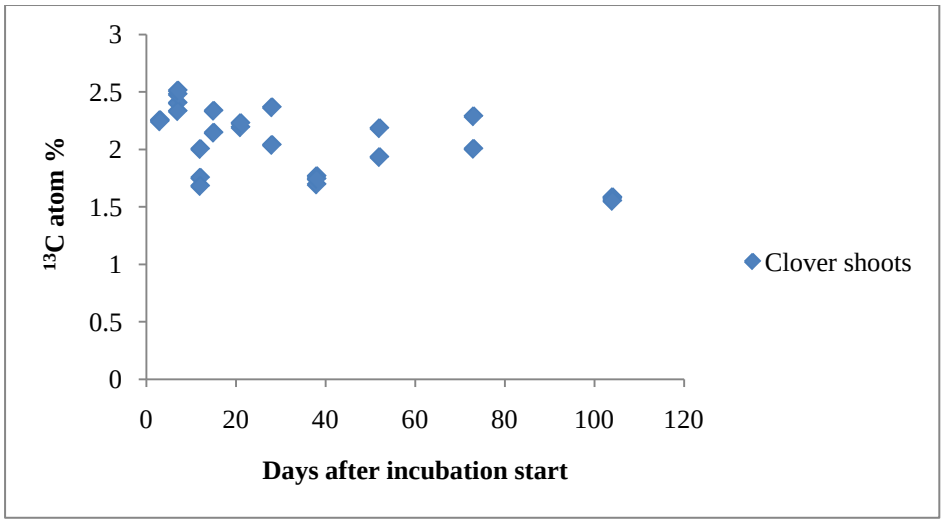

Figure 12. ^{13}C atom % in respired CO_2 from soil supplied with ^{13}C labeled clover shoots.

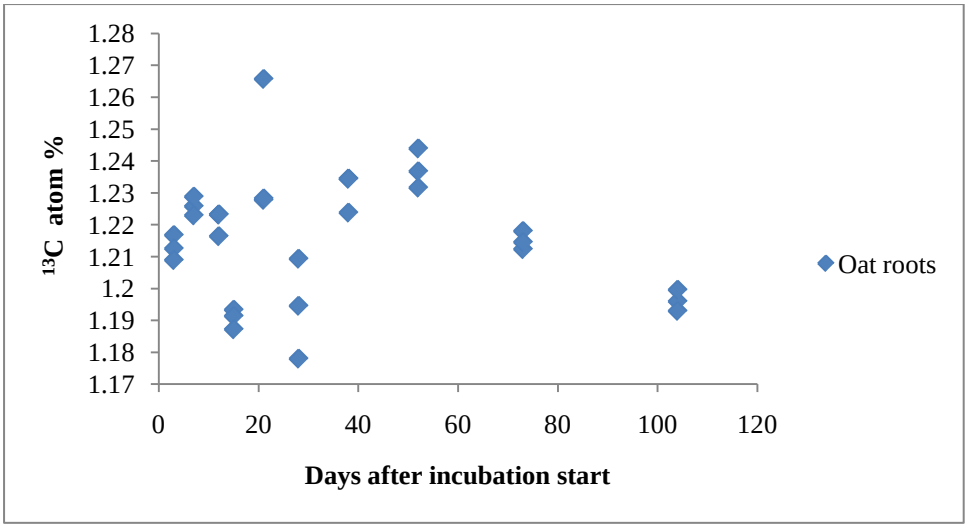

Figure 13. ^{13}C atom % in respired CO_2 from soil containing ^{13}C labeled oat roots.

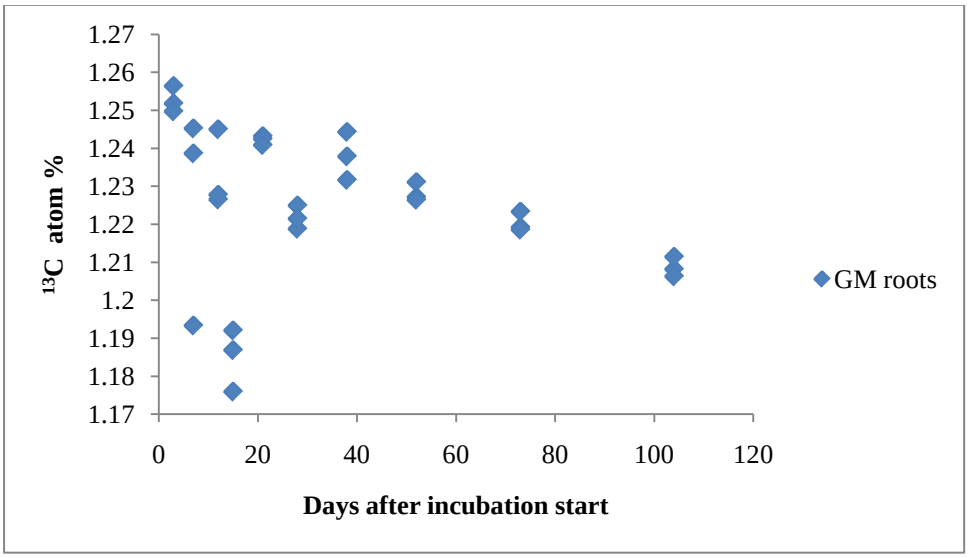

Figure 14. ^{13}C atom % in respired CO_2 from soil containing ^{13}C labeled green manure (GM) roots.

Cumulative soil respiration

The cumulative CO_2 respired from the treatment with labeled oat roots after 104 days, did not differ from the reference treatment which included unlabeled oat roots (T = 2.04 P = 0.178 DF 2). However, the cumulative CO_2 respired from the treatment with labeled green manure roots was significantly higher (T = 7.44 P = 0.018 DF = 2) (Figure 15).

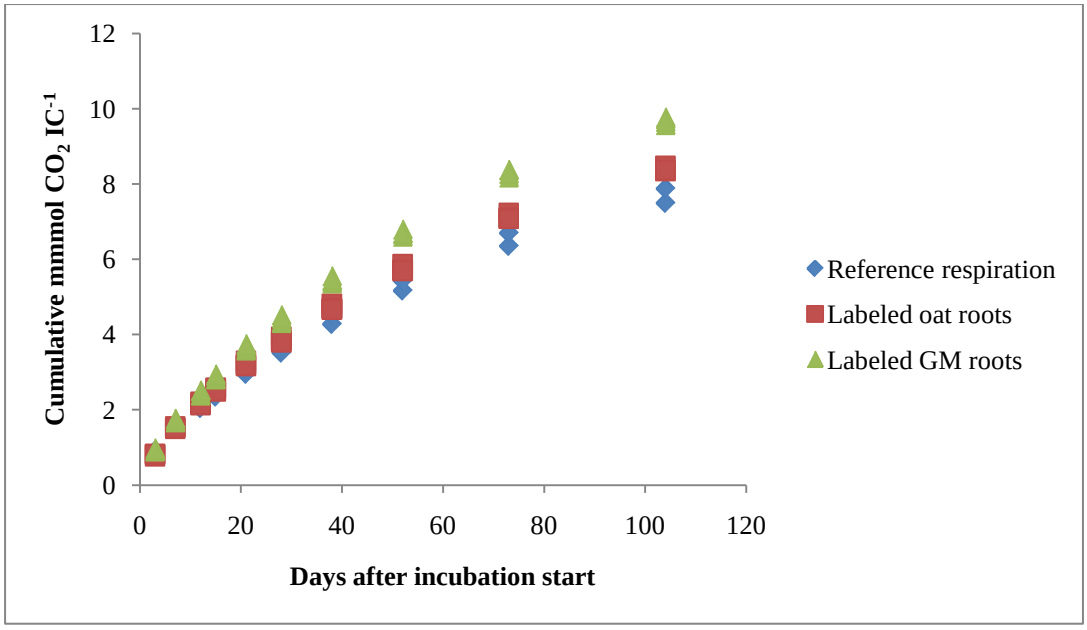

Figure 15. Cumulative mmol CO_2 IC^{-1} respired from: unlabeled soil without addition of shoots (reference respiration), labeled roots from oat without addition of shoots and labeled roots from green manure (GM) without addition of shoots.

The cumulative amount of evolved CO_2 after 104 days was about 6-7 times higher from soil that was added 1.5 g shoots, compared with the soil that was not amended with shoots (Figure 16).

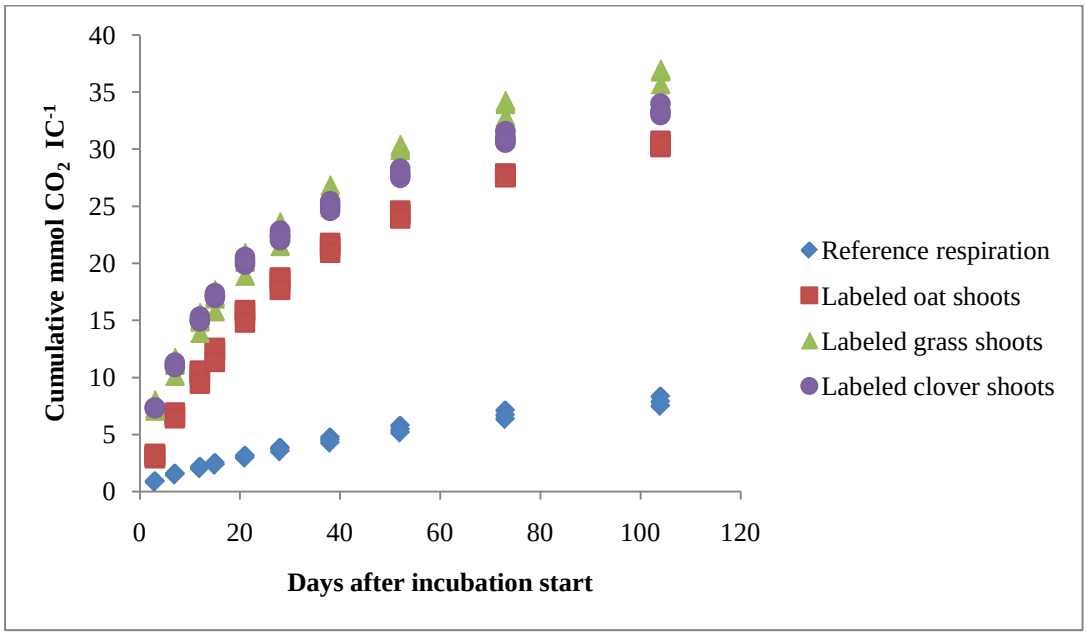

Figure 16. Cumulative mmol CO_2 IC^{-1} respired from: unlabeled soil without addition of shoots (reference respiration), unlabeled soil added 1.5 g oat shoots, unlabeled soil added 1.5 g grass shoots and unlabeled soil added 1.5 g clover shoots.

Estimated apparent respiration of shoots

By subtracting the mean amount of CO_2 respired from the soil that was not amended with shoots, from the amount of CO_2 respired from the treatments where 1.5 g of shoots had been added to the soil, the apparent amount of CO_2 derived from shoots was estimated to be 22.49 ± 0.023 mmol, 28.69 ± 0.06 mmol and 25.48 ± 0,043 mmol, from soil amended with oat straw, grass and clover respectively (Figure 17). Differences between shoots were significant (ANOVA, F = 520.59 P = 0.00 DF = 8).

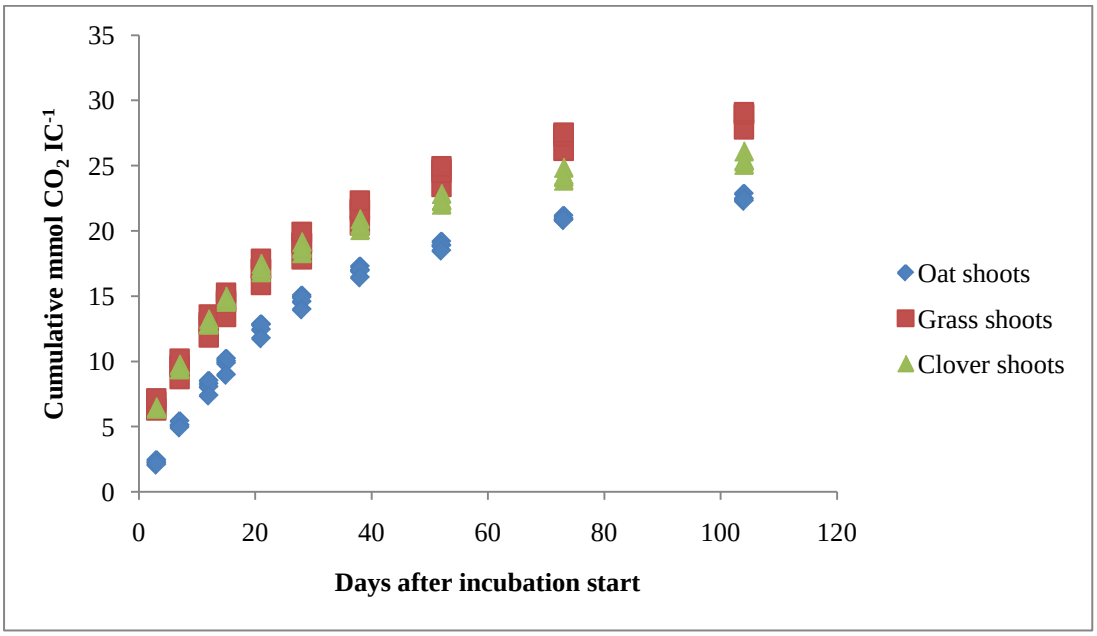

Figure 17. Cumulative mmol CO_2 IC^{-1} apparently derived from shoots given as mmol CO_2 collected in soil amended with shoots minus reference respiration (from soil without shoot amendment).

Estimated respiration of shoots using ^{13}C atom %

Near significant difference was found between total CO_2 derived from oat, grass and clover (n = 2 for all) was found (ANOVA, F = 8.2 P = 0.061 DF = 5). The data were best fitted by a logarithmic trend line. After 104 days of incubation 16.82 ± 0.072 mmol CO_2, 18.77 ± 0.15 mmol CO_2 and 14.45 ± 0.082 mmol CO_2 of shoot origin had been recovered from oat, grass and clover respectively (Figure 18). This corresponds to 55 %, 51 % and 43 % of the total soil respiration from oat- , grass- and clover shoots, respectively

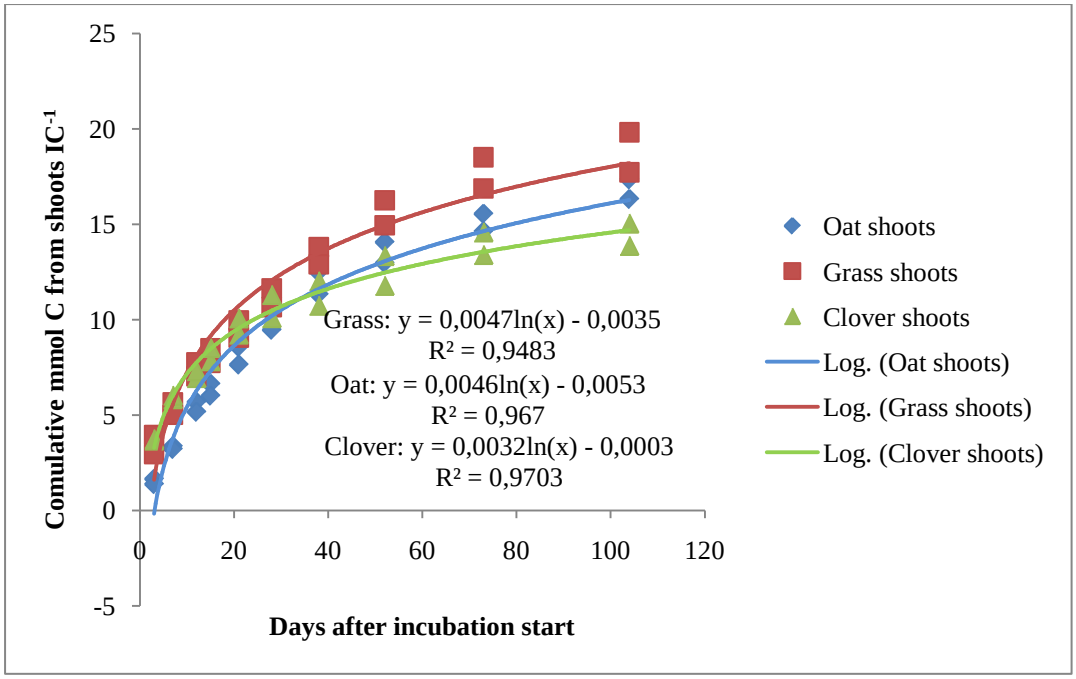

Figure 18. Cumulative amount of CO_2 (mmol IC^{-1}) derived from added shoots (oat, grass and clover) calculated using ^{13}C atom % in total CO_2, and the relative contribution of shoots to the total CO_2. Logarithmic trend line.

Relative amount of added shoot C mineralized

The estimated relative amount of added C that had been respired after 104 days using the total CO_2 accumulated in treatments with amended shoots, minus CO_2 obtained in the reference treatment (apparent shoot respiration) was 40.09 ± 0.42 %, 51.76 ± 1.09 % and 46.21 ± 0.78 % for oat shoots, grass shoots and clover shoots respectively. Using ^{13}C atom % values in a two source mixing model it was calculated that 29.97 ± 1.29 %, 33.86 ± 2.67 % and 26.22 ± 1.49 % of the original C had been respired from oat shoots, grass shoots and clover shoots, respectively (Table 4).

Table 4. Carbon content of added shoots, added C in shoots (mmol IC^{-1}), apparent (Ap.) mmol C respired of shoot origin (cumulative CO_2 minus cumulative reference CO_2), apparent percentage of shoot C respired, mmol C respired based on recovered ^{13}C in total CO_2 and percentage of the C in shoots respired estimated using the latter approach.

	Mean % C in shoots	Added mmol C IC^{-1}	Ap. mmol C respired IC^{-1} difference	Ap. % C respired difference	mmol C respired ^{13}C atom %	% C respired ^{13}C atom %
Straw	44.38	56.12	22.49 ± 0.23	40.09 ± 0.42	16.82 ± 0.072	29.97 ± 1.29
Grass	44.93	55.43	28.69 ± 0.06	51.76 ± 1.09	18.77 ± 0.15	33.86 ± 2.67
Clover	44.15	55.14	25.48 ± 0.043	46.21 ± 0.78	14.45 ± 0.082	26.22 ± 1.49

Total root derived C and relative root C respired

Using the same ^{13}C atom % approach as for shoots, after 104 days of incubation it was estimated that 0.56 ± 0.018 mmol C of green manure origin and 0.44 ± 0.007 mmol C of oat root origin had been recovered as CO_2 (Figure 19). This accounts for 5.3 % and 5.86 % of the total SOC respiration for green manure roots and oat roots, respectively. The data fitted a polynomial trend line. The total mol CO_2 respired was significantly (T = 11.0 P = 0.008 DF = 2) higher from green manure roots as compared with oat roots.

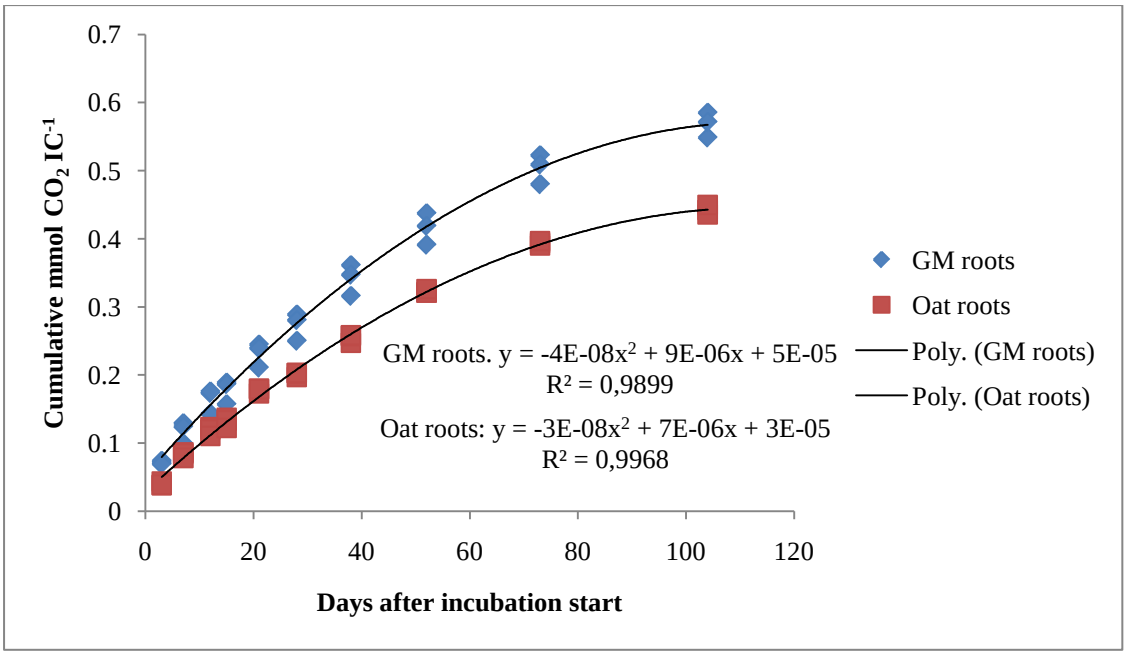

Figure 19. Cumulative amount of CO_2 (mmol IC^{-1}) derived from oat and green manure (GM) roots. Polynomial trend line.

The total root C was estimated to constitute 1 % and 0.9 % of the total SOC content for oat roots and green manure roots, respectively. The calculated amount of labeled roots

and root derivates (from the growing season 2009) is 0.067 g IC^{-1} and 0.069 g IC^{-1} for green manure and oat, respectively. The relative amount of original root derived C respired after 104 days of incubation is 10.25 ± 0.33 % and 7.67 ± 0.12 % for oat roots and green manure roots respectively (Table 5).

Table 5. Total amount of C contained in green manure (GM) roots and oat roots (g IC^{-1} and mmol IC^{-1}), share of labeled roots of total SOC, cumulative mmol root derived CO_2 after 104 days of incubation and percent respired root C of original root C.

	Tot g root C IC^{-1}	**Tot mmol C in roots IC^{-1}**	**Share root C of SOC**	**Cumulative mol CO_2 from roots**	**% roots respired**
Roots GM	0.067	5.53	0.01	0.56 ± 0.018	10.25 ± 0.33
Roots oat	0.069	5.76	0.009	0.44 ± 0.007	7.67 ± 0.12

Respiration rates

The mean daily respiration rate, given as respired mol CO_2 mol C^{-1} day^{-1}, declined rapidly after the onset of incubation for both roots and added shoots (Figure 10 and 21) thus indicating a first order kinetic relationship of the decomposition of litter.

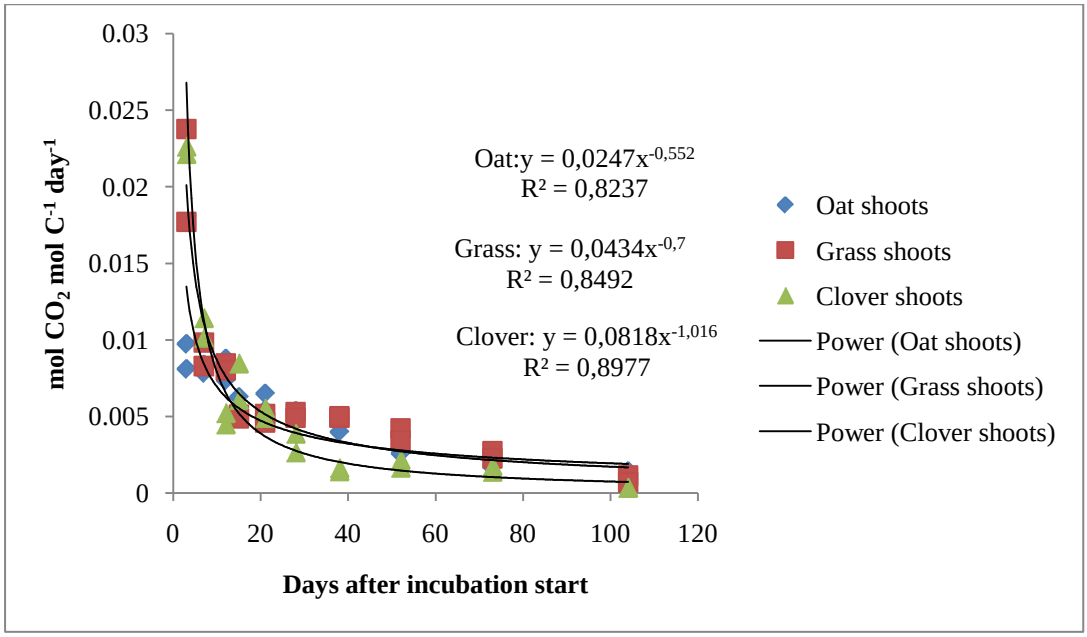

Figure 20. Mean daily respiration rate for added oat straw, grass shoots and clover shoots (mol CO_2 mol C^{-1} day^{-1}). Power trend line.

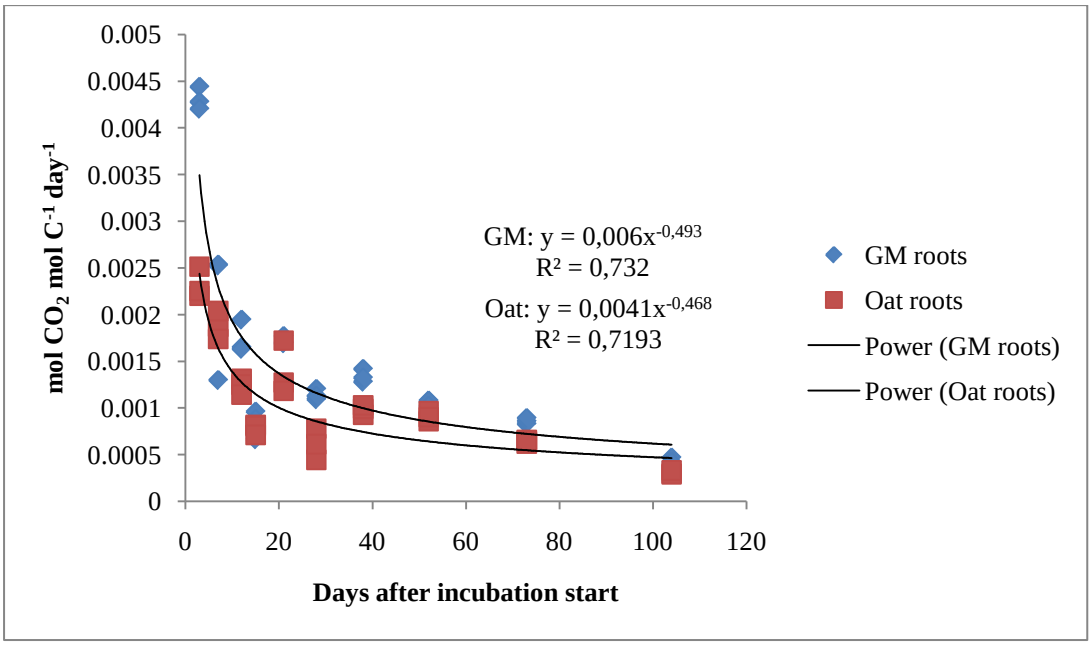

Figure 21. Mean daily respiration rate for green manure (GM) roots and oat roots (mol CO_2 mol C^{-1} day^{-1}). Power trend line.

Early in the incubation process incubation chamber 1 (control soil) was compromised as 2 ml 2 M NaOH was accidentally spilled on the soil. The CO_2 respired in incubation chamber 1 deviated substantially from the other three replicates and was therefore considered an outlier and excluded from the calculations. The total amount of CO_2 collected in the empty control incubation chamber (IC 17) was marginal, and was not considered in the calculations.

Discussion

Field labeling with $^{13}CO_2$

Organic material with distinct isotope ratios offer unique properties in terms of tracking the fate of organic material as it is subjected to the actions of the biosphere. All organic material contains some proportion of the carbon isotopes ^{14}C (radioactive) and ^{13}C (stable), and it has been established that the natural ^{13}C abundance of C_3 plants is 1.1 atom percent (-27 $\delta^{13}C‰$) (Ostle et al. 2000). The natural ^{13}C abundance in C_4 plants are somewhat higher (-12 $\delta^{13}C‰$) and by introducing C_4 plants into a field for the first time it is possible to exploit the difference in natural ^{13}C abundance to reveal the fate of both the old (C_3 ^{13}C signature) SOC and the newly added C_4 ^{13}C signature (Clapp et al. 2000). However, introducing C_4 crops like maize to the cropping system for experimental purposes might be constrained by climatic conditions (i.e. difficult to grow in Norway) or it may be undesired to introduce such a crop according to the object of the experiment. In order to investigate the fate of C_3 plant material under all C_3 plant conditions, some of the C_3 pool has to exceed the natural abundance of ^{13}C, and this calls for a different approach.

By exposing plants to CO_2 that is enriched in either ^{14}C or ^{13}C it possible to artificially increase the $^{14}C/^{13}C$ content to above natural abundance and thus achieve two carbon pools, both having a distinct isotope ratio. If the plants are enriched with ^{13}C the contribution of each pool (natural ^{13}C abundance pool and enriched ^{13}C pool) to the overall pool might be quantified by using a mixing model, as in this study. The method relies on quantifying the ^{13}C signature of the different carbon pools in question and this implies labor demanding and costly isotope ratio analyses using mass spectrometer. Because ^{13}C is a stable isotope, any health hazards are avoided using this approach.

A totally different approach is the use of the radioactive carbon isotope ^{14}C as a tracer (Buyanovsky & Wagner 1987; Gale & Cambardella 2000; Martens et al. 2009). Due to its radioactive nature, the amount of ^{14}C can be quantified using a Geiger counter and the radioactivity of the substrate is thus used as a proxy for the quantity of the organic pool in question. The method allows rapid quantification of the ^{14}C amount and it can be used to measure the radioactivity directly from *in situ* incubation sites. However, the very same radioactivity that makes the method feasible may also exert a health hazard to the researchers, and the more labor demanding ^{13}C approach might well offset this risk.

In general, the principle of incorporating the $^{14}C/^{13}C$ enriched CO_2 into plant tissue is achieved by enclosing the plants in a closed atmosphere (labeling chamber) and insert $^{14}CO_2/^{13}CO_2$ into the labeling chamber. The $^{14}C/^{13}C$ carbon that is photosynthetically assimilated into organic compounds is either incorporated into structural components (roots or shoots) or is respired shortly after assimilation. Ostle et al. (2000) found that 76.4 % and 61.65 % of the added ^{13}C was respired within 24 hours from shoots and roots respectively. However, after the initial rapid decline the loss ceased and this effect is by the authors attributed to that the carbon had been stabilized into structural components like cellulose, hemicelluloses and lignin (Ostle et al. 2000). Thus it could be expected that some 70 percent off all the added ^{13}C in this experiment has been lost trough rapid plant respiration, probably occurring mostly the night after the day of labeling.

A similar approach to labeling plants with ^{13}C as the one used in the present study was used by Puget and Drinkwater (2001). The method is rather simple and easy to manage. The plants are exposed to highly variable CO_2 levels, from high (> 600 ppm) at the instant moment after insertion of acid, to low (<100 ppm) as the photosynthetic activity of the plants exhausts the CO_2 within the labeling chamber. This might cause some stress to the growing plants and this stress factor might be further increased as the temperature inside the chamber rises to above 40 °C. The expired CO_2 rich air that was added to the labeling chamber served two purposes; it both enhanced the utilization of the added $^{13}CO_2$ as the photosynthesis was boosted by the addition of CO_2 and it relieved the plants from the stress of being exposed to low pp. CO_2. During the course of the labeling process, no detectable effects of the exposure to any labeling related stress factors were observed.

Ostle et al. (2000) developed a system (stable isotope delivery system) for labeling multiple plots at the same time with both constant total CO_2 and $^{13}CO_2$ levels. The system was used to label grassland. The same system was also used by Foereid et al. (2006) to label grassland. Such a system for adding $^{13}CO_2$ eliminates the perturbations in CO_2 level during the course of the labeling, however it is expensive and complicated to handle. Labeling chambers has also been used in the field (Buyanovsky & Wagner 1987; Martens et al. 2009) and in greenhouses (Gale & Cambardella 2000) to label plants with $^{14}CO_2$.

The pulse measurements of pp. CO_2 that was carried out at least once during each labeling event revealed that there was some variation amongst the microplots in terms of ability to sink CO_2. The same variation was later found in the isotope ratio of the plant material (data not shown). However, the ^{13}C atom % was higher in harvested plant material from the microplots that showed least ability to sink CO_2 (low biomass), thus indicating that the low amounts of added ^{13}C could be accumulated at a greater extent in the microplots having the lowest biomass. The difference in ^{13}C atom % does not affect the *ex situ* incubation experiment, as the crops from the different microplots were mixed. However, in the *in situ* incubation this might have some implications.

***Ex situ* incubation**

The use of lye (NaOH) as a CO_2 trap to accumulate CO_2 between samplings is a widely used technique in association with incubation trials (e.g. Gale et al. 2000; Martens et al. 2009) and soil respiration measurements (Hendrix et al. 1988). Ideally the CO_2 collected in the lye represents the total amount of CO_2 respired between the insertion and exertion of lye from the system. A prerequisite for this to be accomplished is that the lye has capacity (i.e. enough quantity and surface area to ensure sufficient contact between the gas and liquid face) to handle the respired CO_2. In the present experiment only 10 ml lye was applied and the surface area of the lye was small. The gas samples taken during the course of the incubation (data not shown) revealed that the lye had insufficient capacity to handle all the CO_2 evolved at the early phase of the incubation. This has implications for the cumulative CO_2 curve, as the CO_2 respired in the early phase is underestimated. However, as the respiration diminished the lye became capable of absorbing all the respired CO_2 and the total cumulative CO_2 level after 104 days are thus unaffected.

The destruction and subsequent change of the valves fitted to the incubation chambers lead to some inlet of atmospheric air and thus CO_2. This effect was not observed on the total amount of CO_2 collected in the lye. The isotope ratio analyzes however, revealed that a decrease in atom percent ^{13}C concurred with this event, and this might be taken as an indication towards a possible effect of the CO_2 inlet.

Most studies of SOC dynamics are conducted in the field and so are many incubation trials using $^{14}C/^{13}C$ as markers (Puget & Drinkwater 2001; Clapp et al. 2000; Foereid et al. 2006; Buyanovsky & Wagner; Ostle et al. 2000; Martens et al. 2009). Such an

approach allows the soil to be studied undisturbed *in situ* and this might improve the reliability of the results. The respiration is measured based on the occurrence of labeled material at different occasions. This only allows insight to what has happen between two abrupt sampling events and does not offer any continuity in the respiration data. However, *in situ* incubation makes it possible to follow the fate of the SOC as it is incorporated into the soil structure, and allows the nature of physical protection to be investigated (Puget & Drinkwater 2001). Laboratory incubation with a fixed temperature and continuous accumulation of respired CO_2 and the use of ^{13}C labeled plant material facilitates unique properties that may supplement the use of *in situ* incubation.

The mineralization course of unlabeled litter to soil can be calculated by subtracting the respired CO_2 from a control soil from the CO_2 respired in the amended soil. This method gives an indication of the CO_2 that is apparently derived from the added litter. However, some of the CO_2 apparently originating from mineralized litter may actually be of SOM origin, which implies priming effect of the added litter, as described by Nottingham et al. (2009).

The "litter amended soil minus control soil" approach is not an adequate method of measuring the CO_2 derived from roots, unless the quantity of added roots is known and the control soil is without roots. To quantify root biomass without isotope tracers is difficult and implies destructive excavation and subsequent washing of roots (Subedi et al. 2006), and this would compromise the very objective of this study, which is to incubate soil with intact aggregates. One possible *ad hoc* solution would be to quantify the root biomass in one part of the soil and extrapolate the result to also count for the incubation soil. However, because as much as 60 percent of the root derived carbon may be constituted by root exudates (Buyanovsky & Wagner 1987), the reliability of such a method would be rather poor.

When roots and shoots labeled with ^{13}C are incubated, continuous accumulation of CO_2 makes it possible to compare how the mineralization, from roots and shoots added to the soil, progresses. It also distinguishes between shoot derived carbon and SOM derived carbon, and may thus reveal a potential priming effect. This could also be accomplished using field incubations, but priming effect would be difficult to detect due to the relative low additional reduction of the total SOM pool priming effect would induce.

$\delta^{13}C$ ‰ values and assumptions

Mean $\delta^{13}C$ ‰ values for unlabeled plants collected from the BYGGRO trial (-30.61± 1.22 ‰) is in line with the expected $\delta^{13}C$ ‰ for C_3 plants (-27). The $\delta^{13}C$ ‰ values of the CO_2 derived from the acid treated soil (-12.24 ± 2.18) was slightly lower than the 2.1‰ reported by Ostle et al. (2000) for $CaCO_3$. The mean $\delta^{13}C$ ‰ measured from the unlabeled soil (MP 9-20) was -25.35, thus slightly above the natural abundance of plant material and the difference could arise from the higher $\delta^{13}C$ ‰ in the inorganic carbon (carbonate) in the soil, or from a possible microbial isotope discrimination.

Some labeled root samples were collected at the time of preparation for *in situ* incubation. However, the few samples taken are to be used at a later occasion and were not analyzed for $^{12}C/^{13}C$ isotope ratio. Because the plants were continually labeled with ^{13}C throughout the growing season, it may be assumed, especially for oat which has been labeled since sowing, that the isotope signature in the roots equals that of the shoots. After 2 pulse labeling events lasting 10 hours each, Ostle et al. (2000) found that the $\delta^{13}C$ ‰ was 84.3 ± 28.7 (1.26 ± 1.2 ^{13}C at %) and 45.8 ± 14.72 (1.21 ± 1.18 ^{13}C at %) in shoots and roots respectively. This implies that the accordance in ^{13}C atom % values between roots and shoots are some 96 %. The green manure plants were well established prior to the onset of labeling and a proportion of the roots thus remain unlabeled, indicating that the true $\delta^{13}C$ value of the roots does not equal the $\delta^{13}C$ value of the shoots.

The ^{13}C signature of the roots is crucial in quantifying the amount of roots and root derived carbon in the soil at the beginning of the incubation. If the actual ^{13}C signature is lower than the assumed one, this would imply that the estimated amount of root carbon is underestimated. The calculated amount of root carbon was only some 1 percent of the total SOC content for both oat roots and green manure roots. This might be an underestimate and the estimated total quantity of root carbon should not be trusted to represent the real quantity.

However, the objective of this experiment is to establish the relative amount of mineralized root derived carbon and not the original quantity of root carbon as such. The estimate of the share of root mineralized during the incubation period is still correct even

if some root growth in the very early spring is not labeled. This is because the unlabeled part of the roots is neither included in the initial carbon, nor in the CO_2 derived from the labeled part of the roots. It is thus not necessary to label all roots in order to fulfill the objective of this experiment. In the treatment where shoots are added, both the $\delta^{13}C$ ‰ value and the quantity of added carbon are known and this issue is thus absent.

Estimated mineralization of shoots and roots

Using the "litter amended soil minus control soil" approach it was calculated that 51.1 %, 40.1 % and 46.4 % of the added C in shoots had been mineralized from grass, oat and clover respectively. These apparent values deviate profoundly from the 33.9 %, 30 % and 26.2 % that was estimated based on isotope ratio calculations for grass, oat and clover, respectively. This either implies that the calculations based on isotope ratios is wrong (i.e. the obtained $\delta^{13}C$ ‰ is incorrect), that ^{13}C-discrimination has taken place during microbial decomposition, that atmospheric CO_2 has entered the incubation chambers, that a priming effect has been induced by the addition of litter, that some of the collected CO_2 is of carbonate origin or a combination of these factors.

During the second week of the incubation many of the valves fitted on the incubation chamber was damaged, due to material intolerance to NaOH. Both during this time, and when the valves were changed with the new system, some atmospheric air entered the incubation chambers. Due to a high respiration activity within the incubation chambers amended with shoots, a substantial below-ambient pressure developed as the evolved CO_2 was trapped in the NaOH. This resulted in a greater inlet of atmospheric air in the amended incubation chambers compared to the control soil. This diluting effect, manifested as a decrease in $\delta^{13}C$ values during the affected period might explain some of the deviation between the two approaches to estimate the mineralized shoots.

If decomposing microorganisms discriminate between ^{12}C and ^{13}C in terms of the proportion respired as $^{12}CO_2$ and $^{13}CO_2$ this could potentially lead to false estimates of the real quantity of mineralized ^{13}C enriched substrate. Ekblad et al. (2002) tested whether there was a difference in the estimated mineralization of natural ^{13}C abundance glucose and ^{13}C enriched glucose using the isotope ratio of the respired CO_2 as a basis for the

estimation. They concluded that microbial ^{13}C discrimination is minor, and this factor is thus unlikely to explain the observed deviation in this experiment.

The 1.5 g of added shoots to 400 g of dry weight soil equals about 750 g DM m^{-2} in the 17 cm deep layer, or twice the amount of oat straw produced m^{-2} in this field trial, and thus represents a heavy supply of litter. Steinbeiss et al. (2009) observed a doubling in SOC mineralization when yeast derived biochar was supplied at levels corresponding to 30 % of the initial carbon content. In the present study the litter added corresponded to 10 % of the original SOC, and a substantial priming effect would thus be plausible. However, given the differences in substrate quality between yeast derived biochar and the substrates used in this experiment, and the fact that other studies have found no (Martens et al. 2009), or even a negative (Potthast et al. 2010; Torbert et al. 2000) priming effect, a substantial priming effect should not be claimed for this experiment.

If substantial amounts of CO_2 derived from inorganic C (carbonate-C), is emitted from the soil this might explain some of the observed deviation. When incubating peat soil limed with $CaCO_3$ under laboratory conditions, Biasi et al. (2008) found that as much as 50% of the collected CO_2 was of carbonate origin. However, with the soil used in the present incubation (having a pH of 6.7) such a high contribution from carbonate CO_2 is unlikely.

The relative amount of mineralized roots is estimated to be 10.25 % and 7.62 % for green manure roots and oat roots respectively and such a difference could be expected given the more favorable C/N relationship in the green manure (data not shown). The soil containing oat roots was amended with soluble nitrogen fertilizer (0.1 M KNO_3) and this might have evened out some of the expected difference in total mineralization of roots. Also treatments with added shoots of grass and oat was supplied with 0.1 M KNO_3 whereas the treatment with clover shoots was not, and also here the fertilizing seems to have even out the expected difference in total mineralization of the different substrates.

The soil was raw sieved with a 2 mm mesh size sieve, and most of the course roots were thus excluded from the incubation soil. This might explain the low estimated quantity of newly added roots carbon in the soil (ca 1 % of total SOC), most of which might be expected to consist of root exudates and fine roots. Root exudates and fine roots are more

likely to be physically incorporated into microaggregates or chemically associated with clay particles than course roots (Gale et al. 2000a; Golchin et al. 1994 a; Oades 1988; Six et al. 2000). If this is the case, the mineralization rate of roots might be underestimated because if also the course roots (unprotected) were included they would be more prone to mineralization and thus skew the total mineralization towards a higher value.

Possible explanations for the obtained results

This experiment offers no causative explanations for the observed results; however several earlier studies may provide possible explanations for the observed differences in mineralization rate between shoots and roots. The relationship found in this laboratory incubation experiment is in line with findings of many earlier studies. Using ^{13}C labeled hairy vetch (*Vicia villosa*) in an *in situ* incubation experiment with incorporation of shoots into the soil, Puget and Drinkwater (2001) found that after 5 months, 13.3 percent and 48.8 percent of the original C of shoots and roots, respectively was retained in the soil. Buyanovsky and Wagner (1987) used *in situ* pulse labeling of winter wheat with ^{14}C to evaluate the fate of carbon added either as root C or shoot C after incorporation into the soil. After one year, 16 percent of the original C in straw was found as SOM compared to 24 percent for root. However, a larger fraction of the shoots remained as litter compared to roots (35 % and 23 % respectively) and this implies that the potential contribution of shoots to the table SOM pool is greater than what can be found after one year. Gale and Cambardella (2000) evaluated the fate of root C and shoot C in a simulated no tillage laboratory incubation trial at 25 ºC using ^{14}C labeled oat. After one year 66 percent and 56 percent of the initial shoot and root derived carbon respectively had been mineralized. Of the remaining shoot derived carbon 11.4 % was found as surface litter, 1-3 % was found as free POM and 9-14 % was found as clay/silt associated C. In comparison coarse roots constituted 4.2 % of the original root C, POM constituted 11-16 % of the original root C and silt/clay associated C constituted 17-24 % of the original root C. However, only the leaves of the shoots were added to the soil surface, and this might have favored the mineralization compared to the whole shoot. From an experiment with specific root (α,ω-alkanedioic acids) and shoot (mid-chain hydroxyl acids) biomarkers, using natural abundance ^{13}C, it has also been shown that roots contribute more to SOC than shoots (Mendez-Millan et al. 2010). In a review, Rasse et al. (2005) concluded that in the field, shoots on average are mineralized 2.4 times faster than roots.

Puget and Drinkwater (2001) analyzed both shoots and roots and found more of the structural components lignin, hemicelluloses and cell walls in shoots as compared to roots. In their review Rasse et al. (2005) also concludes that in laboratory incubation experiments, where a known amount of roots are added to a soil, the shoots on average were mineralized 1.3 times faster than shoots. This implies that the 1.3 factor may be attributed to the structural and biochemical properties of shoots and roots and that such difference only counts for 30 % of the observed difference found *in situ*.

If contact between soil and litter per se. is important in terms of physical protection it would be expected that shoots incorporated into soil has equal ability to become physically protected as roots that are grown within the same soil. If only 30% of the observed difference in mineralization rate between roots and shoots can be attributed to structural differences, the present study clearly shows that even though shoots are cut into small fragments and incorporated into the soil, shoots are not as physically protected as roots. Hence, there has to be something with the way in which the roots and root derivates interact with the soil matrix that makes them susceptible to physical protection.

Roots continuously release polysaccharides in the root cap region as they grow, and the combined exudates are designated mucilage. The mucilage is sticky and acts as gluing agents, connecting mineral particles and SOM into aggregates (Oades 1984). A proportion of the mucilage is thus incorporated into the soil structure and prevented from being an easy prey for microorganisms. As aggregates are formed, POM may be enclosed within the aggregates that is formed by the gluing actions of mucilage and protect the occluded POM from decomposition (Gale et al. 2000a; Golchin et al. 1994 a; Oades 1988; Six et al. 2000). It is thus not merely the contact between soil and litter that facilitates physical protection, but rather a complex cascade of events where root exudates pay a fundamental role. In addition, roots continuously release organic acids (Grayston et al. 1997) and it has been documented that such acids may adsorb to particles and thus reduce the biodegradability (van Hees et al. 2003).

Respiration trends

Trend lines for daily respiration might suggest that the respiration of roots will continue at a higher rate than shoots in the future, thus evening out the differences over time.
Based on the favorable C/N ratio of the added clover (data not shown), it is expected that the total CO_2 respired from clover should be higher than for oat shoots and grass shoots. The estimated relative respiration of the original added C in clover shoots is 26.2 %. This low value may be caused by an artifact in the analyzed CO_2 collected from sampling date 3, 7 and 10 and this is supported by the low accordance with the other data. However, even if these values are corrected to fit the other values obtained, the estimated respiration is still only 28 % of the original added clover C.

The obtained results are based on microbial respired CO_2 and do not necessary equal the amount of C digested from added shoots as such. Some of the digested C is incorporated into the cells of the microorganism (Vold et al. 1999) and one possible explanation for the observed low mineralization rate might be that more of the C in clover is incorporated into microorganisms as compared to oat C and grass C. If this is the case, the clover originated C harbored within the cells of microorganisms may be respired at a later stage, thus indicating a lag in the observed clover originated CO_2.

Conclusion

Based on the data obtained in the *ex situ* incubation experiment it is concluded that after 104 days of incubation, green manure shoots incorporated into loamy soil are respired about 2.9 times faster than green manure roots and that oat shoots incorporated into loamy soil are respired almost 3.9 times faster than oat roots. The observed phenomenon is believed to be caused either by enhanced physical protection of root derived SOC as a result of close interaction with the soil matrix, differences in substrate quality, a combination of the two, or it may have a more complex cause. The incubation experiment will continue beyond the time and scope of this thesis, and whether the observed relationship holds true over time will be evaluated at a later stage. As the ongoing *in situ* incubation trial progresses, the causative factors governing the observed phenomenon in this experiment will hopefully be revealed.

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Appendix 1

Quantity (g) ammonium-nitrate (NH_4NO_3) and nitrogen (N) added (m^{-2}) and total amount solute added (l m^{-2}) at three different occasions during the growing season 2009.

Mp	Date	NH_4NO_3 g m^{-2}	N g m^{-2}	Solute l m^{-2}
5-20	08.06.09	6.9	2.4	4.8
5-20	16.06.09	6.9	2.4	4.8
5-20	23.06.09	6.9	2.4	4.8
		Σ 20.8	**Σ 7.2**	**Σ 14.3**

Appendix 2

Crop weight (g m^{-2}) and weight distribution between grass, clover and herbs at the respective harvest dates given as g m^{-2} and percentage of total weight.

Mp	Harvest date	Clover g m^{-2}	Grass g m^{-2}	Herbs g m^{-2}	Clover % of total	Grass % of total	Herbs % of total	Σ g m^{-2}
1	03.06.09	146.9	285.6	12.4	33.0	64.2	2.8	445.0
2	03.06.09	184.9	239.7	4.1	43.1	55.9	1.0	428.7
3	03.06.09	124.8	276.4	5.9	30.7	67.9	1.4	407.2
4	03.06.09	72.0	304.3	16.5	18.3	77.5	4.2	392.9
1	14.07.09	246.0	61.5	4.7	78.8	19.7	1.5	312.2
2	14.07.09	252.2	68.9	3.1	77.8	21.3	1.0	324.3
3	14.07.09	210.0	84.1	3.6	70.5	28.3	1.2	297.7
4	14.07.09	171.9	90.6	7.7	63.6	33.5	2.9	270.1
1	24.08.09	283.3	109.7	11.2	70.1	27.1	2.8	404.2
2	24.08.09	274.3	119.0	7.7	68.4	29.7	1.9	401.1
3	24.08.09	235.8	128.2	5.1	63.9	34.7	1.4	369.0
4	24.08.09	256.0	134.5	9.9	63.9	33.6	2.5	400.4

Appendix 3

Total crop weight (g m^{-2}), weight distribution: whole crop, herbs, straw, grain and grain spillage (g m^{-2}) and yield index (YI) including grain spillage.

Mp	Σ g m^{-2}	Whole crop g m^2	Herbs g m^{-2}	Straw g m^{-2}	Grain g m^{-2}	spilled grain g m^{-2}	YI
5	826.7	786.9	19.2	284.1	341.0	20.6	0.45
6	792.0	740.0	24.2	368.3	333.0	27.8	0.47
7	934.1	891.6	15.2	446.5	408.3	27.4	0.47
8	698.7	630.1	40.0	318.1	289.0	28.7	0.48
9-20	685.6	664.8	20.8	–	–	–	–