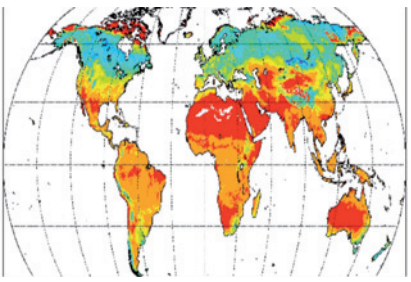

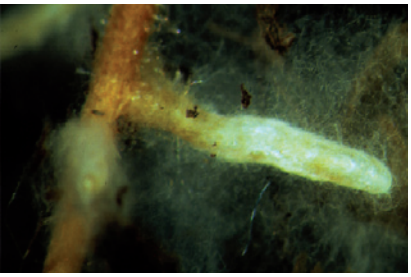

**COST Action FP0803**

**Technical Report**

# Belowground Carbon Turnover in European Forests: Fine Roots, Mycorrhizal Mycelia, Soil Organic Matter and Soil Models

## A Technical Report for National C Reporters, LULUCF Experts and Ecosystem Modellers


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Swiss Federal Institute for Forest, Snow and Landscape Research WSL

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- Soil carbon storage simulated by LPJ-GUESS
- Ectomycorrhiza of Norway spruce with extramatricular hyphae
- Soil profile of a Cryptopodsol at Cima Pianca, Ticino, Switzerland

**Addresses of Authors:**

Ivano Brunner
Swiss Federal Institute for Forest, Snow and Landscape Research WSL
8903 Birmensdorf, Switzerland
ivano.brunner@wsl.ch

Isabella Børja
Norwegian Forest and Landscape Institute
P.O. Box 115
1431 Ås, Norway
isabella.borja@skogoglandskap.no

Lise Dalsgaard
Norwegian Forest and Landscape Institute
P.O. Box 115
1431 Ås, Norway
lise.dalsgaard@skogoglandskap.no

Gaby Deckmyn
Campus Drie Eiken
University of Antwerpen
2610 Wilrijk, Belgium
gaby.deckmyn@ua.ac.be

Alf Ekblad
School of Science and Technology
Örebro University
701 82 Örebro, Sweden
alf.ekblad@oru.se

Douglas L.Godbold
Institute of Forest Ecology
Universität für Bodenkultur (BOKU)
1180 Vienna, Austria
douglas.godbold@boku.ac.at

Irene Guerra Avalos
Servicio de Sanidad Forestal y Equilibrios Biológicos
Dirección General de Desarrollo Rural y Política Forestal
Ministerio de Agricultura, Alimentación y Medio Ambiente
C/Gran vía de San Francisco, 4. 5º planta
28005 Madrid, Spain
at_sgpfd5@magrama.es

Martin Lukac
School of Agriculture, Policy and Development,
University of Reading
Reading RG6 6AR, UK
m.lukac@reading.ac.uk

Karsten Kalbitz
Institute for Biodiversity and Ecosystem Dynamics (IBED)
University of Amsterdam
Science Park 904
1098 XH Amsterdam, The Netherlands
k.kalbitz@uva.nl

Kaie Kriiska
Estonian Environment Information Centre
Mustamäe tee 33
10616, Tallinn, Estonia
kaie.kriiska@keskkonnainfo.ee

Ivika Ostonen
Institute of Ecology and Earth Sciences
University of Tartu
Vanemuise 46
51014 Tartu, Estonia
ivika.ostonen@ut.ee

Andrea Schnepf
Forschungszentrum Jülich GmbH
Agrosphere (IBG-3)
52425 Jülich, Germany
a.schnepf@fz-juelich.de
and
Institute of Hydraulics and Rural Water Management
University of Natural Resources and Life Sciences BOKU
Muthgasse 18
1190 Vienna, Austria

Primož Simončič
Slovenian Forestry Institute
Vecna pot 2
1000 Ljubljana, Slovenia
primoz.simoncic@gozdis.si

Elena Vanguelova
Forest Research, Alice Holt Lodge
Farnham
Surrey GU10 4LH, UK
elena.vanguelova@forestry.gsi.gov.uk

## Foreword

Within the framework of the COST Action FP0803 'Belowground carbon turnover in European forests' several conferences, workshops and Training Schools were held during the period of 2009 and 2013. The first conference was held at our Institute, WSL, in Birmensdorf (Switzerland), as the first event of the COST Action following the kick-off meeting in Brussels (Belgium) in 2009. This conference gathered the knowledge in a 'State of the Art' meeting with contributions in the fields of tree roots, mycorrhizal hyphae, soil organic matter, and biogeochemical modelling. At the conference in Birmensdorf it was also decided that the products of this COST Action should be a series of jointly written review-publications in ISI-referenced international journals.

In the following conferences held at Ljubljana (Slovenia) in 2010, Barcelona (Spain) in 2011, Antalya (Turkey) in 2012, and Bordeaux (France) in 2013, the tasks of the review publications were fixed, the writing process initiated, and its progress tracked. In the course of this process it was also decided that a Technical Report should be produced at the end of the COST Action. This Technical Report should compile all the findings for the National C reporters, the LULUCF (Land Use, Land-Use Change and Forestry) experts and the ecosystem modellers.

In a workshop held in Vienna (Austria) in July 2012, it was decided that such a Technical Report should also contain a questionnaire in order to find out the needs and demands of National C reporters and LULUCF experts regarding fine roots, mycorrhizal mycelia, soil organic matter, and ecosystem models. This questionnaire was sent out in autumn of 2012 to the National C reporters and LULUCF experts of all European countries, to selected countries outside Europe, and to the European Commission's Joint Research Centre (JRC) in Ispra (Italy).

It is our hope that the outcome of this COST Action will be used in the future not only by researchers and modellers who investigate fine roots, mycorrhizal hyphae, and soil organic matter, or by those who apply soil and biogeochemical models, but also by forest managers, who are responsible for a sustainable management of the belowground C pools in forests, and by National representatives and experts of governments, who have to report on their National C budgets under the framework of the Kyoto protocol.

Birmensdorf, in May 2013
Ivano Brunner

## Content

# COST Action FP0803 - Mission of Statement

**Introduction**

The COST Action FP0803 **'Belowground carbon turnover in European forests'** has the aim to improve and to coordinate the methods and the knowledge to measure and calculate belowground carbon (C) turnover rates, and to implement the obtained values in improved biogeochemical models to develop sustainable belowground C management strategies for European forest ecosystems to ensure a maximum of resilience under adverse or gradually changing environmental conditions. The importance of belowground C turnover in the functioning of forest ecosystems is often underestimated. While inputs and outputs of C in the aboveground part of forest ecosystems can be measured relatively easily and continuously, little is known about the mechanisms of belowground C allocation. These include processes affecting the turnover rates of the tree fine roots and of the mycelia of mycorrhizal fungi. Annually, about 0.9 t/ha C flow into the forest soils by the fine root turnover, and about 0.4 t/ha C by the mycelia turnover (Brunner and Godbold 2007, Godbold et al. 2006). Belowground C turnover has also an international dimension, as the changing climatic conditions affect forest ecosystems all over Europe, and as most European countries need to estimate and report the changes of belowground C stocks in forests under the United Nations Framework Convention on Climate Change (UNFCCC) and the Kyoto Protocol (IPCC 2003, 2006, 2007).

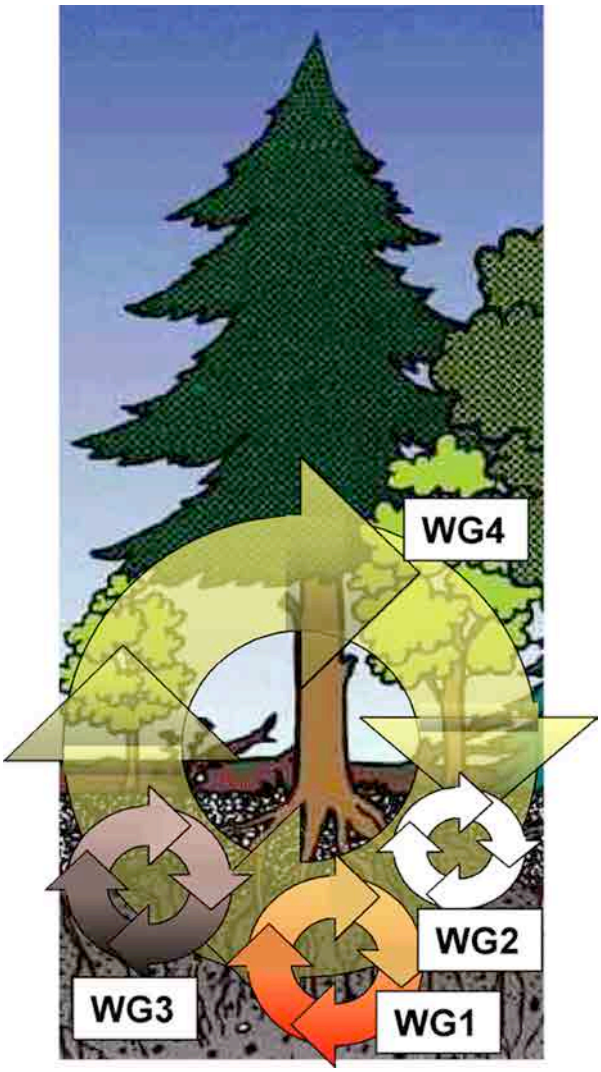
National greenhouse gas inventories involve the estimation of changes in C stock in forests from the five C pools aboveground biomass, belowground biomass, dead wood, litter, and soil organic matter (IPCC 2003, Chapter 3). On the one hand, fine roots of less than 2 mm in diameter are often excluded from the belowground biomass, because these often cannot be distinguished empirically from the litter or from soil organic matter. As a consequence, living fine roots are often included in the litter or with the soil organic matter (IPCC 2003, Chapter 3). On the other hand, the mycelia of ectomycorrhizal fungi are included in the soil organic matter. Soil organic matter refers to a complex of large and amorphous organic molecules and particles derived from the humification of aboveground and belowground litter, and incorporated into the soil, either as free particles or bound to mineral soil particles. It also includes organic acids, dead and living microorganisms, and the substances synthesized from their breakdown products (IPCC 2003, Chapter 3).

Figure 1. Tree roots and forest soils.

It was the aim of the COST Action FP0803 to improve the estimations of belowground biomass, in particular of tree roots (Fig. 1) and of mycelia of ectomycorrhizal fungi, and of the soil organic matter. This included the estimations of the increase (growth) and the decrease (mortality) of all living belowground biomass and their turnover values, as well as the net C stock change in soils. Only good estimations enable a realistic calculation of the overall belowground C stock changes in forests and their integration in appropriate soil and ecosystem biogeochemical models.

**Working Groups**

This Action aimed to harmonise and improve knowledge on the belowground C turnover processes and estimations. The Action focused on four key areas with four Working Groups (WGs, see also Fig. 2),




- WG1: Fine root turnover
- WG2: Mycorrhizal mycelia biomass and turnover
- WG3: Soil C stocks and turnover
- WG4: Biogeochemical modelling

with the intention of linking the different research fields involved, of evaluating the potential of new and innovative methodologies, and of developing new process-based descriptions of belowground C dynamics within biogeochemical models. This is important, as the projections of C dynamics in forest ecosystems under changes in climate and atmospheric C dioxide concentrations need to be improved.

The objectives of this Action has been achieved mainly through the participants of the COST Action by performing Working Group meetings, by reviewing and summarising the current scientific knowledge on belowground C turnover in European forests, by compiling databases, and by promoting Short-Term Scientific Missions STSM.

Figure 2. Simplified scheme of the four Working Groups

In the Working Group meetings the gathered knowledge has been transferred into methodological and technical recommendations, which then had resulted in state-of-the-art reviews on belowground C turnover.

## Tasks of the Working Groups

**The tasks of WG1** were mainly the gathering of knowledge, discussion, and giving common agreements on fine root turnover rates:

- Collecting and itemizing the literature on methods and calculation formulas to estimate fine root biomass, production, mortality, lifespan, decomposition, and turnover rates, e.g. sequential coring, ingrowth cores, mesh bags, max-min method, minirhizotrones, $^{13}C$ and $^{14}C$ isotopes, a.o;
- Comparing and evaluating the current methods for fine root turnover estimations and calculations, summarising the advantages / disadvantages and strengths / weaknesses of the approaches;
- Gathering published turnover rates of European tree species in dependency on e.g. forest ecosystems, soil types, and climatic factors.

**The tasks of WG2** were mainly the gathering of knowledge, discussion, and giving common agreements on the response of the following key processes undertaken by mycorrhizal mycelia: 1) Turnover of hyphae; 2) Decomposition of hyphae; 3) C flux through mycelium; 4) Interactions with soil biodiversity; 5) Incorporation into predictive models:

- Collecting and itemizing the literature on methods and calculation formulas to estimate mycorrhizal mycelia biomass, production, mortality, lifespan, decomposition, and turnover rates:
- Comparing and evaluating the current methods for the mycorrhizal mycelia biomass and turnover estimations and calculations, summarising the advantages / disadvantages and strengths / weaknesses of the approaches;
- Gathering published turnover rates of European fungal species and their dependency on e.g. forest ecosystems, soil types, or climatic factors;
- Evaluating the methods to quantify rates of C allocation to mycorrhizal mycelia and onwards to other soil microorganisms;
- Evaluating the relationship between the responses of mycorrhizal mycelia compared to fine roots.

**The tasks of WG3** were mainly the gathering of knowledge, discussion, and giving common agreements on soil C pools and turnover rates:

- Collecting and itemizing the literature on SOM pools and dynamics in different forest ecosystems, taking into account the variability of climatic conditions, of vegetation and of soil types;
- Comparing the methods to evaluate 1) SOM pools from the qualitative to the quantitative descriptive approaches (i.e. humus forms, chemical fractionation, physical fractionations according to different methods); 2) SOM dynamics (stabilisation, decomposition, mineralisation), taking into account both the pre-treatment step (e.g. fractionation, chemical hydrolysis and oxidation for labile C or lignin evaluation) and the instrumental methods used for the characterization;
- Discussing and evaluating the importance of stabilisation processes, e.g. chemical, physical, biochemical stabilisation;
- Assessing the effects of increasing atmospheric $CO_2$ concentration (through changing biomass input) and changing temperature and moisture on soil processes affecting soil C stabilisation and storage;
- Comparing and evaluating the current methods for the soil C turnover estimations and calculations, summarising the advantages / disadvantages and strengths / weaknesses of the approaches.

**The tasks of WG4** were mainly the gathering of knowledge, discussion, and giving common agreements on biogeochemical models which apply turnover rates:

- Collecting and itemizing the literature on biogeochemical models, and on models which apply e.g. fine root turnover rates;
- Discussing and evaluating models where the incorporation of new modules such as mycorrhizal mycelia turnover rates or soil C stocks and soil C turnover rates can be integrated or incorporated;
- Comparing and evaluating the current biogeochemical models for belowground C or forest ecosystem modelling, summarising the advantages / disadvantages and strengths / weaknesses of the approaches:
- Integrating the outcomes of the WG1, WG2 and WG3 into suitable models which include belowground C turnover rates and running them;
- Sensitivity analyses of BGMs, including alternative process representations;
- Upscaling of belowground C dynamics achieved at the site scale to regional to global scales;
- Communicating the remaining uncertainties to researchers, issue managers, policy makers, and stakeholders;
- Proposing and developing sustainable belowground C management strategies to ensure a maximum of resilience under adverse or gradually changing environmental conditions.

**The tasks of all WGs were:**

- Resolving controversies and giving common agreements on turnover methods, turnover calculations, and on turnover rates to be used by modellers;
- Putting together the outcomes and writing peer-reviewed publications for international journals.

**Results and dissemination**

The short-term expected result was to increase the scientific basis for estimating belowground C turnover rates, in particular for fine roots of trees, of which currently only "assumed" values are applied in biogeochemical models. The long-term expected result was that new model parameters could be introduced into the models which otherwise would overlook relevant C contributions to forest soils, e.g. that of mycorrhizal mycelia. By providing a better understanding of the mechanisms governing soil organic matter pools, the results of the Action allow a scientific link with available soil databases. If the abiotic conditions governing the relative proportion of soil organic matter pools are well known, preliminary information on forest areas not covered by detailed studies can be extrapolated from the knowledge of soil types and climatic conditions.

Dissemination of the knowledge to researchers and modellers has been achieved by the organisation of Training Schools. These Training Schools were held to learn either how to estimate belowground C turnover or how to transfer the knowledge to modellers in order to implement effective turnover values into improved biogeochemical models. Several joint publications (Brunner et al. 2013, Cerli et al. 2012, Deckmyn et al. 2013, Ekblad et al. 2013, Wallander et al. 2013) had contributed to the dissemination of the outcome of this Action. The target groups were not only the researchers and modellers who investigate soils of forest ecosystems and who apply soil and biogeochemical models, but also forest managers who are responsible for a sustainable management of the belowground C pools in European forest, and regional and national representatives of governments, who have to report on the C budgets of their countries due to the Kyoto protocol.

# Belowground C in Forests Soils: Turnover as a Key Process

## Introduction

Globally, forests cover 4 billion ha or 30% of the Earth's land surface. The total amount of carbon (C) stored in these forests are estimated to be about 230 Gt C in the aboveground biomass, 60 Gt C in the belowground biomass, 40 Gt C in dead wood, 20 Gt C in litter, and 400 Gt C in forest soils (Kindermann et al. 2008, FAO 2005). Here, the C in the belowground biomass includes the living biomass of live roots, however, with the exclusion of the fine roots of less than 2 mm diameter, because these often cannot be distinguished empirically from litter or soil organic matter (FAO 2005). In such cases, these fine roots are included either in the litter or in the organic matter of mineral and organic soils (FAO 2005).

Forest soils host large amounts of tree roots. The amount of C incorporated in the tree roots, accounts between 14 and 27 t/ha C (Brunner and Godbold 2007). This amount is small compared to the amount of C, which is included in the forest soil as soil organic matter (SOM), between 65 and 329 t/ha C (Brunner and Godbold 2007), However, between 10% and 20% of the roots in forests are fine roots (Jackson et al. 1997). This is relevant, because these fine roots are delicate (see Fig. 3) and in temperate forests have a relatively short lifespan (=turnover time) of approximately one year. Therefore, fine roots deliver forest soils with significant amounts of C every year throughout the entire depth of the root-zone soil profile (Rasse et al. 2005). The same can be assumed for the extramatricular hyphae of mycorrhizal fungi. The hyphae are an extension of the mycorrhizas of the fine roots, forming both fine foraging hyphae and courser rhizomorphs. The hyphae enlarge their surface area to gather nutrients and water and, thus, act as primary C suppliers throughout the whole rooting zone, similarly to the fine roots. However, the lifespan of the fine hyphae is expected to be much shorter than that of the fine roots, between weeks and months.

Soils, as mentioned above, contain in the form of SOM large amounts of C. However, SOM is mostly stable with residence times (=turnover time) of one to several hundreds of years, with the C trapped in SOM, and most likely, C accumulates in young forests, is in an equilibrium in old forests, or mineralises under a degradation environment.

While the turnover of the fine roots and the hyphae of mycorrhizal fungi supplies the forest soils with C, the turnover of SOM diminishes the C from forest soils. The turnover (=turnover rate; $yr^{-1}$) is the inverse of the turnover time (yr).

Figure 3. Mycorrhizal fine root of Norway spruce with extramatricular mycorrhizal hyphae.

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**Turnover as a key process**

The turnover of roots is traditionally calculated with dividing the annual root production by the maximum standing crop (Gill and Jackson 2000). However, several methods exist to estimate the annual root production, either with the maximum-minimum method, the decision matrix, or with root ingrowth cores (see also Majdi et al. 2005). Alternatively, the lifespan (=longevity) of the roots, of which the turnover is the inverse of the lifespan, is estimated using minirhizotrons observations by monitoring appearance and disappearance of fine roots. Most recently, lifespans of roots are also estimated using 'bomb' radiocarbon ($^{14}C$) measurements or the $^{13}C$ signals in free air $CO_2$ enrichment (FACE) experiments using $CO_2$ with altered $^{13}C$ composition of the root cellulose (see also Majdi et al. 2005).

The turnover of hyphae of mycorrhizal fungi in forest soils is calculated, similarly to roots, by dividing the hyphal production by hyphal biomass (Wallander et al. 2004). Here, the production of hyphae is estimated using mesh bags, and the biomass of the hyphae with measuring the phospholipid fatty acids (PFLA) as fungal biomarkers. Most recently $^{13}C$ signals from FACE experiments were used to estimate the turnover of hyphae (Godbold et al. 2006).

Turnover of SOM is estimated with several methods, by decomposition studies, using the natural labelling of the stable isotope $^{13}C$ from C3 and C4 plant litter, the in situ labelling of SOM with 'bomb' $^{14}C$, and the $^{14}C$-dating using the natural radiocarbon decay (von Lützow et al. 2007). The $^{14}C$ dating with $^{14}C$ having a half-life of 5570 yr has a time frame of 200-40'000 yr. This technique, however, will produce only meaningful results for functional SOM pools with a homogeneous turnover rate (von Lützow et al. 2007). Otherwise, the dating will result in a mixture of old and young SOM (apparent mean $^{14}C$ age), which is then also expressed in the commonly used term mean residence time (MRT) (von Lützow et al. 2007). Therefore, a variety of methods are used to differentiate the SOM pool into functional homogeneous SOM pools that are formed by specific stabilisation mechanisms (e.g. recalcitrance, spatial inaccessibility, organo–mineral interactions) either with physical or chemical methods, or the combinations of fractionation methods (von Lützow et al. 2007).

A simplified scheme of the relevant terms and processes of belowground C turnover is shown in Fig. 4.

**Turnover of roots, hyphae and SOM**

Total fine root mass in forests is estimated between 5 and 8 t/ha (Jackson et al. 1997). About 52% of the total fine root mass are living fine roots (=biomass) with a C content of 49%. Thus, C from living fine roots is about 1.1 to 2.4 t/ha C in forests. With an approximate turnover of tree fine roots between 0.5 and 1 /yr (Gill and Jackson 2000), the input of C accounts for about 0.5 to 2.4 t/ha·yr C. One of the discrepancies of the calculation of the turnover time of the fine roots originate from the situation, that either both the biomass and the necromass are included in the total mass calculations or only the biomass (e.g. 'standing crop', see also Gill and Jackson 2000).

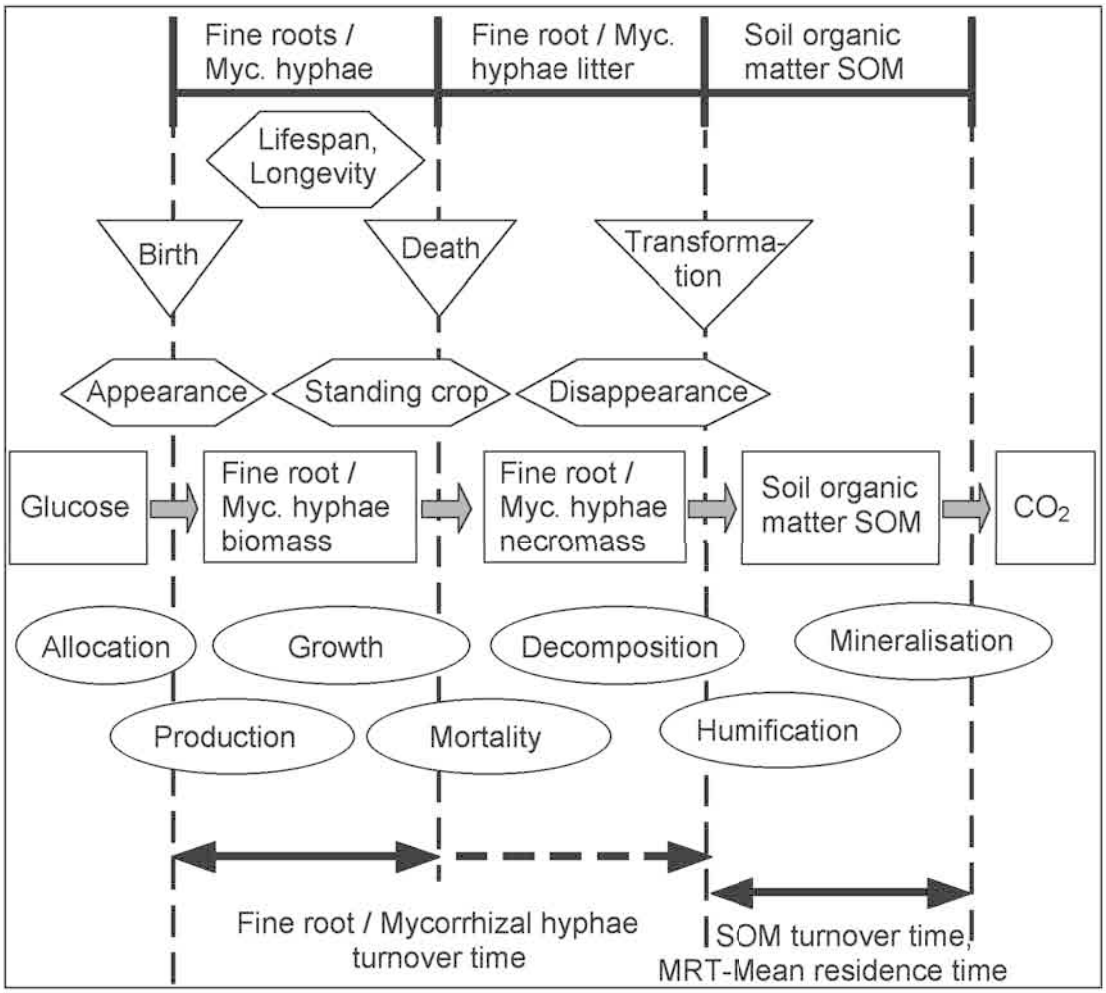


Figure 4. Simplified scheme of the relevant terms and processes of belowground C turnover in forest soils. For the dotted line of the estimation of the root/hyphal turnover time see the comment in the text.

The biomass of mycorrhizal mycelia in forest soils is estimated to be about 0.7 to 5.8 t/ha, with an annual production of about 0.1 to 0.6 t/ha·yr (Wallander et al. 2001, 2004). The lifespan of fine hyphae of mycorrhizal fungi was calculated to be about 9 days using data from a FACE experiment (Godbold et al. 2006), thus, mycelia would have a turnover of about 40 /yr. Godbold et al. (2006) calculated an approximate C input into soils from fungal hyphae of about 5.2 t/ha·yr C, which was in their experiment actually higher than the C inputs from fine roots. This stresses the potential importance of mycorrhizal hyphae in soil C dynamics, but also the need to obtain more data on the turnover of hyphae of mycorrhizal fungi.

SOM has various SOM sub-pools with its characteristic turnover times. Using $^{14}C$ analyses after soil particle size fractioning of a forest soil, the C of the fraction 5-2000 µm had the turnover time of 5910 yr, that of the fraction 2-5 µm 1660 yr, and that of the fraction <2 µm 75 yr (Quideau et al. 2001). $^{14}C$ analyses after soil density fractioning of a podzolic forest soil resulted in turnover times of 70-1200 yr for free particulate organic matter (FPOM), of 120-1880 yr for occluded particulate organic matter (OPOM), and of 180-2170 yr for mineral associated organic matter (MAOM). However, differences between the horizons were evident with increasing ages with increasing soil depths (Schulze et al. 2009). Therefore, despite the large C stock of the forest soil in this study (between 130 and 203 t/ha C) and the high turnover times (or small turnover rates of 0.01 to 0.0005 /yr,

respectively), the decrease of the C due to turnover is small, approximately 0.05-2 t/ha·yr C.

**Conclusions**

A simplified scheme of pools and fluxes is shown in Fig. 5. If pool A would be the roots, then its turnover would result in a delivery of C to the soil organic matter SOM (pool B). The root pool can only increase when the input increases (e.g. increased productivity), or the output would become smaller (e.g. reduced mortality). The turnover of SOM results in $CO_2$ and, thus, in a loss of C from the soil.

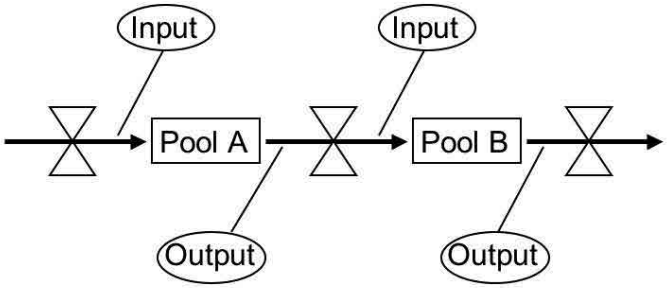


Figure 5. Simplified scheme of pools and fluxes.

The rough estimations on the C inputs into soils deriving from root and hyphal turnover and on the C loss through SOM turnover result more or less in a steady state and in an equilibrium of the system, although relative to the total soil C pool, only small amounts of C are lost due to heterotrophic respiration and the leaching of dissolved organic C and matter (DOC, DOM).

It is the challenge of the future to be able to predict the changes of belowground C in forests soils under altered climatic conditions such as the conditions of global climate change. Elevated mean temperatures, prolonged drought periods, altering precipitation patterns, increased occurrences of storms, more frequent insect attacks, and the appearance of new fungal pathogens are only a few challenges which the forests may face in the future, and which might hamper the predictions on the changes of the belowground C in forest soils. Thus, the understanding of the belowground turnover processes is an issue of highest priority and all efforts need to be done in order to improve its knowledge.

# General Information on the Questionnaire

In October 2012, a letter has been sent to National C reporters and LULUCF experts. This letter contained general information about the COST Action and a questionnaire with eight questions.

**COST FP0803:** The COST Action FP0803 "Belowground C turnover in European forests" aims specifically investigations on the belowground carbon (C) turnover in forests. As an output from the COST Action, we wish to facilitate the clarification of issues associated with the estimation of emissions from the LULUCF sector. We are writing to seek your opinion on potential knowledge gaps related to estimates on C change in forest soils i.e. pools definition (including sub-pools), size and turnover of pools and uncertainties. What knowledge gaps exist and how can they be addressed? As a scientific group supported by the EU COST framework, our aim is to offer guidance to the LULUCF/KP expert community in the form of a Technical Report. To do this we would like you to complete a very short questionnaire attached to this letter.

Below, you will find information on a) what does this COST Action do and why, b) issues where we believe our knowledge and research could be used, and c) a short list of questions for you to answer in order to focus our collating as well as our future research efforts. All questions focus on belowground forest C. We are aware of the fact that answers will depend on the Tier level and method applied.

The objective of the COST Action FP0803 is to improve and to coordinate the methods and knowledge on measuring and calculating belowground C turnover rates. Further, we aim to implement these turnover rates by incorporation into existing biogeochemical models. These, in turn, will facilitate the development of sustainable belowground C management strategies for European forest ecosystems to ensure maximum resilience under adverse or gradually changing environmental conditions.

## Issues

**Belowground biomass models:** At the present there are several biometric models parameterised for the estimation of both coarse and fine root biomass and for the most common European forest tree species (e.g. spruce, pine, beech and oak). Wider use of these models would allow for more accurate estimation of belowground inputs, a process, which has direct relevance for soil C storage and dynamics.

**Dynamic soil carbon models:** A limited number of models are currently used to estimate forest soil C change (CENTURY, RothC, Yasso, Yasso07, ROMUL). These models have been used in a research context for many years. Thus, we as a group, have knowledge and data that could be helpful both in the use and the parameterisation of these models. An overview of the performance of the models in a variety of situations, may be valuable complementary information when national model estimates need to be verified.

**Uncertainty:** In our group, there is a process of reviewing uncertainties in C estimates for forest soils at different scales: from the extraction of the individual soil sample, through steps of chemical analyses and calculation, to the methods of up-scaling to the National level (total forest area). We also aim to provide recommendation of reducing the existing levels of uncertainty. This review will focus on the experience from soil measurements in Europe.

**Reporting rules and definitions:** With reference to the current Kyoto commitment period finishing in 2012, we understand that the reporting rules (Good Practice Guidance for Land Use, Land-Use Change and Forestry, GPG 2003) are in place and cannot be changed.

However, we feel that there is a large amount of ambiguity in the description and classification of the dead organic matter pools (Table 3.1.2, GPG 2003). As an example, fine root biomass below 2 mm diameter is currently considered a part of the dead biomass pool (necromass), which is not appropriate. This issue is important especially when using soil C models (as outlined above). Model input of dead organic material – both above and belowground – needs to be quantified and apportioned correctly to run these models. Further, we believe that we can contribute to the discussion of several crucial points in the process of designing the **new** IPCC Good Practice Guidance for LULUCF.

**Availability of data and research outputs on soil C dynamics:** We are in possession of review papers detailing values of important parameters linked to belowground processes (e.g. fine root turnover). According to the response from the LULUCF expert community, other reviews could be prepared for additional parameters considered crucial to reporting, such as turnover rates of fine roots, amount/dynamics of mycorrhizal hyphae, size of soil C pools. We think that such tables would help in the estimation of input to soil C models. Such parameter lookup tables could be structured based on the four typical biomes found in Europe: boreal, temperate, oceanic and Mediterranean.

**Questions:**

To aid us target the contents of the Technical Report including tables and background knowledge, we would like you to respond to the following questions. Please bear in mind that our group specialises on soil C stocks (litter and organic carbon in mineral and organic soil) and turnover in forests – thus we can only provide data / advice relating to these issues:

1. Would you benefit from guidance in the design of field methods and sampling intensities for (re)measuring soil carbon? You may specify any major challenges you have when coordinating new soil inventories with already existing frameworks (i.e. National Forest Inventories)
2. Would you benefit from information on organic soils (i.e. definition, emission factors, management implications)?
3. Do you report changes in litter and soil organic carbon pools in mineral soil on forest land? If so, are you currently using a soil C model to estimate soil C stock and / or change? If YES, which model? Do you require help with the classification and quantification of belowground living biomass (roots, fungi etc.) and soil organic matter pools?
4. What kind of help (if any) do you require with the parameterisation and input estimation to your model (litter)?
5. What models have you used in the past to estimate changes in litter and / or soil organic carbon, and what were the problems (if any)?
6. What has been done toward a verification of your change estimates in litter and / or soil organic carbon? What are your major challenges in the verification process and what information could be helpful to improve your verification efforts? (This includes any efforts toward the support of “no source”).
7. Indicate what type of data/output you would like to report, but cannot calculate at present and why? (e.g. data, resource, or model unavailability).
8. In your experience, which are the five most important gaps in knowledge that you as a LULUCF expert feel should be addressed?

# Questionnaire with Answers and Comments

## Question 1:

**Would you benefit from guidance in the design of field methods and sampling intensities for (re) measuring soil carbon? You may specify any major challenges you have when coordinating new soil inventories with already existing frameworks (i.e. National Forest Inventories)**

## Answers:

**Austria:** Guidance on the design of sampling is appreciated. We expect that the majority of sites is not undergoing a strong temporal change in soil C stocks. However, we are aware that certain types of ecosystems are particularly vulnerable to soil C losses. Efforts to identify potential hotspots of C dynamics would help to focus the sampling efforts to the most relevant sites (Challenge: harmonization with previous soil assessments).

**Czech Republic:** Yes, any improved guidance would be helpful. However, rather on focusing to new data acquisition, I would welcome new data compilation in form of easy to use reference soil carbon stock values for the common European forest types, including uncertainty. As a LULUCF emission inventory compiler, I would prefer using the end-results of soil-related research rather than being involved in soil sampling myself. In terms of the current soil sampling programs within NFI or other country-lever inventories (e.g., landscape inventory), the obvious challenge is dealing with volumetric (quantitative) soil measurements with reasonable accuracy and working efficiency.

**Denmark:** We can always use more information to guide is regarding inventory designs for repeated sampling of soil C. There have been a few studies on designs of inventories already (France: Saby and Arrouays and US: Palmer). We are currently working on analysis of repeatedly sampled soil data in a 7x7 km grid as well as baseline soil data from a subset of permanent NFI plots. The plots in the first grid (where we rely on existing plot designs) are so large (50x50 m) that spatial variability within plots may hamper assessment of changes.

**Estonia:** Under ICP Forest and BioSoil programs, some soil surveys have been done following sampling methodology determined in before mentioned program manuals, thus Estonia does not need guidance of the field methods. Sampling intensity, on the other hand, is dependent on available financial resources, therefore recommendations from the COST Action FP0803 working group are very welcome, but due to limited resources, the implementation of these recommendations cannot be guaranteed.

**Finland:** According to Mäkipää et al. (2008) soil carbon inventory with adequate sampling size (plots and inside plots) would cost 9 million euro and would take at least 15 years. So this is not an option for Kyoto reporting. We have studies relating to sampling design, but I would really appreciate new methods to be used to estimate soil C content. Something else than coring. The major challenge is money. We will not have money for nationwide soil inventory. Due to this we have to rely on smaller samples and use those for model testing and model development.

**France:** Maybe such guidance would be beneficial, but I am not linked enough with such programs to say that there are needs of guidance. On forest soils, I know three different

programs of soil monitoring:
- The RMQS (Réseau de mesure de la qualité des sols) which is a very large program led during a first period 2000-2010. A second period should begin now, but currently we only have one view of the soil characteristics with this network. My correspondent for this monitoring is Manuel MARTIN (infosol / manuel.martin@orleans.inra.fr).
- The NFI, but descriptions of soils with this survey are less, you cannot have carbon content for example. My correspondent for this monitoring is Antoine COLIN (IGN/ Antoine.Colin@ign.fr).
- The RENECOFOR network, which relates to two consistent periods of measurement. This network is about to be analysed to estimate carbon fluxes on forest lands within the framework to the LULUCF inventory. My correspondent for this monitoring is Manuel NICOLAS (ONF/ manuel.nicolas@onf.fr). I guess that for these monitoring methods and sampling intensities are already defined, but it could maybe be improved.

**Greece:** We believe we would benefit from guidance in the design of field methods and sampling intensities for (re)measuring soil carbon. We have not conducted yet a new soil inventory, however this is within our plans for the near future and we plan to utilize the existing ICP forest sampling network, where soil carbon was measured in the past in some plots of the LEVEL I and all the plots of the LEVEL II (according to the relevant guidance of the ICP forest manual), while additional new plots will be selected.

**Iceland:** Yes. We have a NFI and do sample on each measurement plot a one sample of soil (down to 30 cm) and one collective sample of litter, vegetation under 50 cm height and the humus layer. We revisit each plot with 5 years interval and sample again these two samples. Currently we have not analysed these sample series because of lack of funding.
If someone knows about student(s) who want to analyse these samples and use in his thesis i.e. we would be very happy about that!

**Ireland:** No, there is sufficient guidance on these and establishment of inventories should reflect national circumstances. However, major challenges include a) Lack of historic land use trends with soil carbon databases (20 to 50 m yearsr) b) large variability on soil C stocks; c) lack of country specific data on emission factors from organic soils and how these relate to land use change; d) in sufficient funding for establishment of large soil inventories.

**Japan:** We carried out national forest soil carbon inventory in ca. 2500 points all over the country from 2006 to 2010 in which carbon stocks were measured for dead wood, litter and surface soil. We started a second survey in the same points from 2011. For the measurement of dead wood in these projects, we adopted a method described in the LULUC-GPG (2003) to determine the carbon stock of dead wood.

**Latvia:** We have already forest soil inventory in place, it is not integrated yet with National forest inventory; however, it is question of funding. Sampling is done in 95 plots, the idea is to extend it to another 115 plots. Sampling period is 5-6 years, taking samples at once in all plots. We use composite samples from certain soil depths (litter to a whole depth, 0-10, 10-20, 20-40 and 40-80 cm). The same samples are used for physical and chemical analyses. Comparative analysis shows no significant difference between whole layer and middle of layer sample analysis. The most problematic issue is separation and sampling of litter layer. At the moment litter is considered every dead biomass laying on the ground above soil layer with diameter of less than 2 mm, actually no dead branches are collected. Accounting of dead wood starts with 6.1 cm diameter, therefore considerable part of dead

biomass is not accounted. We would benefit from extrapolation method to calculate small fractions of dead wood as a function of large fractions of dead wood and litter. Sampling depth of litter or border between litter and soil is another problematic issue, especially on organic soils; different soil scientists have different positions on that; thus results of carbon stock change in litter (and actually in upper soil layers) are not comparable between periods, if sampling is done by representatives of "different schools". This issue should be somehow solved, probably in level of mathematics and data processing.

**Lithuania:** We have experiences for measuring soil carbon only on Level I plots (BioSoil project) according to methods of ICP-Forests. The results of BioSoil project were not helpful for LULUCF reporting. Lithuania has not national values for carbon pools in soil and forest litter. We have plans to initiate soil carbon monitoring on NFI plots in 2013.

**New Zealand:** I am sure that it would be helpful to learn from others' experiences. New Zealand has undertaken robust approaches to field campaigns and sampling, but still has issues with data coverage. Soils fall outside of other national inventory efforts (forest inventories often concentrate on biomass measurements and/or only collect surface soil (to 10 cm depth), rather than the 30 cm depth at which reporting occurs). Lack of funding has hampered regular and exhaustive sampling of soils. Due to the variability of field data, and issues involved with modelling significant land-use effects in SOC pools, determining the best focus areas for soil sampling has also been an issue.

**Norway:** For the Norwegian NFI a general remeasuring programme of soil carbon which was measured ca. 1990 (1000 locations / plots) is not currently an issue. This is due to 1) costs, 2) the NFI plots can not be used for destructive sampling of any kind, and 3) methodology in 1990 is difficult to replicate. Furthermore – many plots (typical forest stands in Norway) are in rocky and mountainous terrain and soils are shallow and difficult to sample.

**Romania:** Yes, I would. In my country we have a monitoring system of 16/16 km (about 240 points), in which we take samples of organic C in 1994 and 2004. But, the data are not completely comparable: in 1994 they were only for 0-10 cm and 10-20 cm and in 2004 for 0-10, 10-20, 20-40 and 40-80 cm (like for other countries according to the European rules-BIOSOIL and FOOTMON projects). We want to do another inventory in 2014. But could be some problems:

- If in 2014 will be a drought year? (The amount of organic C could be different). A yearly inventories will be better, but who could afford these, especially for a big country?
- Because of the large number of points there could be problems with the teams (a lot of people, not all specialists in soil etc), with the timing (different periods maybe for some points) etc.

And we have a National Forest Inventory started in 2008 at 4/4 km: more than 3000 plots till now. I think the remeasuring the C content in these points could answer to the question, but there are a lot of uncertainties and till now no complete guidance:

- How I will take the samples for litter? (Together with the humus or the last one is incorporated in the first 10 cm of soil?).
- How many plots are necessary for a country area (our example: 6.5 million hectare of wood and more than 20 soil types).
- How will be took the samples? By fix depth or by pedogenetic horizonts?

I think good inventories for all European countries (with the same methodology) in the future is needed.

**Russia:** Soil carbon measurements in Russian forest soils take places two ways at the moment: 1) From ICP Forest Monitoring plots, but due to very large territory they cover European North-West region only; 2) Collecting the database on soil samples from literature and from scientific organizations and strict nature reserves. They cover larger territory and can be linked with forest typology. Arable soils have better data but they changes very fast due to changes in land-use (for example about 35'000'000 ha of former arable soils in Russia are abandoned now and are exposed to spontaneous afforestation (Kurganova et al. 2010).

**Slovakia:** In most countries only soil data from international or national soil surveys that were not directly linked with requirements of LULUCF reporting are available. In fact, even if we know very much about sampling intensity, variability and uncertainty, the (re)sampling is too costly. However, guidance in the sampling design can be useful.

**Slovenia:** Yes, any additional approach to already existing methods and soil monitoring programs is desirable. Common soil monitoring for all land use categorises for whole countries are more exception than the rule; in Slovenia soil pollution monitoring activities are related to limited areas, soil fertility monitoring was carry out for specific land uses (intensive agriculture LU), in the past basic soil mapping activities (whole country) were done. Beside national there are some international project like ICP Forest activities in 1995/1996 and Biosoil demonstration project, module Soil (Forest Focus; Regulation (EC) No 2152/2003) 2005/2006, forest soil monitoring at systematic 16x16 km grid was performed at national and EU level (level I plots, partly level II plots); In 2007 KP soil monitoring only for forest land (8x8 km grid) was performed. In 2012 at few tens plots of non-forest LU soil sampling and carbon analysis were carry out.

The main problems of soil monitoring activities are comparability of sampling methodologies due to the modifications between different sampling periods and in time, different soil sampling depths, representativeness of sampling areas versus aboveground dendromass, heterogeneity of the forest stands/relief and areal carbon content, difficulties regarding soil bulk density data (no historical data), soil bulk density pedotransfer functions (need to be calculated for specific regions, local areas…), and mainly to the deficiency of funds and interest of (expert, public, political…) founders and non-existing national and EU soil regulation(s). The soil monitoring is expensive activity with a lot of possible aspects and interests like UNFCCC/KP GHG reporting, drought sensitivity calculations, soil fertility / productivity information, biodiversity, etc.

All mentioned soil-monitoring activities are part of long-term process obtaining better soil data, improving individual segments of soil monitoring within the limits of KP Parties capacities.

National Forest Inventories are a good frame for forest soil monitoring activities but in spite of logical link data relations (e.g. soil properties and site productivity, above and below ground carbon content relations), implementation of such approach depends on many factors and is different for Annex I Parties (soil data could be a part of forest management planning practice as a basic site quality information).

**Spain:** Any indicative guidance is always welcome. Although Spain has not considered the creation of a national soil inventory, it could be interesting as an option to develop it in the future.

For reporting it's important to separate between what is called "forest land remaining forest land (FL-FL)" and "forest land in transition" which comes from a land use change.

Currently, we are trying to use data from different nets and inventories (BioSoil, Futmon Large Scale Monitoring, Futmon Intensive Monitoring, Land Use inventories and

some other based on the tree species autecology) designed with different objectives and referred to incomparable cartographies so our challenge is to adapt the measurements of soil carbon to make the results comparable.

In the case of FL-FL only data obtained from BioSoil and Futmon monitoring are comparable for soil organic carbon content change. To solve this problem another option was considered: we have found that although there are lots of profiles analyzed since 70´s is very difficult to make any correlation with the soil organic carbon stocks and the environmental conditions or aboveground vegetation due to the huge variability existing in the Spanish forests.

Summarizing: at first it would be very helpful for Spain to be able to establish a relation between aboveground C content and environmental conditions and belowground C content and fluxes (roots and mineral soil) at the more detail possible level, but at least at general level for Mediterranean countries.

**Sweden:** We already have an inventory of forest soils in place where we just have finalised the field part of the third re-inventory. We have no plans for changing the inventory setup for the fourth re-inventory but are of course interested in participating in discussions with others that plan to start an inventory or have experiences from successive inventories.

**Switzerland:** No, since I'm not involved in field sampling

**Joint Research Centre JRC:** In principle IPCC splits “your” belowground C stock in several pools, as shown in the 1st comment.

1) Clear definition of the C pool which is measured using both qualitative and mainly quantitative criteria (e.g. organic matter dimensions/ranges, type and origin; soil depth on which is measured). This would include quantitatively based definition of mineral soils (C content and soil depth). Also, it should be also clear how the definition is implemented by the sampling method.
2) Is there a continuum between C pools (i.e. what is not reported as root is reported under the soil’s organic matter, i.e. are fine roots (<2 mm) included in any of the pools. How I remember pre-processing of soils samples excludes any living matter, while root biomass pool excludes them because of sampling methods! Sampling method should allow estimation of missing pool.
3) Measuring methods should be able to capture C stock changes for short time intervals (as needed under GHG inventory).
4) Method should be able to capture the trend of C pool change, at least.
5) UNFCCC reporting and KP accounting rules allow use of “not a source”, which means it is enough to demonstrate (including by ecosystem response based reasoning) that the C pool does not decrease in time (so no emissions from that pool), which is reported in tables by notation keys.
6) Since GHG inventory and reporting is due on annual basis belowground C stock change in 5A1 (forest remaining forest) may not be relevant. Meantime, it may be very relevant for any conversions from/to forest (reported under land category 5A2 – conversion to forestland or 5B2, 5C2,… as conversions to Cropland, Grassland,…).
7) Share the information (publication, databases, etc) and join decision making process at any level (national, international).
8) GHG inventories follow reporting requirements: on one side “completeness, comparability, accuracy on all land uses and C pools/GHG sources” and on other side “resources efficiency”. This means “negligible” sources/sinks are in practice

disregarded and focus is on key sink/source categories. How much is negligible is not clear, but in general changes in soils related pools (SOM, LT…) are here!

9) Ability of method to derive “actual emissions”, i.e. emission in the moment it occurs rather than “potential emissions” generated by linear interpolation of the 2 moments in time (i.e. in deforestation, SOM emissions are much higher in first years)
10) Repeated sampling looks very promising (in theory), although in practice even 10 years is a lot of time and risks of inconsistency are very high, not only because staff changes and technical progress but ecosystemic changes, like litter presence on afforested lands (i.e. in afforestation on cropland) so layer separation problems
11) Implementation of standardized methodologies and trainings offer best way for reducing uncertainties, harmonization is helped by cooperation.

## Overall comments:

**JRC:** Some of old JRC highlights are still relevant (with more highlights on definitions, training and development of ways for better statistical processing). Almost all countries have in place some kind of soil inventory, usually related to NFI, but all seem to have problems / or at least afraid of resampling. Sampling and resampling methodologies are very heterogeneous across the countries and further guidance seems to not be of very high interest. Reasons are mainly related to costs and risk of methodological inconsistencies due to long cycles. Empiric data seem to be interesting for validation of modelling and simulation exercises. For example 2006 Guidelines tries to standardize the definition of DOM and SOC layers, by defining a 2mm threshold for particulate to be classified under one or the other (Table 3.1.2 in GPG 2003 and 1.1 in 2006). We all know that this is not possible, but IPCC provides way out allowing reporting on “national circumstances”, realistically actually…

## Question 2:

**Would you benefit from information on organic soils (i.e. definition, emission factors, management implications)?**

## Answers:

**Austria:** We would somewhat benefit from this information. Organic soils (peat-lands) are not widely distributed in Austria, they are not actively managed. We expect that these soil types have no significant impact on the national GHG budget. Nevertheless, additional information would help for testing our hypothesis.

**Czech Republic:** Not that much as the share of organic soils is limited in our country in contrast to others and forest management is usually restricted on organic soils.

**Denmark:** Definitely! Currently we have had to align our definitions in Denmark across land uses agriculture and forestry for comparability (>12% C in 0-25 cm). Forest soil definitions do no always match those used for agricultural sols. Moreover, definitions may be very different among countries. As long as this is not resolved it makes little sense to give common emission factors.

**Estonia:** Estonia would greatly benefit from additional information on organic soils, since country-specific data regarding this soil type is very scarce.

**Finland:** Yes, process based emission factors for drained forested peat-lands would be very nice.

**France:** Currently, organic soils are considered as negligible in the French national inventory, the uncertainty on other sources/removal is still very large and this issue is not a priority. Then of course if easy guidance and data were available it could be improved in the French inventory. So yes, according to me it could be beneficial.

**Greece:** We have few organic soils in Greece (and mainly on agricultural land), thus information on them would not add significantly to our needs on information on forest soil carbon stocks and changes.

**Iceland:** Yes. Small portion of the forest areas are located on organic soils that can be very different from one plot to another. Some have been drained other not.

**Ireland:** Yes, but these should reflect national circumstances.

**Japan:** The distribution of organic soils is limited in Japan. We did not use the information on organic soils in LULUCF-GPG.

**Latvia:** We are going to elaborate methodology on estimation of carbon stock changes in drained forest on organic soils. However, changes of emissions of $N_2O$ and $CH_4$ due to drainage in different organic soils is considered to be too costly to obtain reliable results, therefore we would like to take part in an international initiative to estimate changes of fluxes of these gases depending of fertility of soils and stand characteristics. Considering abundant amount of the emissions factors on $N_2O$ and $CH_4$ in scientific publications differing thousands of times, we would like to evaluate internally the range of emissions in different forest stand types.

The same situation is with swamps and peat-lands cleared for peat production and with croplands and grasslands on organic soils. We need emissions factors of $CH_4$ and $N_2O$ also for these areas. Particularly we need to evaluate impact of different management approaches. The emissions factors of $CH_4$ and $N_2O$ should be also climatic conditions specific, respectively. Average temperatures and precipitation should be used as factors in emissions' equations.

**Lithuania:** Lithuania has not problems with definition and area identification of organic soils for LULUCF reporting. The national value of emission factors have not detected yet. We are using emission factors from IPPC guidance.

**New Zealand:** Yes. Currently New Zealand uses Tier 1, although we are interested in moving to Tier 2. This of course, requires country-specific data, so would not directly benefit from European efforts in forest soils. (Additionally, NZ does not report on organic forest soils, but does report organic soils for other land categories.) Still, more elucidation of definitions, emission factors, and management implications could improve the coarse-level global defaults currently used.

**Norway:** Yes. Specifically in the boreal zone, drained vs. non-drained soils. Longevity of the effect of drainage. Any factors that may be important (site index/vegetation type/climate/water table…). Also non-$CO_2$ emissions. In NFI the definition of organic soil is the depth of the organic layer and not the carbon content. What is the error / effect of this field based / pragmatic definition vs. definition based on chemical analyses.

**Romania:** No. The surface of organic soils in Romania is small (1967 ha in forests, from a total area of 6.5 mio ha).

**Russia:** Definition and corresponding measurements of fine roots are the most important problem for the models application. Lack of an agreement about separating of roots in fractions, I met fine roots definitions as <2 mm, <1.2 mm, <0.6 mm. It is different to verify models with such variety of definitions.

**Slovakia:** No. The area of organic soils in Slovakia is negligible.

**Slovenia:** Slovenian soils are mainly mineral soils, there are few organic soils areas in forests; BUT certainly all clear definitions are welcome to avoid any further LULUCF reporting problems; different soil types/LU due deriving from different soil database could provoke misunderstandings in soil GHG reporting (subparts), especially in case that different institutions (also data basis) are responsible for different UNFCCC/KP reporting chapters (Agriculture - Land use and land use changes and forestry/LULUCF).

**Spain:** Spanish forest soils are mainly mineral soils; less than 0.1% of the total surface corresponds to organic soils, therefore, the benefit for Spain would be limited. We would prefer prioritizing other reservoirs; nevertheless, any information will be welcome if it is recounted to the south of Europe.

**Sweden:** Yes, we currently discuss how to improve the reporting of emissions from organic soils and also the implications of the new activity “Wetland drainage and rewetting” that may be reported and accounted under the Kyoto protocol during the second commitment period.

**Switzerland:** No, not currently. The current focus is on mineral soils.

**Joint Research Centre JRC:** Having in mind that countries implement continuous development of surveying systems (i.e. NFI) there is practically an automatic harmonization (often very slow) or, at least, awareness on definitions, parameters/proxies, thresholds used by others. Impact of forest management is particularly important. Harmonization of emission factor measurement methods for organic soils (e.g. subsidence, oxidation rate estimation) and area (e.g. vegetation type, K40 emission) is needed.

## Overall comments:

**JRC:** This is one of the GHG emissions hotspots in Europe no matter land use, and much more knowledge is needed.

## Question 3:

**Do you report changes in litter and soil organic carbon pools in mineral soil on forest land? If so, are you currently using a soil C model to estimate soil C stock and / or change? If YES, which model? Do you require help with the classification and quantification of belowground living biomass (roots, fungi etc.) and soil organic matter pools?**

## Answers:

**Austria:** Austria reports these changes. The Yasso07 model is used. Currently the below-ground living biomass is confined to roots. Roots are discarded prior to the chemical analysis and are not further investigated in the standard soil analysis laboratory protocol. We are not differentiating fungi from other living parts.

**Czech Republic:** We do not report changes for 5A1 (Land remaining Forest land), while we do report changes associated with land use conversions (e.g., 5A2 Land converted to Forest land). Some of our soil-related argumentation under UNFCCC and KP reporting is based on YASSO modelling, but we do not use it for any quantitative estimates for land use categories converted to other land use categories. Any help in improving soil C stock change would be beneficial provided if is free of charge (with own funding).

**Denmark:** Yes we do report changes. No we do not apply specific soil C models for reporting at present. Forest floor (the term litter in GPG is very inappropriate) C stocks are estimated based on measured forest floor depth in all NFI plots based on simple empirical linear models by soil type and tree species type. Mineral soil C stocks have not been documented to change in a repeated soil inventory, so mineral soil C stocks only change as a result of decreases in FRF area.

**Estonia:** Currently Estonia does not report changes in litter or mineral soil carbon pools due to lack of data (litter is not involved in national forest monitoring). For SOM in mineral soil, tier 1 is used, which means that carbon stock in mineral soil is assumed to remain constant so long as forest remains forest. Estonia comprehends the impreciseness of this assumption and realizes the need to develop more accurate models for estimating SOM carbon pools. At present, relevant data is collected and analysed with the aim to apply Yasso07 model in the future for estimating changes in forest soil carbon stocks.

**Finland:** Yes we report, with Yasso07 model. We have been also comparing this model with Romul model. Our fine root quantity estimates are based on Helmisaari et al. (2007) work, while turnover rates are based on various studies (Brunner et al. 2013 being latest).

**France:** Currently, litter and soil organic carbon are considered balanced in the LULUCF inventory on forestlands remaining forestlands. Trials were led with INRA infosol to estimate carbon fluxes on forestlands but this was not completely satisfying. RothC model was used at that time but I think that they are now developing new methodologies with other models. I guess help could be beneficial on these issues but personally I cannot say what would be necessary.

**Greece:** Under the UNFCCC NIR we report no changes in the litter and soil organic carbon pools in the mineral soil on forestland (TIER 1). None model has been used for the estimation of soil C stock changes. Solely, a model to calculate bulk density has been

used. This model is based on soil organic carbon and texture analysis. The quantification of below living biomass would be of great help.

**Iceland:** Yes. We report increase in C in the litter and soil only on afforestation areas (Land converted to Forest Land) and only for the first 50 year since afforestation. We use simple linear regression models to estimate C-sequestration in these pools (annual increase factors). These factors are findings from research results.

**Ireland:** Yes (CARBWARE, a National C flow model). No help required unless funding streams can be identified for implementation of an extensive soil C monitoring system to further improve models. Some national research is being carried out.

**Japan:** Yes. We used a model, which was modified from the CENTURY, for the calculation of carbon stock changes for dead wood, litter and soils. The belowground living biomass and their dead organic matter are crucial for the model.

**Latvia:** We are reporting mineral soils and litter as not a source according to results of the forest soil inventory. We would like to use Yasso model, because it doesn't need soil data and it can be used in agriculture as well, and, what is the most important, it can be used in forecasting of carbon stock in changing climatic conditions; however, considering that 50% of our forest soils are naturally wet or drained, application of Yasso in current status is rather limited and we need extensions for evaluation of effect of hydrological properties of the area. We don't need help in classification and quantification of below ground biomass; however, we are interested to work on tree species, which are not very common in our conditions, but can be used in plantations, to make projections of carbon stock change under different management scenarios.

**Lithuania:** Lithuania state that carbon changes in soil and forest litter is close to 0 (Forest land remaining Forest). Total carbon change in soil and litter is closely connected with changes of total forest-land through afforestation and deforestation. Lithuania is not using C models for carbon assessment in soil and litter, but this experience will be helpful for us. We have not experience with the classification and quantification of belowground living biomass (roots, fungi etc.) and soil organic matter pools, and we think that it will be helpful for us too.

**New Zealand:** New Zealand has explored both Tier 1 and Tier 2 options for reporting soils. The Tier 2 approach implements a custom-built regression model (soilCMS) to provide an unbiased national-scale estimate of soil carbon stocks based on New Zealand soil data. There is interest in exploring the applicability of process-based models, such as Century and RothC. I am not clear what you mean by “Do you require help with the classification, quantification of belowground living biomass (roots, fungi etc.), and soil organic matter pools".

**Norway:** Up till now we have used Yasso to estimate changes in litter and soil carbon on mineral soil (forest remaining forest). 2013 will be the first year using Yasso07. We expect to be able to split the Yasso07 estimate to pools using either NFI registrations of dead wood or assuming certain chemical soil pools (and size fractions) in the model make up specific reporting pools. However, the methodology for this is under development i.e. it is always interesting to see how others have done it.

**Romania:** I didn't report changes but I want to do that in the near future on the base of successive inventories. I think everyone needs help with the classification and quantification of belowground living biomass (roots, fungi etc.) and soil organic matter pools at this moment.

**Russia:** First evaluation of changes in litter and soil organic takes place this year at preparation of Second Evaluation Report on Climate Change for Russian Federation. Due to large territory models apply for some case studies at European part of Russian Federation. Necessity to separate models applications for arable and forest soils. Evaluation of $CO_2$ emission from arable soils has been done by Smith et al. (2007) with application of the RothC model (Coleman et al. 1997) but results are far from reality. They have been done without adding mentioned afforestation processes and taking into account extreme climatic scenario of HadCM3 climate model which looks inapplicable for the whole territory of European Russia and Ukraine.

Analysis of forest soils dynamics for Russian soils has been done for some regions of European Russia by CBM_FS3 model, which is developed in Canadian Forest Service and is applied worldwide (Kurz et al. 2009) and by the modell system EFIMOD (Komarov et al. 2003) included soil organic matter and soil nitrogen dynamics ROMUL (Chertov et al. 2001) developed in Russia and based on structure and databases of Russian forest inventory. Results of the EFIMOD-ROMUL system application for Russian forests are published in (Komarov and Shanin 2012).

**Slovakia:** We do not report changes in litter and soil organic pools in mineral soils on forest-land.

**Slovenia:** No, we don't report changes in litter and soil organic pools in mineral soil on forest land, but for testing we are using Yasso07 to estimate changes carbon storage in forest soils; due to the lack of specific data we test models only for pine stands; beside Yasso07 with tested also Romul model.

Difficulty regarding $C_{org}$ content assessment for forest soils is forest sites spatial variability: the expected time changes are lesser than the spatial variability of the stock of $C_{org}$ in forests. Alternative solutions are model calculations which are often used for assessment of the stock of $C_{org}$ in forest soil and the changes thereof.

For soil organic pools in mineral soil layers we tested forest soil monitoring data (1995/96 versus 2005/06) and data from few long-lasting research plots data to get idea about carbon content changes process; due to lack of specific data, unreliable and problematic sampling design we got different results; for Yassa07 model the carbon content (total, litter and organic carbon in mineral soils) slightly increases (1986-2012; 0.22 t C / ha per year), in case of other approaches the carbon content slightly decrease. Therefore, we concluded that on the basis of such results we stay at the so-called conservative position regarding FM and forest soils UNFCCC/KP reporting - forest soils are not a source of greenhouse gases.

Yes; any exchange of data and results especially for input data for Yasso07 model or others models are highly desirable; important is to increase database model input parameters for example for central European site conditions and tree species (beech, fir, spruce,…). It is important to include different forest management practice, which is different from country to country or region to region. It is also important to have for regions common approach regarding so-called "evidence" to support claims that putting the forest floor and non-forest soils are a source of emissions that are needed for the preparation of LULUCF reporting and national reports statutory reviewers.

**Spain:** Spain doesn't report about changes in carbon content in litter and mineral soils. Currently, the Inventory Team is working in verifying that Tier 1 can be used for these pools, and that we can assume that there are no variations in the carbon stocks. We could be able to report carbon stocks, but due to the lack of re-measurements, the carbon stock changes are difficult to assess. A model to estimate carbon stock changes that adapts to Mediterranean conditions could be an applicable tool for Spanish reports on SOC and litter. With regard to belowground living biomass, we apply the default factors by the IPCC (2003 GPG for LULUCF) to calculate the carbon stocks in roots. If more detailed information was available for Mediterranean specificities that would be very welcome.

**Sweden:** Yes, we report changes in litter and soil organic carbon pools in mineral soil on forest land using repeated measurements on permanent sampling plots. At the moment we see no need to revise our sampling program but are of course interested in participating in discussions that may lead to improvements.

**Switzerland:** The model Yasso07 is used to estimate fluxes. Model outputs (i.e. combined C stock in soil, litter and deadwood) were modified to account for the pools separately. Currently the inputs to the model include only material from trees. A quantification of Inputs from herbs and shrubs would be valuable.

**Joint Research Centre JRC**: So far for reporting 5A1 – "forest remaining forest", only few countries use Tier 3 model (AT, FI, CZ, CH – yasso/07, UK, IE, SW own models). Assumption of Tier 1 (assumption of "no change" in the C pool in time) is applied for DW (dead wood) pool by 11 MS – member states (i.e. BG, CY, CZ, ES, GR, IE, LV, LU, NL, PT and RO) and for LT (litter) pool by 16 MS (i.e. BE, BG, CY, CZ, DE, ES, EE, GR, LT, LU, LV, MT, NL, PL, RO and SI). LT and DW data come usually from different data sources (i.e. DW from NFI or sometimes from biodiversity studies). Seven MS estimate C stock change in soil organic matter (SOM) pool either by repeated measurements (i.e. forest health programs by PT; national forest inventories by SE; research projects by BE); model based (i.e. AT, FI, GB) and expert adjustment of default values (i.e. PL). All other countries report no change in SOM. Not all NFI have a soil program, although just started such programs within latest cycles. Regarding organic soils 88% of total area is reported by models (50%) or estimation based on repeated measurements (38%) (by Nordic countries), other use IPCC default actors.

## Overall comments:

**JRC:** Reporting of changes of SOC is particularly difficult under stable land use. For land conversions reporting is easier, but not clear how much uncertain. Fact is that reporting rules "facilitates" reporting under UNFCCC inventory (by allowing use of Tier 1 – no change in the SOC, DOM pool) and accounting against emission reduction target under KP (by "not a source" supported by "sound reasoning"). This reduced the pressure for quantitative reporting, so I would say a disincentive for modelling effort, poor resampling. Looks meaningful to involve NFI outputs. For organic matter pools, especially litter pool, the situation is worse than for SOC. Dead wood is better because is subject of measurement by NFI.

## Question 4:

**What kind of help (if any) do you require with the parameterisation and input estimation to your model (litter)?**

## Answers:

**Austria:** Yasso07 has the above- and belowground litter-fall as input parameters. Challenges aboveground: estimation of litter-fall from branches belowground: estimator of coarse root biomass; estimator of fine root biomass (are they functions of tree diameter and height or are other factors of high relevance (e.g. dependence of fine root mass on soil fertility / elevation); should fine root biomass be estimated as a function of total root mass, total tree biomass, or needle/leaf mass?). Such information could be easily incorporated in the Yasso07 parameterization procedure. The most pertinent question is the turnover rate. The review paper of Brunner et al. (2013) gives guidance on the turnover of fine roots. Are there additional site factors that constrain the choice of tau within the given range? How is the turnover of coarse roots and stumps?

**Czech Republic:** None under the current reporting approach.

**Denmark:** As we do not use models for reporting (so far) there is no immediate need. But based on our experiences with YASSO (see question 5) we would need litter input. While we have relatively good information on aboveground litter-fall (quantity and to some extent quality though we would need more info on organic composition – lignin, cellulose etc.), more information is needed for belowground litter input. Regarding YASSO, it is also a drawback for reporting (and process understanding) that forest floor and mineral soil cannot be distinguished.

**Estonia:** For using Yasso07 more data about chemical composition of different litter fractions of different tree species is needed.

**Finland:** The annual variation of fine root litter input would be very valuable information. Also the role mycorrhizas in the soil C cycle would be needed to be evaluated.

**France:** I don't know. This could be seen with Infosol.

**Greece:** We have not used any model yet. The main reason has been the lack of some parameters for running one of the most popular models (CENTURY, ROTH C, YASSO). However, we would be very interesting to work with a model suitable for Mediterranean forest soils and that would not require too many parameters.

**Iceland:** At the moment we don’t use models and the goal is to use in the future stock change methods build on the litter and soil samples from the NFI plots. In the meantime we use the rather simple factors described in the answer to question 3. Nevertheless has our interpretation of these research results been reviewed both by domestic and international expert review teams from UNFCCC without criticism. Further development of more sophisticated models will always be a goal for us but one have to take into account firstly rather special environment in Iceland with volcanic soils of basaltic origin, not a common soil type in other parts of Europe and secondly sparse budget to do further research on the development of the carbon in these two pools (litter and soil).

**Ireland:** None.

**Japan:** We have no available data about decomposition rate of roots, so we are using parameters in the original Century model. Data of root decomposition will help for improvement of the model.

**Latvia:** We would like to cooperate on elaboration of projections' models for different forest management types to estimate carbon stock change in litter. We have rather limited information of litter-fall (we just now, that nutrients and carbon turnover in litter is much bigger than in living biomass on the base of some monitoring plots and depth of litter layer, and thus carbon stock in litter too, may change several times within a few years in some forest stands); therefore, we are interested to gain more information about turnover of litter, decomposition periods and factors affecting decomposition in different stand types. Deforestation and afforestation are another group of questions on decomposition and formation of litter layer. Fertilization of forest and drainage is one more bunch of lack of knowledge. We would like to cooperate in joint project on implementation of Yasso or similar transparent and open source solution on projections of carbon stock in litter depending from land management practices. I would like to highlight word projections, because evaluation of short-term temporal changes has very high uncertainty level and litter is more interesting in long-term forest management planning with 50-100 years forecast period. Considering previous experience with implementation of ready to use solutions, we are not interested at all to use something that is not tested and evaluated in Latvia with participation of our researchers.

**Lithuania:** Firstly, we need experience and understanding how models (CENTURY, RothC, Yasso, Yasso07) works. Afterwards, to select the model, which is useful for us. Finally - parametrisation and input estimation to model.

**New Zealand:** We are not yet parametrising a process-based soil model, but I am sure guidance could be helpful if we get to this point…. Since we use only Tier 2 (and not Tier 3), we do not use litter as an input to soils.

**Norway:** Turnover rates for various tree components (roots, branches, foliage) for boreal forest. To some extent covered by the paper already published.

**Romania:** My answer is not about a model but about the methodology of calculating the C stocks. It is made with the formula:

$$\text{C-stockmin} = \text{C-conc} \times \text{BD} \times \text{d} \times \text{CFst} \quad (1)$$

where C-stockmin is the C stock in the mineral soil (kg/m$^2$ ×10 = t/ha), d is the depth class/horizon thickness (m), C-conc is the concentration of organic carbon (g/kg), BD is the bulk density (kg/dm$^3$) and CFst is the correction factor for stoniness, 100x (% stones / 100). But, if someone wants to apply that formula could have some problems, especially because the samples of soils are taken from different depths.
I made some calculation for more than 7000 samples using three possibilities of calculation:
- On pedogenic horizons
- On fixed depths
- Using regression equations.

The differences, for the same data are big.
Only that formula is not sufficient. Examples of calculation are needed.

**Russia:** Litter flow, first of all fine roots litter. It is possible to use some regression models based on different conversion coefficients and allometric equations but they are absent for most tree species cereal crops and, moreover, for meadows herbaceous plants.

**Slovakia:** No answer.

**Slovenia:** In the case of Yasso07 we need more data for chemical composition of different litter fractions for typical national species; we are looking for help regarding specific meteorological input data, which are not available for stand level (sub-models). Further workshops are needed where modellers could exchange information with soils scientists and national reporters with presence of JRC experts. If one of the data providers is missing we lose comprehensive insight information about problematic. There is also a need to inform authors of LULUCF IPCC Good Practice Guidance regarding specific problems about litter and organic carbon in mineral soil reporting requirements (how many Annex I parties could meet proposed requirements without avoidance the data reliability, over-simplification of simplifying the calculation of carbon stock changes in soils, .. costs calculation of proposed approach?…).

**Spain:** Spain doesn't report about changes in carbon content in litter and mineral soil: Spain is not using any model as it was explained above, but a model with a correlation between living biomass (or carbon content) and the amount of litter/SOC would be very useful (if adapted to Mediterranean context, as mentioned).

**Sweden:** We do not use a model (see above)

**Switzerland:** Any

**Joint Research Centre JRC:** Summer schools on the use of models and how to sample/process parameters (although global parameters seems available for some models).

## Overall comments:

**JRC:** Risk of bias of estimations of C stocks, but annual changes may not necessarily be over/underestimated (this is a benefit for using models in reporting GHG inventories).

## Question 5:

**What models have you used in the past to estimate changes in litter and / or soil organic carbon, and what were the problems (if any)?**

## Answers:

**Austria:** Yasso07 was chosen for its simplicity. Due to the few required parameters it can be applied to many sites that are not intensively used. The model comparison in Palosuo et al. (2012) lists the other models. DNDC, Century, RothC use more parameters, that are not necessarily available. We adopted the opinion that the use of complex model is not justified when the required parameters are not available and when default values are used. We also explored the COUP model. In terms of versatility it was most promising. However, the challenge of identifying the required parameters was considerable.

**Czech Republic:** YASSO model linked with EFISCEN.

**Denmark:** An MSc thesis tried to apply the YASSO07 model to SOC change based on repeated sampling in certain beech and Norway spruce plots. However, the YASSO07 model generally overestimated SOC change rates substantially.

**Estonia:** No models have been used.

**Finland:** Repola and Marklund for biomass and various works that report litter turnover rates, see Liski et al. (2006) for details.

**France:** Maybe Manuel Martin from INRA Infosol could give details on the difficulties met by modelling soils in France.

**Greece:** We have not used any model

**Iceland:** For the litter we use the same increase factor as described in the answer to question 3. For forest on drained wetland we use default IPCC factors for emission of $N_2O$ and $CO_2$ from organic soils on drained wetlands.

**Ireland:** As above.

**Japan:** No answer.

**Latvia:** No models were used in the past considering soil organic carbon stock in forest constant. Stock change method on the basis of soil analyses were used in Soviet period for croplands; however methodologies are hardly comparable with nowadays and results were not maintained during last 20 years. Default emissions factors from the IPCC GPG LULUCF were used for drained organic soils. In future we want to integrate estimation of temporal changes in soil organic material and forecasts with the National forest inventory as a national level (GHG inventory and operative projections) planning and with stand wise forest inventory for stand level planning (forest management plans).

**Lithuania:** Any models.

**New Zealand:** Litter has not been a main focus of modelling. Field data are used to estimate litter carbon stocks, and it has been found to be a small portion of the overall

forest biomass. This perspective, obviously, approaches litter as a component of the forest biomass pools rather than an input to soil.

Modelling the soil organic carbon pool has been challenging from the perspective of demonstrating different land-use effects. While there are different levels of SOC across land uses, showing statistically significant effects of land use (while also accounting for biophysical factors such as soil type and climate), proves to be challenging. Researchers are better able to model SOC at the regional level than at the national level, indicating that we are missing something when scaling up across the country. More soil data would always help modelling efforts (soils research is not well supported currently). Robust and accessible modelling approaches would also be helpful.

**Norway:** Both Yasso and (at least expected) Yasso07 underestimate the carbon stock of forest soils in Norway (based on the relatively few measurements we have). We believe this is, at least partly, due to a wet climate in many regions, poorly drained soils. Conditions not specifically accounted for by the model (and the lack of data used in the model parameterization representing such conditions..?).

**Romania:** Answer: my colleagues used $CO_2$Fix model and now they want to use the Carbon Budget Model (CBM-CFS3), made in Canada by Dr. Werner Kurz and the team from Canadian Forest Service.

**Russia:** RothC for arable soils (Romanovskaya 2006), ROMUL for forest soils.

**Slovakia:** No models used yet for forest soils. RothC tested for agricultural soils

**Slovenia:** Yasso07 and Romul, but for limited forest area/plots.

**Spain:** Spain is not using any model by now.

**Sweden:** We do not use a model for the reporting. However, we use the Q-model for other purposes (i.e. for reporting predictions) and our experiences is that for litter estimates we lack good under-storey models, lack broad leaved litter estimates and have moreover quite uncertain fine root litter estimates. For the Q model we experienced that the model lack a description on climate variability affecting SOC decomposition and decomposition functions on leaf litter. Climate variability is already incorporated in the model and the calibration for broad-leaf forests of the model has already started.

**Switzerland:** No previous experience.

**Joint Research Centre JRC:** I have limited knowledge on Yasso.

## Overall comments:

**JRC:** Use of models in other countries and eco-regions brings benefit in developing/improve the models. For GHG inventory national scale models are necessary. Protocols and analysis necessary to develop local parameters must be shared and networking encouraged.

## Question 6:

**What has been done toward a verification of your change estimates in litter and / or soil organic carbon? What are your major challenges in the verification process and what information could be helpful to improve your verification efforts? (This includes any efforts toward the support of "no source").**

## Answers:

**Austria:** Verification via field truth with data from BioSoil. Major challenges: how trustworthy is a field measurement? We found that Yasso07 describes soil C changes quite well, but soil C stocks are not well described.

**Czech Republic:** We have mostly used our own modeling study (Cienciala et al. 2008) using EFISCEN and YASSO, for supporting "no source" for the emission categories concerned. In terms of verification data, we have conducted country-level landscape inventory (CzechTerra) that included soil component (limited to forest soils). We try to secure funding for a repeated assessment in a near future. This would represent empirical evidence on soil C stock change on forest land. What would be helpful? Trustable reference values based on compiled empirical evidence, simplified reporting approaches and more expert insight when i) negotiating reporting obligations (currently rather unrealistic), as well as when ii) preparing methodological guidance for reporting.

**Denmark:** Our inventory project aimed at supporting "no source". We also did not detect any overall changes in soil C stocks at 124 forest sites between 1990 and 2008. However, it appears that the "no source" requirements have been changed in the sense that countries now need to prove that there is in fact a sink (i.e. significant positive change in SOC stock) rather than just not being able to detect a source or a sink. This is a point for discussion I think.

**Estonia:** No verification has been done yet.

**Finland:** See attached paper (Palosuo et al. 2012). Here we compared Yasso, Yasso07 and Romul against BioSoil measurements. See Fig. 3 for comparison. The major challenge is the huge uncertainty (variation) of soil carbon measurements.

**France:** No answer.

**Greece:** We have not carried out a second survey yet.

**Iceland:** As mentioned earlier we have some research results we are using in a rather simple manner. As for the mineral soil our interpretations of in country research results and use of default emission factor has been evaluated and proved by an UNFCCC ERT. For the organic soil the major challenges is to get better estimate on the emission (fluxes) of GHG from forest on drained wetlands, the magnitude and the development of the emission with time. At the moment we use as early mentioned an IPCC default emission factors with no time limit. There are some research going on in Iceland on the GHG-budget of wetlands and drained wetlands but reviewed results are lacking. We at the Icelandic Forest Research are planning a research in a poplar plantation on drained wetland where a flux-, chamber- and tree-measurements will be done. Our expectations are high on the results of this research project.

**Ireland:** Litter models have been verifies for major forest types through COFORD funded research programmes (e.g. CARBiFOR), but not for less common forest types such as broadleaf or semi natural forests. Soil carbon: a) Mineral soils are currently demonstrated not to be a source (see NIR 2011) but more data is required. This is currently being addressed in a new research project (ForCRep), b) Soil emission factors currently use Tier 2, but these are being refined in the ForD Rep project.

**Japan:** We are promoting a national forest soil inventory in which carbon stocks of dead wood, litter and mineral soil (0-30cm) are measured in ca. 2500 forest plots. This project started in 2006 and the first round was finished in 2010. Data provided in this project has been used for the verification of the CENTURY-IFOS model.

**Latvia:** Extension of the forest soil inventory (at least 210 sample plots with 5 years period of sampling), expanding forest soil inventory to grasslands and croplands with at least 100 plots in grasslands and 100 plots in croplands. Development of methodology for laser scanning based evaluation of changes of carbon stock (fluctuations of surface) on peat-lands. Evaluation of decomposition of peat layer in drained forest on organic soils. Elaboration of methods for estimation and forecasting of $N_2O$ and $CH_4$ fluxes in naturally wet and drained forests and in swamps and peat-lands drained for peat extraction. We are mostly interested in joint development of climatic conditions and management driven long term forecast models, not so much in evaluation of temporal changes, which nowadays have very high level of uncertainty.

**Lithuania:** Firstly we need to create carbon (in soil and litter) monitoring system, which provide real national values (litter biomass, carbon stock in mineral and organic soils) in Forest land remaining Forest land. We have plans to initiate studies for soil and litter carbon stock change in area of afforestation.

**New Zealand:** We have conducted studies to validate the results of the soil CMS model: both looking at paired sites (to look at the difference in SOC in grassland and forest land use types), and collecting field data from the most common ”cells” (soil-climate-land uses).

**Norway:** A remeasuring of level II plots from ca. 1990 has been initiated. However, as methodology was very different at that time and not ideal for sampling carbon – it is not yet known if these measurements will be useful to estimate change. Projects have been initiated to resample on 2 sites with relatively well-documented time series. However, these 2 sites only represent one region (near Oslo) – not the country as a whole. We have only little data for verification (model validation) – studies in other regions of the world with climate similarly to Norway, particularly the northern and western part of the country would be useful (either model comparisons or remeasurements)

**Romania:** I like better to see data or reports from other countries for comparison, but it seems to me that are not available. I calculated the C stocks in Romanian forest and I want to compare with other countries, but I found only very few data. A database will be very good.

**Russia:** Verification could be done at application of different models at the same data at different case-studies across Europe. The main problem is lack of repeated measurement of carbon and nitrogen pools for forest soils for a long time (50-100 years). Chronosequences are also useful but they are usually not well documented especially in

concern changes of land-uses and/or forest fires or insect attacks, which change carbon and nitrogen dynamics.

**Slovakia:** Change not reported.

**Slovenia:** Additional soil sampling and soil analysis (carbon content, bulk density) but for limited number of plots (only plots with archived soil samples older than 15 years) were done to asses changes in soil carbon stocks for forest remaining forests. Unreasonable is to repeat soil sampling within short periods in the case of sustainable forest management practice due to the greater soil carbon stocks variability in the area that between two sampling events, particular for litter. Because reporting commitments we need to add to report evident facts that forest/forest soils are “not a GHG source”. In the moment we continue with logical explanations and confirmations that the forest floor are not additional a greenhouse gas emissions source (equilibrium or functioning of the ecosystem in the direction to balance of GHG sinks/emissions is a natural process in the forests for longer periods, especially in the case of no forest management and extreme events).

**Spain:** Verification is not needed if the change of C is not calculated, which is the case of Spain. There is something that will be very useful; it’s the justification that the reservoir (litter, SOC) represents less than 25% of the total C stock of the category forest land. Currently the experts are working in that issue.

**Sweden:** We have used two models: Yasso07 and Q, to verify the estimates of carbon stock changes based on inventoried data. The experience is that the results from the models and the measured changes are comparable and according to the uncertainty not significantly different. Another issue is the scale of the inventory data and the model applicability when verifying inventory data. There is a trade-off between uncertainty of large-scale inventory data and model parameter uncertainty. We hope to improve these exercises, comparing models and measurements of carbon stock changes in soil when more data become available from the soil inventory.

**Switzerland:** Whenever possible estimates were verified with independent data. For stocks more and extensive data were found, incl. soil database of WSL. For fluxes less information could be found (e.g., NABO data) and ancillary data had to be used (e.g. comparison with data from other countries). Challenges include data availability part, time series of fluxes, cumulative uncertainty assessments.

**Joint Research Centre JRC:** National GHG inventories report poor verification of dead organic matter pools, more often such verification are reported for soil C pool (e.g. when national estimates are verified/validated against regional, local data). Models output is usually verified against empiric databases.

## Overall comments:

**JRC:** It worth continuing checking modelled data both against empiric data and outputs from other models. Combined process – empiric models might be more useful.

## Question 7:

**Indicate what type of data/output you would like to report, but cannot calculate at present and why? (e.g. data, resource, or model unavailability).**

## Answers:

**Austria:** Effect of land use change is currently not represented in models although LUC is responsible for stronger C stock changes than climate change alone (result of CLIMSOIL).

**Czech Republic:** In my opinion one should develop simpler, rule-based, more robust reporting schemes for soil and litter C stock changes based on compiled empirical evidence. Their application would increase reporting transparency and avoid new, useless and costly sampling programs in many countries. It should ultimately harmonize soil reporting across countries of the same region.

**Denmark:** E.g., nitrous oxide fluxes from drained forest soils and due to land use changes. Also we need better information (possibly aided by modelling) on carbon dynamics within organic soils in which SOC stock changes are difficult to assess by sampling.

**Estonia:** Litter-lack of currently available country-specific data and lack of resources for collecting field data, also no human resources or know-how for model testing.

Carbon stock changes in mineral and organic forest soil-carbon stock is known, but how to calculate carbon stock changes? Nation-wide re-sampling is not feasible due to high costs. Yasso07 could be used for mineral soils, but as mentioned above, there is not enough litter data, also lack of human resources and know-how for model testing.

**Finland:** We don't report emission of undrained forested peat-land soils. We don’t have methods / models for that. Also methods are lacking for soil emissions due to land-use change.

**France:** No answer.

**Greece:** We would like to acquire more data on belowground biomass of forest species, since there have been too few studies conducted up to present.

**Iceland:** We have problem with one report. That is to report on the changes in the litter and soil pools on deforested land (forest converted to settlements). I would appreciate to hear about possible solutions there.

**Ireland:** In relation to which pools? Soils are the most difficult due to lack of national specific data.

**Japan:** No answer.

**Latvia:** We would like to report $CH_4$ and $N_2O$ fluxes in forest, farmlands and wetlands, particularly, impact of management activities on these gases. High uncertainty level is hampering enthusiasm on reporting of temporal carbon stock changes in soil and litter; we would like to avoid speculative solutions just to report something for these pools. According to our understanding the land use change is factor driving visible carbon stock changes in soil and litter, respectively, these changes have to be highlighted in reporting

and not so much efforts should be contributed to lands having relatively constant land use characteristics.

**Lithuania:** Yearly carbon stock change in soil and forest litter in Forest remaining Forest, Land converted to Forest, Forest management, ARD.

**New Zealand:** We would like to report the robust results from a national soil model, and we work towards doing so. Uncertainties in land use effects remain high due to the nature of scaling a limited amount of field data up to national level estimates across very diverse landscapes.

We have limited soil data in planted forests and natural forests. We still need to better understand SOC transition after land-use change (currently we use a 20 year linear transition, but we recognize that actual change may occur differently). We also assume steady states after 20 years under consistent land use, but field studies are indicating that this is not the case in all situations. We currently cannot model this. We lack data in "settlements" (which in many cases are a class of forests themselves), wetland, and "other" land types, following the GPG land categories.

**Norway:** 1) Differentiation of soil carbon into pools of litter, dead wood and soil is a challenge. 2) Emission factors for organic soils are not based on Norwegian conditions i.e. presumably very uncertain. 3) A tier 2 method for soil on afforestation / deforestation lands could be tricky as the C stocks for forest soil are presumably underestimated by the model (see above). This makes the direction of change for carbon in soil "counterintuitive" – where you expect increase in soil carbon due to afforestation the estimates may give you a decrease. The methodology is here also under development. Use of measured stock (1990 data) could be used instead.

**Romania:** 1) The dead wood (no available data). 2) The variation of C stocks in litter (no available data in different time period – more than 7 years difference)

**Russia:** Data on forest ground vegetation turnover in forests. Actually there are no good estimates of their carbon and nitrogen turnover for different forest types. Such data are crucially important at description of strong external impacts i.e. forest fires, clear-cut. In this case biological turnover is concentrated in ground vegetation and carbon and nitrogen dynamics (including leaching, emission etc,) is defined by herbaceous plants and dwarf shrubs.

**Slovakia:** No answer.

**Slovenia:** 1) Soil carbon storage for all LU: need to improve soil data quality (data, resources, quality control, and harmonization of analytical and sampling methods, data recalculation …); 2) Carbon content/storage changes (t C / ha per period) in soil: model adaptation at regional / national level (additional data; models modification & adaptations, how to run models, data preparation…); 3) Land use changes and GHG emissions: national emissions factors calculation (tier III...).

**Spain:** Spain would like to present estimations for all the information requested by UNFCCC and the Kyoto Protocol. As explained above, we have some problems in reporting some pools, mainly because of the lack of data or the existing difficulties for adapting existing ones to GHG inventories needs. There is also the problem of the inadequacy of existing models to the particularities of the countries in the south of Europe

(the existing models overestimate the stock and do not reflect well the real evolution of the Spanish forest).

Spain would like to move to a Tier 3 (top level of information, the most precise that includes the model utilization and measurements) by means of the application of an own model with specific parameters, but for that, activity data need to improve.

**Sweden:** Currently we calculate all required data for the reporting. Most urgent is the need to improve the estimates associated with land that change management or land use (for instance Land converted to Forest land and Forest land converted top other land use categories). Today we use quite rough emission/removal factors in combination with area estimates for all conversion categories reported under the UNFCCC and the Kyoto protocol.

**Switzerland:** As indicated in question 3, Yasso07, reports estimates of the C stock as the total of the soil, litter and deadwood stocks. The attempted separation of the Yasso07 results into the three pools is an approximation only and an additional source of uncertainty

**Joint Research Centre JRC:** In general, it is not clear if C stock in fine roots (<2 mm) is included in the estimates associated to deforestation, which may lead to underestimation of emissions.

In general, changes in C stocks in land remaining under same category are very uncertain (just note that the estimates of GHG inventory are aggregated at national level, so increasing biomass stands cover >95% of national forest compensated by <5% clear cut).

## Overall comments:

**JRC:** Implementation of continental grids (harmonized, like LUCAS in the EU or ICP Forests) or repeating sampling, developing simpler schemes of reporting.

## Question 8:

**In your experience, which are the five most important gaps in knowledge that you as a LULUCF expert feel should be addressed?**

## Answers:

**Austria:** 1) Effect of land use change on soil C (magnitude of effect and quantity).
2) Reliable information on soil bulk density, if not measured on the site. Explanation: We calculate C pool as f (bulk density, C content, rock content). Much effort is put on C content estimations; the multiplier bulk density is not fully addressed.
3) Effect of disturbance on soil C inherent question: are disturbance events periodically replenishing the soil C pool or would we reach a steady-state even without disturbance?
4) Relevance of organic soils (even when scarce).
5) Appropriate fractionation methods for SOM.

**Czech Republic:** 1) Effect of long-term soil disturbance associated with land conversions.
2) General comprehension adaptation and mitigation measures in forestry (classical believe that forest may endlessly accumulate carbon, inadequate understanding of long-term adaptation measures that may be more important than short-term carbon gains etc.)
3) Lack of expertise in the topic of large-scale (regional and country-level) carbon stock change estimates and approaches as compared to experience at (research) plot level. Associated lack of understanding of economical effect of decisions under KP – specifically that related to soil sampling implicitly requested.

**Denmark:** 1) “Non-source” requirements: a significant sink (increase in SOC stocks) or just no significant difference over time?
2) Land-use change effects (afforestation and deforestation).
3) How to best report $N_2O$ and $CH_4$ fluxes.
4) Better knowledge of SOC dynamics in organic soils (i.e., soil high in C because of moisture regime).
5) Better information on belowground process rates for C input (roots).

**Estonia:** The major question is the impact of land use changes to soil organic matter pool, both in mineral and organic soils. Also impact of felling and different forest management practices to soil carbon stock.

**Finland:** 1) Role of fine roots, processes that drive the quantity and turnover.
2) Soils models, more testing is needed.
3) Drained peatlands don’t have models for emissions.

**France:** As inventory compiler, I would say that our biggest challenge is to manage dealing with land use changes. Because carbon fluxes are often linked with the past land uses, it is always difficult to link the work from other researchers who don't use the land use in their estimates. When researchers speaks of Forest they don't split forest between forest remaining forest and land becoming forest which is the case in the UNFCCC reporting. This is a big difficulty in nearly all the LULUCF inventories.

Then there is a lot of criticism on the annual reporting for these processes under the UNFCCC and a lot of unjustified pressure too. Everybody knows that it is very difficult to track carbon fluxes from forest soils and because of the requirements under the UNFCCC

reporting, estimates are implemented by countries with very different protocols (not always very reliable). Most of them are using models but depending on the parametrisation the used results can be very different. A few years ago I tried to compile methodologies used by a few countries among the world in their UNFCCC reporting and I was finally not convinced by the different trials from these countries. For example I remember that Denmark wrote in its report that they doubt that actual trend can be monitored in forest soils on such a small period although they have a large network and few forests. Finally I would also say that to treat correctly this subject it would be necessary to define clearly the role of the meteo data in the estimates, which is not the case according to me.

**Greece:** The estimation of belowground biomass and its changes as well as the estimation and changes in the dead wood mass.

**Iceland:** 1) Changes in carbon in the mineral soil pool for Forest Land remaining Forest Land.
2) Changes in carbon in the litter pool for Forest Land remaining Forest Land.
3) Models for annual fluxes of GHG of organic soils, both drained and un-drained soils and both for Land Converted to Forest Land and Forest Land remaining Forest Land.
4) Changes in carbon in the mineral soil pool for Forest Land converted to Settlement (Deforestation).
5) Changes in carbon in the litter pool for Forest Land converted to Settlement (Deforestation).

**Ireland:** 1) Development of spatial land use tracking systems and inventories able to detect land use change at a scale which is consistent with the forest definition and one able to accurately detect historic land use change.
2) Information of soil emission factors associated with land use change, drainage and management practice.
3) Information of mineral SOC stock changes following land use transition and in land remaining forest land, crops remaining cropland etc.
4) Emission and removal associated with fires following disturbances and during recovery
5) Establishment of reference levels for cropland and grazing land management under art 3.4, no good historic data.
6) Soil models for non-forest soils.

**Japan:** I am not LULUCF expert but I feel that uncertainty is huge in the estimation of carbon stock change in dead wood and mineral soil.

**Latvia:** 1) Land-use changes (afforestation and deforestation, croplands to grasslands and vice versa).
2) Long term impact of land use practices (climatic and management factors driven modelling for farmlands and forests) including possibilities of formation of deposits of organic carbon in deeper soils layers.
3) $N_2O$ and $CH_4$ fluxes (forests, wetlands, farmlands).
4) Transformations of dead wood (fractional structure in different stand types, decomposition periods, impact of extreme events – periodic large loads of dead biomass).
5) Lack of funding to be able to follow up and to evaluate new methodologies, to be able to separate speculations from real innovations and to be able to take part in development process.

**Lithuania:** 1) Models for soil and litter C.
2) More information about classification and quantification of belowground living biomass (roots, fungi etc.) and soil organic matter pools and how it to use for LULUCF reporting.
3) Experience of other countries for carbon assessment in soil and forest litter.
4) Methods of calculation and reporting for LULUCF.

**New Zealand:** I'm not sure whether this question pertains specifically to soils and/or to forests. Assuming that it is permissible to be a bit more expansive…
1) Gaps in soil data. Beyond forests, it is necessary to have good soil data for all six major land-use categories in order to have good understanding and to properly understand land-use transitions.
2) Robust and efficient means for scaling field data to national extent. This is especially difficult in diverse landscapes (where there is a big variety of soil types, climate zones, and landform).
3) SOC transition profiles for soils that have undergone land-use change (and whether steady state assumptions should apply).
4) Urban settlement carbon pools (applies to all 5 carbon pools covered by the GPG). For the most part, carbon in cities is ignored, and often assumptions that carbon decreases in cities (or that it is a void) are made. This is actually not true in many locations (where there can be quite a lot of carbon stored, or at least a lot of carbon flux).
5) Capturing disturbance effects upon SOC (in New Zealand's case, erosion), which should also be accounted for as part of "human-induced change". More generally, this point would pertain to getting past tidy model results and assumptions and ensuring estimates reflect reality.

**Norway:** 1) Information on why the model underestimates stock (in the Norwegian case).
2) Processes in afforestation and deforestation – boreal conditions.
3) Emissions from organic forest soils

**Romania:** 1) The depth for calculating the C stocks in soils (in many works is 30 cm because the roots of trees are there. My opinion is that the real reason for that depth is the missing data for depths more than 30 cm). Organic C is also to larger depths than 30 cm (in smaller amount, is true). 100 cm will be better?
2) I didn't see in the IPCC Good Practice Guidance differentiation between the litter and the humus layer. The amount of organic C is very different between these two pools.
3) A model for estimating the changes of organic C in soil only (the actual models try to solve too many problems: soil, litter, biomass etc). The inputs for that model must be very obvious and simple.
4) A simple methodology to take into account the quantification of belowground living biomass (roots, fungi etc.).
5) If there is a response concerning the variation in time of C stocks in forest remaining forest for some European countries it will be good that that answer is extrapolating to all countries (field effort and calculation is too difficult).

**Russia: 1)** Quantitative data on carbon and nitrogen pools in forest ground vegetation and in grasslands.
2) Model calculation of $CH_4$ and $NO_x$ fluxes (latest is partially done but requires a lot of parameters); basic cations and aluminium dynamics models.
3) Quantitative evaluation of the role of fine roots and mycorrhiza in soil and ecosystem processes that drive turnover.
4) Models of DOC and DON fluxes at watersheds.

5) Age and species-specific belowground biomass share in total biomass (more data on biomass partitioning among plant compartments, BEF etc.).

**Slovakia:** 1) Representative site-specific datasets related to changes of soil carbon stocks after land use change.
2) "Country specific" input data related to carbon balance in agricultural soils.

**Slovenia:** 1) Quality of national reporting regarding litter and organic carbon changes in soils – a) common approach robust enough (realistic reporting methodology Annex I parties); comparability for all parties, QA/QC, assessment of data reliability..; If not b) other options ….
2) Lack of knowledge, skills, expertise, modelling, **especially country specific** regarding litter, dead wood and organic carbon content in mineral soils (UNFCCC/KP LULUCF carbon storage pool) as a basis of LULUCF and future EU "LULUCF" reporting.
3) Soil monitoring; common approach (agriculture and forestry) at national and international (EU?) level (methodology, sampling design, harmonization of analytical methods), use of already existing methods, programs and database, modification and upgrade of existing systems; lack of EU soil protection and monitoring legislation/….
4) National specific emission factors for LU changes.
5) Land use (LU) changes, country specific methodology; how to combine different methods, data updating... and - Improvement (modification) of IPCC GPG LULUCF instructions, - Clear soils definitions/classification – UNFCCC/KP IPCC LULUCF, WRB, ICP Forest, Forest soil Manual, NFI (FAO… reporting), national definitions, others… (are we really speaking the same language?)…

**Spain:** There are many gaps in the different land uses, but concerning forest and specially taking into account the aim of the COST action in which you are involved:
1) Changes in dead organic matter pools (litter and dead wood) both for existing forests and newly established forests.
2) Forest mineral soils carbon stocks and changes both for existing forests and newly established forests.
3) Due to the importance of forest fires in the Mediterranean countries, it would be interesting to know about the influence of fire in the evolution of carbon content in forest soils and belowground biomass.
4) Once having this information (points 1, 2 and 3), associate it to different types of forests, and to Mediterranean climatic conditions.
5) Information about these pools on Mediterranean shrub-lands would also be welcome. There is an important lack of information about carbon content in shrub-lands, and this can be an important pool for Mediterranean countries.

**Sweden:** Carbon stock changes related to Land use change, emissions from organic soils, uncertainties related to carbon stock change estimates.

**Switzerland:** 1) mechanisms of C stabilization in soils.
2) Estimates of decomposition rates from C in litter (and deadwood in forests).
3) Estimates of fluxes and their detection (high spatial and temporal variability).
4) System boundaries (e.g. soil layer depth).
5) Uncertainty estimation.
6) Integrating effects of major disturbance e.g. wind-throw.

**Joint Research Centre JRC:** 1) Fine roots (<2 mm) biomass quantity (important to accurately estimate emissions from deforestation), possibly as stand dependent parameters (i.e. allometric equations).
2) Age specific belowground biomass share in total biomass (i.e. factors - root to shoot, BEF, BCEF), although on short term (annual reporting on large scale) it can be reasonably assumed as no change. On medium-long term the change in such pools under adaptation of forest management to climate change is more relevant (regionally more important).
3) Emission factors from organic soils under forest management (in relation to stand management, drainage and rewetting) need lot of effort.
4) C stock change in soils in Mediterranean and eastern European forests.
5) Change of belowground C stocks under (fire, non-fire) disturbances.
6) Change of spatial soil classification from qualitative to quantitative criteria (i.e. giving up to soil type/subtype as determinant if the area occupied by a soil and relay on quantitative data and proxies easier to collect, like C content or land use….).
7) Much more on cropland and grassland is also necessary (also having in mind that share of conversions from lands with high C stock to small)!
8) Harmonization of measurement methods (pool definitions, sampling, processing …).
9) A lot is needed on how to process data in a sound statistical way, having also in mind often scarce representation or spatial heterogeneity, and setting harmonized verification procedures.

## Overall comments:

**JRC:** Point 9) from JRC above is still very relevant, as long as the countries may already have large databases on SOC, but need expertise/guidance on how to process statistically and extract meaningful conclusions at national level. UNFCCC reporting rules are settled down, so we have to find ways to report it … I am surprised to see many countries worried about GHG emissions in land use change, since in the UNFCCC reports they are all very sure on their estimates….

## Authors of answers:

Austria: Robert Jandl*
Czech Republic: Emil Cienciala
Denmark: Lars Vesterdahl
Estonia: Kaie Kriiska
Finland: Aleksi Lehtonen
France: Etienne Mathias
Greece: Panagiotis Michopoulos, Argyro Zerva
Iceland: Arnór Snorrason
Ireland: Kevin Black
Japan: Shinji Kaneko
Latvia: Andis Lazdins
Lithuania: Ricardas Beniusis
New Zealand: Nancy Golubiewski
Norway: Lise Dalsgaard
Romania: Lucian Dinca
Russia: Alexander Komarov
Slovakia: Pavel Pavlenda
Slovenia: Primož Simončič
Spain: Irene Guerra Avalos, Cristina García Díaz
Sweden: Mattias Lundblad
Switzerland: Markus Didion
Joint Research Centre JRC: Viorel Blujdea

*Note: personal ad-hoc opinion of R. Jandl; not harmonized with colleagues in charge of soil C reporting.

# Soil Carbon Models used for Kyoto Protocol Reporting

Soil carbon reporting under the Kyoto protocol falls under the category Land Use, Land-Use Change and Forestry (LULUCF), defined in articles 3.3 and 3.4 (optional). Article 3.3 includes only those land areas where afforestation, reforestation or deforestation took place in the reporting period. Other activities such as forest management are contained in article 3.4.

The Intergovernmental Panel on Climate Change (IPCC) defines guidelines for quantifying yearly change in soil carbon stock. It recognizes methods of 3 levels of complexity: Tier 1, Tier 2, and Tier 3. The use of mathematical models falls under the most complex Tier 3 method. Mathematical models are tools to increase our understanding of the underlying mechanisms of soil carbon cycling. They also facilitate predictions of the impact of climate or land use change on soil carbon sequestration.

Below, there is a UNFCCC-list of the Annex I countries, which have used mathematical models for their soil carbon reporting in the national inventory reports of the year 2012. We did not distinguish for which land use change category the model was used but only recorded whether a model was used at all and which model it was. The resulting list is shown in Table 1.

Table 1. Mathematical models used for soil carbon reporting by Annex I countries in 2012 (http://unfccc.int/national_reports/annex_i_ghg_inventories/national_inventories_submissions/items/6598.php)

| Model | Country | # |
|---|---|---|
| Yasso/Yasso07 | Austria, Finland, Norway, Slovenia, Switzerland | 5 |
| Century | Canada, Japan, USA | 3 |
| RothC | Australia, Russia | 2 |
| ROMUL* | Russia, Bulgaria | 2 |
| C-Tool | Denmark | 1 |
| Introductory C Balance Model | Sweden | 1 |
| Dynamic C Flow Model | UK | 1 |

* not in the UNFCCC list; personal communication A.S. Komarov

Out of 43 Annex I countries, 14 countries used or planned to use a mathematical model for soil carbon reporting. Three European countries, i.e., Denmark, Sweden and UK, based their reports on their own national models while ten countries used publicly available established soil carbon models. The Rothamsted Soil Carbon Model (Roth C, Coleman and Jenkinson 1996) was used by Australia and Russia, the Century model (Parton et al. 1992) was used by Canada, Japan and USA. Remaining countries using models, Austria, Finland, Norway, Slovenia, Switzerland, used the model Yasso/Yasso07 (Tuomi et al. 2011). Thus, Yasso/Yasso07 is the most widely used mathematical model for soil carbon reporting by European countries. Other soil carbon models that have not been used for reporting so far include ROMUL (Chertov et al. 2001) and SOILN (Eckersten et al. 1998).
All soil carbon models are dynamic and consider several carbon pools in the soil. Yasso/Yasso07 and Roth C are relatively simpler than the Century model that also has a spatial component.

Analyses of forest soils dynamics for Russian soils has been done for some regions of European Russia by the Century model (Kurz et al. 2009) and by the model system EFIMOD (Komarov et al. 2003). EFIMOD includes also soil organic matter and soil

nitrogen dynamics (ROMUL; Chertov et al. 2001), which has been developed in Russia and is based upon structure and databases of Russian forest inventory. Results of the EFIMOD-ROMUL system application for Russian forests are published in Komarov and Shanin (2012).

**The Yasso07 model**

Yasso07 is a model on the cycle of organic carbon in soil (Fig. 6). It has been developed to be used across the world to estimate the stock of soil organic carbon (SOC), changes in SOC and heterotrophic soil respiration (Liski and Tuomi 2010).

Yasso07 is an advanced version of an earlier Yasso model (Liski et al. 2005). The applicability of the model has been widened by using more extensive data sets when developing the model. The reliability of model-calculated estimates has been improved by advanced mathematical methods. Uncertainty estimates are an essential part of Yasso07 output. Applications of Yasso07 include greenhouse gas inventories, Earth System Modelling and scenario analyses. Yasso07 can be linked to various other calculation systems (Liski and Tuomi 2010).

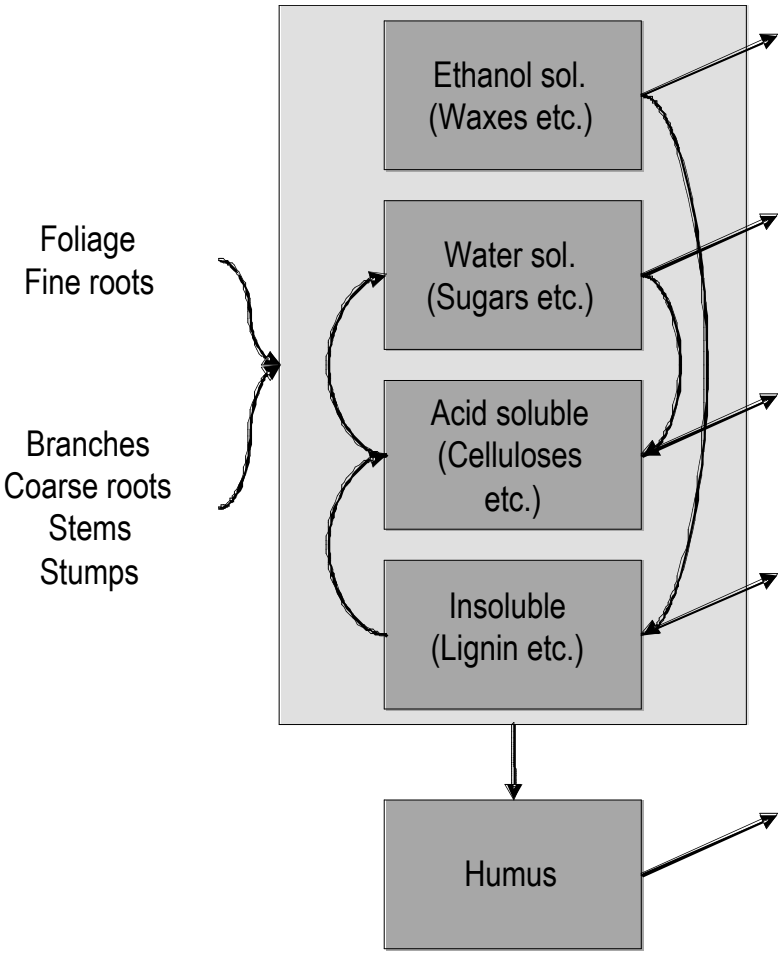


Figure 6. Flow chart of Yasso07 soil carbon model. The boxes represent soil carbon compartments, the arrows carbon fluces; only carbon fluxes deviating significantly from zero are show (from Liski and Tuomi 2010).

**The ROMUL model**

ROMUL is a model of soil organic matter dynamics based on the assumption that there is a consequent change of communities of destructors in the course of soil organic matter decomposition and humification (Chertov et al. 2001). The amount and species composition of destructors depend on the biochemical properties of organic residues, hydrothermic conditions and soil texture. Thus, it is possible to calculate the decomposition and humification rates as functions of biochemical properties of litterfall, soil temperature and moisture (pers. communication A. Komarov).

ROMUL describes the dynamics of the three main pools of soil organic matter (SOM) and corresponding pools of nitrogen: total SOM of the forest floor, labile humus of mineral horizons (originating from decomposing root litter) and stable humus of mineral soil consisting of SOM combined with mineral particles with a slow rate of decomposition (Fig. 7, Chertov et al. 2001). Additionally ROMUL delivers pool of mineral nitrogen available for plant nutrition. It is assumed that this pool fully consumed for forest growth, surplus nitrogen is immobilized in soil. Simple procedure of leaching nitrogen from soil to ground water is also included into nitrogen cycle in the model (pers. communication A. Komarov).

The extended ROMUL model was implemented in the FEMMA model (Laurén et al. 2005), in which spatial flow, solute transport and plant nutrient uptake was modelled. Then Romul was extended to simulate the dynamics of the intermediate and end products of C and N during decomposition succession (pers. communication A. Komarov).

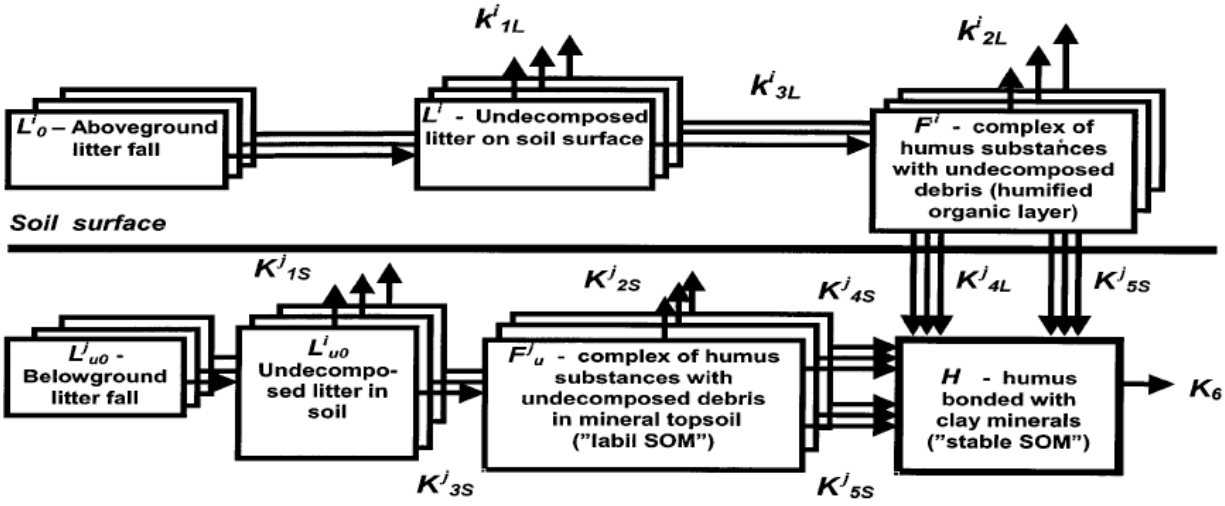


Table 7. Flow chart of ROMUL model (from Chertov et al. 2001)

# Definitions of Soil Organic Matter

**Definitions by the IPCC Guidelines**
Table 3.1.2 in the IPCC Guidelines provides a generic representation of the pools occurring in a terrestrial ecosystem. In total, three pools are distinguished: Living biomass, dead organic matter, and soils. The living biomass pool and the dead organic matter pool are divided each into two further pools: The living biomass pool into above- and belowground biomass, and the dead organic matter pool into dead wood and litter. Each of these pools is discussed in the IPCC Guidelines, although in some cases with only minimal guidance.
(http://www.ipcc-nggip.iges.or.jp/public/gpglulucf/gpglulucf_contents.html; Chapter 3.1.3. Definitions of Carbon Pools, Table 3.1.2)

## Pools relevant for soils:

**Living biomass: Belowground biomass**
All living biomass of live roots. Fine roots of less than (suggested) 2 mm diameter are often excluded because these often cannot be distinguished empirically from soil organic matter or litter.

**Dead organic matter: Dead wood**
Includes all non-living woody biomass not contained in the litter, either standing, lying on the ground, or in the soil. Dead wood includes wood lying on the surface, dead roots, and stumps larger than or equal to 10 cm in diameter or any other diameter used by the country.

**Dead organic matter: Litter**
Includes all non-living biomass with a diameter less than a minimum diameter chosen by the country (for example 10 cm), lying dead, in various states of decomposition above the mineral or organic soil. This includes the litter, fumic, and humic layers. Live fine roots (of less than the suggested diameter limit for below-ground biomass) are included in litter where they cannot be distinguished from it empirically.

**Soils: Soil organic matter (SOM)**
Includes organic carbon in mineral and organic soils (including peat) to a specified depth chosen by the country and applied consistently through the time series. Live fine roots (of less than the suggested diameter limit for below-ground biomass) are included with soil organic matter where they cannot be distinguished from it empirically.

## Definitions of soil organic matter – reasons for misunderstandings and irritations (from K. Kalbitz)

The estimation of carbon stocks in forested ecosystem is an important part of the annual greenhouse gas inventories. The different pools of terrestrial ecosystems are defined in the “IPCC Guidelines for National Greenhouse Gas Inventories…”. Unfortunately, these definitions differ to a certain extent from definitions used by soil scientists and the guidelines of ICP forest describing similar pools. These different definitions might be the reason for some misunderstanding between soil scientists and the “carbon reporting community”.

In text books soil organic matter is described as the sum of all of the organic matter on and in the soil exclusively the living biomass, i.e. soil flora and fauna, roots (e.g. Blume et al. 2010). Soil organic matter also comprises all of the synthetic compounds as pesticides and black carbon. That means, dead organic matter and litter as defined in guidelines for carbon reporting are integral components of soil organic matter. According to the glossary of the Soil Science Society of America soil organic matter is the organic fraction of the soil exclusive of undecayed plant and animal residues. In the European Soil Portal (JRC-Joint Research Centre) "organic soil material consists of organic debris that accumulates at the surface under either wet or dry conditions…". This definition described organic layers/horizons of forest soils only and should not be used.

The term 'humus' might be the reason for most of the irritations. Humus and soil organic matter are used as a synonym by many soil scientist as already suggested by Waksman (reference 142 in "Soil Organic Matter and Biological Activity" edited by D. Vaughan, Kluwer, 1985). However, forest ecologists use the term 'humus' to describe the Oa horizon of the organic layer of forest soils. Sometimes the complete organic layer is described as 'humus'. Therefore, we recommend to not use the term 'humus' anymore in order to avoid misunderstanding.

A further reason for misunderstanding is the use of the term 'litter' in the current guidelines for carbon reporting. This definition includes all of the organic layers/horizons in forested ecosystems whereas soil scientists and forest ecologists use litter for the definition of (mainly) undecomposed leaf/needle and root litter (aboveground: Oi/Ol horizon).

Unfortunately, roots as a main contributor to organic matter in mineral soils are not adequately considered in the current guideline. Larger roots will be sorted out by hand before drying and sieving the soil and another portion will be partly removed during sieving (2 mm). Fragments smaller than 2 mm are considered as soil organic matter. We recommend to keep the material sorted out by hand and all of the remaining material after sieving. These fractions should be weighted separately to improve our estimation of the contribution of (fine) roots to organic matter in the mineral soil.

We would like to recommend an adaptation of the definition of the carbon pools in the "IPCC Guidelines for National Greenhouse Gas Inventories…" in order to uniform the different definitions to a certain degree. We suggest to use the terminology of the WRB (2006) and the manual of IPC forest for sampling and analysis of soils (2010) for the definition of organic layers/horizons.

Organic matter in the following pools should be considered:
- Aboveground biomass
- Belowground biomass (including fine roots in all depths of the soil profile)
- Dead wood
- Organic layers/horizons (including litter, using the definitions used by WRB and ICP forest for the different layers, i.e. Oi, Oe, Oa)
- Mineral soil

To convert the carbon content into soil organic matter a conversion factor of 2.0 should be used (Blume et al. 2010). Sometimes, a conversion factor of 1.724 was used assuming an average carbon content of 58%. However, the large variability of soil organic matter composition does not support such average carbon content.

## Some definitions of Soil Organic Matter:

**Glossary of statistical terms:**
(http://stats.oecd.org/glossary/detail.asp?ID=2505):
Soil organic matter is carbon-containing material in the soil that derives from living organisms

**Wkipedia:**
(http://en.wikipedia.org/wiki/Organic_matter):
Soil organic matter comprises all of the organic matter in the soil exclusive of the material that has not decayed

**University of Wisconsin:**
(http://www.soils.wisc.edu/courses/SS325/organic.htm):
Soil Organic Matter: Natural C-containing organic materials living or dead, but excluding charcoal

**Scheffer/Schachtschabel (Blume et al. 2010):** "Zur organischen Substanz der Böden gehören alle in und auf dem Mineralboden befindlichen abgestorbenen pflanzlichen und tierischen Streustoffe und deren organische Umwandlungsprodukte. Auch die durch menschliche Tätigkeit eingebrachten, z. T. synthetischen organischen Stoffe (z. B. Pestizide, organische Abfälle) werden dazu gerechnet. Die lebenden Organismen (das aus Bodenflora und –fauna bestehende Edaphon) sowie lebende Wurzeln gehören nicht zur organischen Substanz der Böden."
(Translation in English: "Soil organic matter comprises all of the organic matter on and in the soil exclusively the living biomass, i.e. soil flora and fauna, roots. Soil organic matter also comprises all of the synthetic compounds as pesticides and black carbon").

**JRC – Joint Research Centre, European Soil Portal:**
(http://eusoils.jrc.ec.europa.eu /ESDB_Archive/glossary/Soil_Terms.html):
Organic soil material: Consists of organic debris that accumulates at the surface under either wet or dry conditions and in which any mineral component present does not significantly affect the soil properties. Organic soil material must have organic carbon (organic matter) contents as follows: (1) if saturated with water for long periods (unless artificially drained), and excluding live roots, either: 18 % organic carbon (30 % organic matter) or more if the mineral fraction comprise 60 % or more clay; or 12 % organic carbon (20 % organic matter) or more if the mineral fraction has no clay; or a proportional lower limit of organic carbon content between 12 and 18 % if the clay content of the mineral fraction is between 0 and 60 %; or (2) if never saturated with water for more than a few days, 20 % or more organic carbon.

**Glossary of soil science terms of the Soil Science Society of America SSSA:**
(http://www.isric.org/):
**Soil organic matter:** The organic fraction of the soil exclusive of undecayed plant and animal residues.
It means humus and soil organic matter should be used interchangeably including soil microbial biomass but excluding macrofauna and macroflora (i.e. also roots).
**Forest floor:** All organic matter generated by forest vegetation, including litter and unincorporated humus, on the mineral soil surface.

**Litter:** The surface layer of the forest floor which is not in an advanced stage of decomposition, usually consisting of freshly fallen leaves, needles, twigs, stems, bark, and fruits.
**Oe horizon (F layer):** A layer of partially decomposed litter with portions of plant structures still recognizable (hemic material). Occurs below the L layer on the forest floor in forest soils. It is the fermentation layer. See also soil horizon and Appendix II.
**Oi horizon [L layer (litter)]:** A layer of organic material having undergone little or no decomposition (fibric material). On the forest floor this layer consists of freshly fallen leaves, needles, twigs, stems, bark, and fruits. This layer may be very thin or absent during the growing season. See also soil horizon and Appendix II.
**Oa horizon (H layer):** A layer occurring in mor humus consisting of well-decomposed organic matter of unrecognizable origin (sapric material). See also soil horizon and Appendix II.

**World Reference Base for Soil Resources WRB (IUSS Working Group WRB 2007):**
(http://www.fao.org/nr/land/soils/soil/wrb-documents/en/):
Sample preparation: “Samples are air-dried or, alternatively, oven-dried at a maximum of 40 °C. The fine earth fraction is obtained by sieving the dry sample with a 2-mm sieve. Clods not passing through the sieve are crushed (not ground) and sieved again. Gravel, rock fragments, etc. not passing through the sieve are treated separately.” That means organic material (e.g. fine roots, coarse roots) won’t be considered for C accounting in the mineral soil

**Guidelines for soil description (2006):**
(ftp://ftp.fao.org/agl/agll/docs/guidel_soil_descr.pdf):
**O horizons or layers** (table 85, page 72 – description of Oi, Oe, Oa horizons) These are layers dominated by organic material consisting of undecomposed or partially decomposed litter, such as leaves, needles, twigs, moss and lichens, that has accumulated on the surface; they may be on top of either mineral or organic soils. O horizons are not saturated with water for prolonged periods. The mineral fraction of such material is only a small percentage of the volume of the material and is generally much less than half of the weight.
**An O layer** may be at the surface of a mineral soil or at any depth beneath the surface where it is buried. A horizon formed by illuviation of organic material into mineral subsoil is not an O horizon, although some horizons formed in this manner contain much organic matter.
**Oi:** In organic soils and used in combination with H or O horizons, it indicates the state of decomposition of the organic material; slightly decomposed organic material has in more than two-thirds (by volume) visible plant remains.

# Abstracts of Key Publications

**Brunner et al. (2013): Fine-root turnover rates (see also Annex)**
Forest trees directly contribute to carbon cycling in forest soils through the turnover of their fine roots. In this study we aimed to calculate root turnover rates of common European forest tree species and to compare them with most frequently published values. We compiled available European data and applied various turnover rate calculation methods to the resulting database. We used Decision Matrix and Maximum-Minimum formula as suggested in the literature. Mean turnover rates obtained by the combination of sequential coring and Decision Matrix were 0.86 $yr^{-1}$ for Fagus sylvatica and 0.88 $yr^{-1}$ for Picea abies when maximum biomass data were used for the calculation, and 1.11 $yr^{-1}$ for both species when mean biomass data were used. Using mean biomass rather than maximum resulted in about 30% higher values of root turnover. Using the Decision Matrix to calculate turnover rate doubled the rates when compared to the Maximum-Minimum formula. The Decision Matrix, however, makes use of more input information than the Maximum-Minimum formula. We propose that calculations using the Decision Matrix with mean biomass give the most reliable estimates of root turnover rates in European forests and should preferentially be used in models and C reporting.

**Cerli et al. (2012): Separation of light and heavy organic matter fractions in soil**
Density fractionation is frequently applied to separate soil organic matter according to the degree and the mode of interaction with minerals. Density fractions are operationally defined by density cut-off and sonication intensity, which determine the nature of the separated material. However, no tests or general agreements exist on the most appropriate density cut-off as well as on method and intensity of dispersion. Numerous variants have been proposed and applied, with results often contrasting each other and being hard to interpret. Here, we aimed at separating two light fractions (free and occluded into aggregates) composed of almost pure organic material, and one heavy fraction comprising the organic–mineral associations. We tested effects of different density cut-offs and sonication intensities, in combination and separately, on fraction yields, as well as on the fractions' organic carbon, total nitrogen and lignin-derived phenols. We tried to find optimum density cut-offs and sonication intensities, providing light fractions with maximum organic material and minimum contamination by mineral material. Under the test conditions, a density of 1.6 g $cm^{-3}$ gave best results for all test soils, allowing for separation of maximums amounts of almost pure organic material. The density cut-off at 1.6 g $cm^{-3}$ is well in line with previous studies and theoretical considerations, therefore, we recommend the use of this density as most suitable for separation of organic debris. Sonication levels for aggregate disruption to achieve complete separation of occluded light organic matter varied amongst soils. The necessary intensity of dispersion relates to the type of soil, depending on the stability of contained aggregates. The application of one single dispersion energy level to different soils may result either in mineral contamination or in incomplete separation of light and heavy fractions as well as in redistribution of organic material amongst fractions. This means there is no single sonication level that can be applied to all soils. Thus, obtaining a meaningful light fraction residing within aggregates (occluded light fraction) requires assessment of the dispersion energy necessary to disrupt the aggregate system of a given soil without dispersion of organic–mineral associations. This can be done in pre-experiments where the soil is fractionated at different sonication levels. The appropriate dispersion is determined by mass yields and OC content of the obtained occluded fractions.

**Ekblad et al. (2013): Production and turnover of mycelium (see also Annex)**
There is growing evidence of the importance of extramatrical mycelium (EMM) of mycorrhizal fungi in carbon (C) cycling in ecosystems. However, our understanding has until recently been mainly based on laboratory experiments, and knowledge of such basic parameters as variations in mycelial production, standing biomass and turnover as well as the regulatory mechanisms behind such variations in forest soils is limited. Presently, the production of EMM by ectomycorrhizal (EM) fungi has been estimated at ~140 different forest sites to be up to several hundreds of kg per ha per year, but the published data are biased towards Picea abies in Scandinavia. Little is known about the standing biomass and turnover of EMM in other systems, and its influence on the C stored or lost from soils. Here, focussing on ectomycorrhizas, we discuss the factors that regulate the production and turnover of EMM and its role in soil C dynamics, identifying important gaps in this knowledge. C availability seems to be the key factor determining EMM production and possibly its standing biomass in forests but direct effects of mineral nutrient availability on the EMM can be important. There is great uncertainty about the rate of turnover of EMM. There is increasing evidence that residues of EM fungi play a major role in the formation of stable N and C in SOM, which highlights the need to include mycorrhizal effects in models of global soil C stores.

**Kaiser and Kalbitz (2012): Dissolved organic matter in soils**
Dissolved organic matter has been recognized as mobile, thus crucial to translocation of metals, pollutants but also of nutrients in soil. We present a conceptual model of the vertical movement of dissolved organic matter with soil water, which deviates from the view of a chromatographic stripping along the flow path. It assumes temporal immobilization (sorptive or by co-precipitation), followed by microbial processing, and re-release (by desorption or dissolution) into soil water of altered compounds. The proposed scheme explains well depth trends in age and composition of dissolved organic matter as well as of solid-phase organic matter in soil. It resolves the paradox of soil organic matter being oldest in the youngest part of the soil profile e the deep mineral subsoil.

**Palosuo et al. (2012): A multi-model comparison**
We simulated soil carbon stock dynamics of an Austrian coniferous forest stand with five soil-only models (Q, ROMUL, RothC, Soil$CO_2$/RothC and Yasso07) and three plant-soil models (CENTURY, Coup-Model and Forest-DNDC) for an 18-year period and the decomposition of a litter pulse over a 100-year period. The objectives of the study were to assess the consistency in soil carbon estimates applying a multi-model comparison and to present and discuss the sources of uncertainties that create the differences in model results. Additionally, we discuss the applicability of different modelling approaches from the view-point of large-scale carbon assessments. Our simulation results showed a wide range in soil carbon stocks and stock change estimates reflecting substantial uncertainties in model estimates. The measured stock change estimate decreased much more than the model predictions. Model results varied not only due to the model structure and applied parameters, but also due to different input information and assumptions applied during the modelling processes. Initialization procedures applied with the models induced large differences among the modelled soil carbon stocks and stock change estimates. Decomposition estimates of the litter pulse driven by model structures and parameters also varied considerably. Our results support the use of relatively simple soil-only models with low data requirements in inventory type of large-scale carbon assessments. It is important that the modelling processes within the national inventories are transparently reported and special emphasis is put on how the models are used, which assumptions are applied and what is the quality of data used both as input and to calibrate the models.

**Komarov et al. (2012): Modelling of soil organic matter - ROMUL**
Soil organic matter (SOM) dynamics is an important pool in biological turnover of carbon and other elements of plants nutrition in forest ecosystems. Problems at its modelling are: quantification of SOM fractions which are decomposing with specific rates; description of humification, obtaining dependencies of SOM rates of transformation in dependence on external factors. Different methods of fractionating of SOM are now in use based on different solubility of fractions/ This approach presents difficulties at model's initialization. We expanded a model of SOM dynamics ROMUL based on successive stages of mineralization and transformation of fresh litter, which correspond to SOM pools in horizons L, F and H of forest floor. A1 et al. are horizons in mineral soil. Rates of transformation of one SOM pool into another in these horizons can be obtained from experimental data. The dynamics of N, Ca and Mg is described using main equations of SOM transformations with inserting of additional constants or functions as independent multipliers for rates of transformation. Pools of elements available for plants nutrition, and some intermediate pools such as secondary soil minerals have been added to the model. New ROMUL has been successfully applied to ICP Forest plots in Russia.

**Tuomi et al. (2011): Soil carbon model Yasso07**
In this article, we present a graphical user interface software for the litter decomposition and soil carbon model Yasso07 and an overview of the principles and formulae it is based on. The software can be used to test the model and use it in simple applications. Yasso07 is applicable to upland soils of different ecosystems worldwide, because it has been developed using data covering the global climate conditions and representing various ecosystem types. As input information, Yasso07 requires data on litter input to soil, climate conditions, and land-use change if any. The model predictions are given as probability densities representing the uncertainties in the parameter values of the model and those in the input data e the user interface calculates these densities using a built-in Monte Carlo simulation.

**Wallander et al. (2013): Methods to estimate mycelium (see also Annex)**
Mycorrhizal fungi constitute a considerable sink for carbon in most ecosystems. This carbon is used for building extensive mycelial networks in the soil as well as for metabolic activity related to nutrient uptake. A number of methods have been developed recently to quantify production, standing biomass and turnover of extramatrical mycorrhizal mycelia (EMM) in the field. These methods include minirhizotrons, in-growth mesh bags and cores, and indirect measurements of EMM based on classification of ectomycorrhizal fungi into exploration types. Here we review the state of the art of this methodology and discuss how it can be developed and applied most effectively in the field. Furthermore, we also discuss different ways to quantify fungal biomass based on biomarkers such as chitin, ergosterol and PLFAs, as well as molecular methods, such as qPCR. The evidence thus far indicates that mycorrhizal fungi are key components of microbial biomass in many ecosystems. We highlight the need to extend the application of current methods to focus on a greater range of habitats and mycorrhizal types enabling incorporation of mycorrhizal fungal biomass and turnover into biogeochemical cycling models.

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# Annex:
# COST FP0803 Joint Review-Publications - Open Access

**Fine-root turnover rates of European forests revisited: an analysis of data from sequential coring and ingrowth cores.**
I. Brunner, M.R. Bakker, R.G. Björk, Y. Hirano, M. Lukac, X. Aranda, I. Børja, T.D. Eldhuset, H.S. Helmisaari , C. Jourdan, B. Konôpka, B.C. López, C. Miguel Pérez, H. Persson, I. Ostonen.
Plant and Soil 362, 357-372 (2013).

**The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling.**
A. Ekblad, H. Wallander, D.L. Godbold, C. Cruz, D. Johnson, P. Baldrian, R.G. Björk, D. Epron, B. Kieliszewska-Rokicka, R. Kjøller, H. Kraigher, E. Matzner, J. Neumann, C. Plassard.
Plant and Soil 366, 1-27 (2013).

**Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils - A review.**
H. Wallander, A. Ekblad, D.L. Godbold, D. Johnson, A Bahr, P. Baldrian, R.G. Björk, B. Kieliszewska-Rokicka, R. Kjøller, H. Kraigher, C. Plassard, M. Rudawska.
Soil Biology and Biochemistry 57, 1034-1042 (2013).

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REVIEW ARTICLE

# Fine-root turnover rates of European forests revisited: an analysis of data from sequential coring and ingrowth cores

**I. Brunner · M. R. Bakker · R. G. Björk · Y. Hirano · M. Lukac · X. Aranda · I. Børja · T. D. Eldhuset · H. S. Helmisaari · C. Jourdan · B. Konôpka · B. C. López · C. Miguel Pérez · H. Persson · I. Ostonen**

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**Abstract**
*Background and Aims* Forest trees directly contribute to carbon cycling in forest soils through the turnover of their fine roots. In this study we aimed to calculate root turnover rates of common European forest tree species and to compare them with most frequently published values.
*Methods* We compiled available European data and applied various turnover rate calculation methods to

Responsible Editor: Alexia Stokes.

I. Brunner (✉)
Swiss Federal Institute for Forest, Snow and Landscape Research WSL,
8903 Birmensdorf, Switzerland
e-mail: ivano.brunner@wsl.ch

M. R. Bakker
University of Bordeaux, UMR1220 TCEM,
33175 Gradignan, France

M. R. Bakker
INRA, UMR1220 TCEM,
33883 Villenave d'Ornon, France

R. G. Björk
Department of Biological and Environmental Sciences,
University of Gothenburg,
P.O. Box 461, 405 30 Gothenburg, Sweden

Y. Hirano
Graduate School of Environmental Studies,
Nagoya University,
Nagoya 464-8601, Japan

M. Lukac
School of Agriculture, Policy and Development,
University of Reading,
Reading RG6 6AR, UK

X. Aranda · C. Miguel Pérez
Ecophysiology, IRTA Torre Marimon,
08140 Caldes de Montbui, Spain

I. Børja · T. D. Eldhuset
Norwegian Forest and Landscape Institute,
P.O. Box 115, 1431 Ås, Norway

H. S. Helmisaari
Department of Forest Sciences, University of Helsinki,
P.O. Box 27, 00014 Helsinki, Finland

C. Jourdan
CIRAD, UMR Eco&Sols,
2, Place Pierre Viala,
34060 Montpellier, France

B. Konôpka
Forest Research Institute, National Forest Centre,
96001 Zvolen, Slovak Republic

B. Konôpka
Faculty of Forestry and Wood Sciences, Czech University of Life Sciences,
Kamýcka 129, Suchdol,
16521 Prague, Czech Republic

the resulting database. We used Decision Matrix and Maximum-Minimum formula as suggested in the literature.

*Results* Mean turnover rates obtained by the combination of sequential coring and Decision Matrix were 0.86 $yr^{-1}$ for *Fagus sylvatica* and 0.88 $yr^{-1}$ for *Picea abies* when maximum biomass data were used for the calculation, and 1.11 $yr^{-1}$ for both species when mean biomass data were used. Using mean biomass rather than maximum resulted in about 30 % higher values of root turnover. Using the Decision Matrix to calculate turnover rate doubled the rates when compared to the Maximum-Minimum formula. The Decision Matrix, however, makes use of more input information than the Maximum-Minimum formula.

*Conclusions* We propose that calculations using the Decision Matrix with mean biomass give the most reliable estimates of root turnover rates in European forests and should preferentially be used in models and C reporting.

**Keywords** Annual production · Decision Matrix · Fine-root turnover rates · Ingrowth cores · Maximum-Minimum formula · Sequential coring

**Abbreviations**

| | |
|---|---|
| B | Biomass |
| BGC | Biogeochemical cycles |
| C | Carbon |
| DM | Decision Matrix |
| GPP | Gross primary production |
| GUESS | General ecosystem simulator |
| LPJ | Lund-Potsdam-Jena model |
| MM | Maximum-Minimum |
| MRT | Mean residence time |
| N | Necromass |
| NPP | Net primary production |
| P | Production |
| SOM | Soil organic matter |
| T | Turnover rate |

B. C. López
CREAF Center for Ecological Research and Forestry Applications, and Ecology Unit of the Department of Plant and Animal Biology and Ecology, Edifici Ciències UAB, 08193 Bellaterra, Spain

H. Persson
Department of Ecology, Swedish University of Agricultural Sciences SLU,
P.O. Box 7044, 750 07 Uppsala, Sweden

I. Ostonen
Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46,
51014 Tartu, Estonia

## Introduction

Turnover of tree fine roots is one of the major carbon (C) pathways in forests. The cause of the large C flux through this biomass pool is the rather limited lifespan of tree roots less than 2 mm in diameter. Given the estimated size of the C flux associated with the limited lifespan (synonyms: 'longevity' or 'turnover time', inverse of 'turnover rate') of fine roots, thought to reach 0.5 to 3 tC $ha^{-1}$ $yr^{-1}$ in steady-state forest ecosystems (Gill and Jackson 2000; Brunner and Godbold 2007), we clearly need to have a good understanding of the turnover rate at which fine roots die and contribute to soil C pools. Indeed, in the light of ongoing and projected climate change and the implementation of C reporting in many countries, belowground C dynamics have to be taken into account. Given the role of scientists in this debate, it is down to those who study root dynamics to provide the knowledge basis that permits modellers and C reporters to utilise the most realistic turnover values. Currently, root turnover rates are commonly utilised to parameterise biogeochemical models, which require fine root turnover rate data input e.g. Biome-BGC, LPJ, or LPJ-GUESS (e.g. Pietsch et al. 2005; Sitch et al. 2003; Smith et al. 2001). The turnover rates as input can be derived from published scientific literature, based on supposed relationships between leaf lifespan and fine root lifespan (i.e. lower turnover rates for evergreen species with long-lived leaves) with values between 0.18 and 1.02 $yr^{-1}$ (Pietsch et al. 2005; Cienciala and Tatarinov 2006; Tatarinov and Cienciala 2006) or simplified to just one value 0.7 $yr^{-1}$ as suggested recently by Hickler et al. (2008). Using the most appropriate turnover rates will improve the capacity of these models to assess the change in belowground C pools in forests.

Fine root turnover rate is dependent on the fine root biomass and the annual production of fine roots, but also on the various methods and calculations (e.g. Jourdan et al. 2008; Gaul et al. 2009; Finer et al. 2011; Yuan and Chen 2010). However, there is quite some uncertainty regarding which fine root turnover rates would be most suitable for end users. This is illustrated by the ongoing debate among scientists about how the turnover rate of

the fine roots can be estimated best and which method is the most suitable (e.g. Strand et al. 2008; Trumbore and Gaudinski 2003; Majdi et al. 2005; Jourdan et al. 2008). Starting from the most recent developments, stable C-isotopes and radiocarbon ($^{13}C$, $^{14}C$) may be used to estimate root carbon longevity, either by using labelling techniques or natural abundances in the atmosphere (e.g. Matamala et al. 2003; Gaudinski et al. 2001, 2010; Endrulat et al. 2010). A more widely used method to estimate the lifespan of fine roots is the use of minirhizotrons (e.g. Johnson et al. 2001; Majdi and Andersson 2005). This technique allows for a direct observation of individual roots and their development. Both methods suffer from several drawbacks, the main weakness of isotopic analysis for root age determination is the uncertain age of organic compounds used to construct fine roots (Sah et al. 2011). Meanwhile, minirhizotron studies are not able to determine the exact time of root death. In addition, the installation of the minirhizotron tubes can change water and temperature regimes as well as soil matrix resistance to root penetration. Moreover, fine root growth is often stimulated by the conditions along the minirhizotron tube. Unsurprisingly, direct comparisons of these two methods result in a discrepancy in root longevity estimates (Tierney and Fahey 2002; Strand et al. 2008; Gaul et al. 2009), sometimes explained by different fractions of fine roots under observation, i.e. the short-lived and the long-lived fine roots, likely to be recorded by these two methods (Gaudinski et al. 2010).

Alternatively, instead of direct observations of individual root longevity, the mean lifespan can be calculated by dividing the 'pool' (biomass) by its 'input' (annual production). Because the turnover rate is the inverse of lifespan, it can be calculated by dividing the 'annual production' by the 'belowground standing crop' (=biomass) (Gill and Jackson 2000). There are several methods used to obtain estimates of annual fine root production. A widely used method to directly measure the production of fine roots is the use of ingrowth cores (e.g. Persson 1980a, 1980b; Vogt and Persson 1991). This method measures the amount of fine roots which grow into a defined volume of root-free soil over a defined period of time. The advantage of this method is its relative ease and speed of application when estimating root production (Vogt and Persson 1991). More recently, root nets were applied instead of ingrowth cores to minimise soil disturbance during the installation (Hirano et al. 2009; Lukac and Godbold 2010). An alternative method to indirectly measure the production of fine roots is the sequential coring technique (e.g. Stober et al. 2000; Ostonen et al. 2005). Here, several series of soil cores are sampled at defined intervals over a period of at least 1 year. Fine roots are extracted from the soil cores and the differences of the dry mass of living (biomass) and dead (necromass) fine roots between two time points recorded. Taking advantage of data generated by sequential coring, several methods exist to calculate the production from the change of the fine-root biomass and necromass data. The production can be calculated by the 'Maximum-Minimum' formula (McClaugherty et al. 1982), by the 'Decision Matrix' formula (Fairley and Alexander 1985), or by the 'Compartment Flow' formula (Santantonio and Grace 1987). Whereas the 'Maximum-Minimum' formula uses only biomass data, the other two methods require both biomass and necromass data. The 'Compartment Flow' formula further requires decomposition data of fine root litter (e.g. Silver et al. 2005, Osawa and Aizawa 2012). Thus, the values of fine root turnover rates can vary not only due to measurement methods but also due to calculation methods applied (e.g. Publicover and Vogt 1993; Vogt et al. 1998; Strand et al. 2008). A true comparison of the various turnover rates may only be possible by using observations from same sites where various methods were applied (e.g. Haynes and Gower 1995; Ostonen et al. 2005; Hendricks et al. 2006; Gaul et al. 2009). As for the popularity of different measurement methods, many more estimates of root turnover rates are available from sequential coring and ingrowth cores than from the minirhizotron method (Finer et al. 2011).

We took advantage of the European COST network FP0803 "Belowground carbon turnover in European forests" bringing together root researchers from 30 European countries, to investigate on the sources of variation in turnover estimates available in the literature. In particular, as our group covered most of the European research groups that have worked on fine root turnover in the last decades, we were able to reunite/mine detailed datasets needed to evaluate the effect of calculation methods on fine root turnover rates in the European context. This implies that the implications of our work are restricted to European tree species and growth conditions. Our objectives were 1) to evaluate the pure effect of calculation methods on mean turnover rates of European forests and their ranges; 2) to evaluate how other factors such as soil stratification contribute to the calculated/perceived variation in turnover rates of European forests, and 3) to propose turnover rates and ranges for end users for the most common European forests.

## Materials and methods

### Data origin

Our study was carried out on data of fine root biomass and necromass of European forest tree species, extracted from published studies, found through regular literature research in library databases or supplied by members of our COST network. A large proportion of the data originates from doctoral theses due to the availability of raw data in this type of publication. We only included datasets where data collection was carried out for at least one full year. Fine root production was measured either directly by the use of the ingrowth core method or indirectly by the use of the sequential coring method (see Ostonen et al. 2005). Fine root biomass was defined as the amount of living fine roots occurring in the soil at any given time. Sequential coring was used to establish fine root biomass in most studies, apart from the case of the ingrowth core method where biomass usually was estimated from a single coring. We did not consider data originating from minirhizotron studies as these are reviewed elsewhere (Børja et al. in preparation). Finally, the dataset created for this study included 17 studies with 31 datasets for sequential coring and 7 for ingrowth core studies. The most abundant data sets obtained by sequential coring were available for *Fagus sylvatica* and *Picea abies* with 13 and 11 data sets, respectively (Table 3). Data sets of other tree species, e.g. *Pinus sylvestris*, *Populus* spp., and *Quercus* spp., were present only in three or fewer data sets. More than 80 % of the data were from forests with adult trees ('steady state' conditions). Data sets originating from ingrowth cores were available only for *F. sylvatica*, *P. abies*, and *P. sylvestris*, and with only two to three data sets per tree species (Table 4).

### Calculations of fine-root production

Fine root production was calculated either with the 'Maximum-Minimum' formula or the 'Decision Matrix'. The 'Compartment Flow' method was not applied because decomposition data of root litter were not sufficiently available. As a pre-requisite of annual fine root production calculation, a single sampling campaign must have lasted at least 12 months. Studies of less than 12 months (e.g. one vegetation period) or not of required level of detail were not considered (e.g. Konôpka et al. 2005; Konôpka 2009; López et al. 2001). At least two measurements from the same month in two consecutive years are the minimum requirement for the calculation of root production.

The Maximum-Minimum (MM) formula calculates the annual fine-root production ($P_a$) by subtracting the lowest biomass ($B_{min}$) from the highest biomass value ($B_{max}$) irrespectively of other biomass values recorded during a full year (McClaugherty et al. 1982). Necromass data are not required for this method:

$$P_{a(MM)} = B_{max} - B_{min} \quad (1)$$

The Decision Matrix (DM) calculates the annual fine-root production ($P_a$) by summing all calculated productions (P) between each pair of consecutive sampling dates throughout a full year:

$$P_{a(DM)} = \sum P \quad (2)$$

The production (P) between two sampling dates is calculated either by adding the differences in biomass ($\Delta B$) and necromass ($\Delta N$), by adding only the differences in biomass ($\Delta B$), or by equalling P to zero (Fairley and Alexander 1985). The conditions with which of the P formulas to be used are as follows:

$$P = \Delta B + \Delta N \quad \begin{array}{l} \text{a) if biomass and necromass have increased} \\ \text{b) if biomass has decreased and necromass has increased, but } |\Delta B| \text{ lower than } |\Delta N| \end{array} \quad (3)$$

$$P = \Delta B \quad \text{if biomass has increased and necromass has decreased} \quad (4)$$

$$P = 0 \quad \begin{array}{l} \text{a) if biomass and necromass have decreased} \\ \text{b) if biomass has decreased and necromass has increased, but } |\Delta B| \text{ higher than } |\Delta N| \end{array} \quad (5)$$

The Decision Matrix used as the basis for calculations is shown in Table 1. To calculate the annual production, all production values from interim periods are summed up from the start of sequential coring until the same time point in the following year (see also Table 2a, b). In the present study, all differences in biomass and necromass were taken into account during the calculation, assuming that the living and dead pool are continuously changing. However, some authors suggest summing up only the statistically significant differences (e.g. Stober et al. 2000). We propose that accounting for all differences between root biomass in two sampling dates constitutes a better approach. The size (and therefore the significance) of the difference is clearly dependent on the duration of the interim period, as well as on the season. Including significantly different observations would skew the data coverage towards long-gap observations only.

**Table 1** Decision Matrix according to Fairley and Alexander (1985). (B = Biomass, N = Necromass, P = Production)

| | Biomass increase | Biomass decrease |
|---|---|---|
| Necromass increase | $P = \Delta B + \Delta N$ | $P = \Delta B + \Delta N$ [a] or $P = 0$ [b] |
| Necromass decrease | $P = \Delta B$ | $P = 0$ |

[a] if $|\Delta B| < |\Delta N|$

[b] if $|\Delta B| > |\Delta N|$

### Calculations of fine-root turnover rates

The turnover rate $T_{Bmax}$ of fine roots was calculated by dividing the annual fine root production ($P_a$) by the highest biomass value (maximum biomass $B_{max}$) according to Gill and Jackson (2000) (compare also Table 2c):

$$T_{Bmax} = P_a / B_{max} \quad (6)$$

As an alternative, the turnover rate $T_{Bmean}$ was calculated by dividing the annual fine root production ($P_a$) by the mean biomass ($B_{mean}$) according to McClaugherty et al. (1982) (compare also Table 2c):

$$B_{mean} = \sum B/n \quad (n = \text{number of samples per year}) \quad (7)$$

$$T_{Bmean} = P_a / B_{mean} \quad (8)$$

### Assessment of other factors generating variation in fine-root turnover rates

Utilizing the raw datasets in our database, we analysed several other factors for their influence of fine root turnover, based on subsamples of the database for sequential coring only. These factors included 1) soil stratification, 2) soil depth, 3) root diameter, 4) observation length, 5) start of observation period and 6) number of samplings per year. For the soil stratification approach we used 13 sites with detailed root data for the various soil layers. Briefly, for the layer-per-layer approach we computed fine root production per layer and summed this as a fine root production for the entire profile. Turnover rate was then computed as production divided by average fine root biomass for the entire profile. For the whole profile approach, instead, we used the summed bio- and necromass values for the entire profile to compute fine root production and then divided by average fine root biomass to calculate the fine root turnover rate. For this comparison of the two approaches, sequential coring data, decision matrix calculations and mean biomass values were taken from Hertel (1999), Richter (2007), Makkonen and Helmisaari (1999), Bakker (1999), and Ostonen et al. (2005). In the reports relative to seven sites we had fine root data separately assessed for sub diameter classes (<1 mm, 1–2 mm) and so we could do the calculations for each diameter class and compare them with the total diameter class (<2 mm). And, utilizing studies with longest data series, we explored whether and how different observations lengths (1 yr, 1–2 yr, 2–3 yr), the start of the observation period (spring, summer, autumn, winter), and the number of samplings per year may influence the turnover values.

### Statistics

For statistical analyses, simple linear regression and Mann–Whitney *U* test, the software StatView 5.0 (SAS Institute, Cary, NY, USA) was used, with the significance level of $p<0.05$. The data was tested for normal distribution and for homogeneity of variances among groups.

## Results

### Fine-root turnover rate

Turnover rates obtained by the combination of sequential coring, Decision Matrix method, and the maximum biomass data varied from 0.19 to 2.04 $yr^{-1}$ for *F. sylvatica* and from 0.44 to 1.36 $yr^{-1}$ for *P. abies* (Table 3), with mean values for *F. sylvatica* and *P. abies* of 0.86 and 0.88 $yr^{-1}$, respectively (Table 5). Using the mean biomass instead of the maximum biomass, the turnover rates varied from 0.23 to 2.92 $yr^{-1}$ for *F. sylvatica* and from 0.56 to 1.77 $yr^{-1}$ for *P. abies* (Table 3), with mean values of 1.11 $yr^{-1}$ for both *F. sylvatica* and *P. abies* (Table 5). For other tree species, less than three data sets were available, e.g. only 2 data sets were available for *P. sylvestris*, and both had turnover rates higher than 1.5 $yr^{-1}$ (Table 3).

Turnover rates obtained by the combination of sequential coring, Maximum-Minimum method, and maximum biomass data were consistently below 0.7 $yr^{-1}$ for *F. sylvatica* and *P. abies* (Table 3), with mean turnover rates of 0.41 $yr^{-1}$ and 0.44 $yr^{-1}$, respectively (Table 5). The mean turnover rate of *P. sylvestris* was 0.48 $yr^{-1}$ and did fall in a similar range (Table 5). Using the mean biomass instead of the maximum biomass, the turnover rates ranged from 0.26 to 0.95 $yr^{-1}$ for *F. sylvatica* and *P.*

**Table 2** Worked sample with a data set from sequential coring (data from Ostonen et al. 2005). Formula [3] ($P = \Delta B + \Delta N$) and [4] ($P = \Delta B$) are according to Fairley and Alexander (1985). Other formula are according to the Material and Methods section. (P = Production, B = Biomass, N = Necromass, T = Turnover rate)

a) Calculation of the production P using the Decision Matrix.

| Sampling date | Biomass ($g\ m^{-2}$) | Necromass ($g\ m^{-2}$) | Formula | Calculation | Production P ($g\ m^{-2}\ t^{-1}$) |
|---|---|---|---|---|---|
| June 1996 | 127 | 130 | | | |
| July 1996 | 161 | 178 | [3] | (161-127)+(178-130) | 82 |
| Aug. 1996 | 166 | 114 | [4] | 166-161 | 5 |
| Sept. 1996 | 165 | 174 | [3] | (165-166)+(174-114) | 59 |
| Oct. 1996 | 199 | 198 | [3] | (199-165)+(198-174) | 58 |
| Nov. 1996 | 64 | 159 | [5] | 0 | 0 |
| June 1997 | 110 | 125 | [4] | 110-64 | 46 |
| Mean (±SE) [7]: | 141 (±17) | | | | Sum [2]: 250 |

b) Calculation of the annual production $P_a$.

| Method | Formula | Calculation | Annual production $P_a$ ($g\ m^{-2}\ yr^{-1}$) |
|---|---|---|---|
| Decision Matrix | [2] | 82+5+59+58+0+46 | 250 |
| Maximum-Minimum | [1] | 199-64 | 135 |

c) Calculation of the turnover rate T (using mean biomass $B_{mean}$ or maximum biomass $B_{max}$).

| Method | Formula | Calculation | | Turnover rate T ($yr^{-1}$) |
|---|---|---|---|---|
| | | Using $B_{mean}$ | Using $B_{max}$ | |
| Decision Matrix | [6] | 250 / 141 | – | 1.77 |
| Decision Matrix | [8] | – | 250 / 199 | 1.26 |
| Maximum-Minimum | [6] | 135 / 141 | – | 0.95 |
| Maximum-Minimum | [8] | – | 135 / 199 | 0.68 |

*abies* (Table 3), with mean turnover rates of 0.53 $yr^{-1}$ for *F. sylvatica* and 0.57 $yr^{-1}$ for *P. abies* (Table 5).

Using the ingrowth core method, in maximum three data sets were available per tree species (Table 4). Mean turnover rates obtained by ingrowth cores, the Decision Matrix method, and the maximum biomass were 1.00, 0.72, and 0.76 $yr^{-1}$ for *F. sylvatica*, *P. abies*, and *P. sylvestris*, respectively (Table 5). Using the Maximum-Minimum method and the maximum biomass, the mean turnover rates were with 1.00, 0.62, and 0.72 $yr^{-1}$, respectively, in a similar range (Table 5). Using the mean biomass instead of the maximum biomass, the mean turnover rates were higher, 2.58, 1.15, and 1.40 $yr^{-1}$ for *F. sylvatica*, *P. abies*, and *P. sylvestris*, respectively, using the Decision Matrix, and 2.58, 0.98, and 1.31 $yr^{-1}$ for *F. sylvatica*, *P. abies*, and *P. sylvestris*, respectively, using the Maximum-Minimum formula (Table 5).

We compared the difference in turnover rate estimates based on maximum or mean biomass as the denominator. On average in our dataset, using mean biomass rather than maximum resulted in about 30 % higher estimate of root turnover rate T ($T_{Bmean} = 1.3\ T_{Bmax} - 0.001$; $r^2 = 0.98$, $p<0.001$; Fig. 1).

**Table 3** Sequential coring: Mean and maximum biomass, annual production, and turnover rate of tree fine roots recorded with sequential coring. The annual production is calculated with the 'Decision Matrix' or the 'Maximum-Minimum' formula, and the turnover rate is calculated by dividing the annual production by the mean biomass ($B_{mean}$) or by the maximum biomass ($B_{max}$). (B = Biomass)

| Country | Site | Mean annual temp. (°C) | Depth (cm) | Stand age (yr) | Biomass (B) | | Decision Matrix | | | Maximum-Minimum | | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean ($g\ m^{-2}$) | Max. ($g\ m^{-2}$) | Production | Turnover rate | | Production | Turnover rate | | |
| | | | | | | | ($g\ m^{-2}\ yr^{-1}$) | $B_{mean}$ ($yr^{-1}$) | $B_{max}$ ($yr^{-1}$) | ($g\ m^{-2}\ yr^{-1}$) | $B_{mean}$ ($yr^{-1}$) | $B_{max}$ ($yr^{-1}$) | |
| *Fagus sylvatica:* | | | | | | | | | | | | | |
| Switzerl. | Entleb. | 6.7 | 0–25 | >100 | 422 | 580 | 395 | 0.94 | 0.68 | 290 | 0.69 | 0.50 | Richter (2007) |
| Switzerl. | Krauch. | 8.2 | 0–25 | >100 | 480 | 710 | 476 | 0.99 | 0.67 | 356 | 0.74 | 0.50 | Richter (2007) |
| Switzerl. | Nieder. | 8.7 | 0–25 | >100 | 413 | 501 | 281 | 0.68 | 0.56 | 217 | 0.53 | 0.43 | Richter (2007) |
| Switzerl. | Walter. | 7.4 | 0–25 | >100 | 348 | 441 | 193 | 0.55 | 0.44 | 171 | 0.49 | 0.39 | Richter (2007) |
| Switzerl. | Vordem. | 8.8 | 0–25 | >100 | 807 | 957 | 597 | 0.74 | 0.62 | 356 | 0.44 | 0.37 | Richter (2007) |
| Switzerl. | Zofing. | 8.2 | 0–25 | >100 | 517 | 600 | 144 | 0.28 | 0.24 | 142 | 0.27 | 0.24 | Richter (2007) |
| Germany | Götting. | 8.7 | 0–15 | 120 | 177 | 219 | 41 | 0.23 | 0.19 | 75 | 0.42 | 0.34 | Hertel (1999) |
| Germany | Lüneb. | 8.1 | 0–5 | 100 | 279 | 312 | 458 | 1.64 | 1.47 | 97 | 0.35 | 0.31 | Hertel (1999) |
| Germany | Solling | 6.9 | 0–5 | 150 | 134 | 149 | 226 | 1.68 | 1.51 | 45 | 0.33 | 0.30 | Hertel (1999) |
| Germany | Ziegel. | 8.6 | 0–10 | 120 | 70 | 100 | 203 | 2.92 | 2.04 | 46 | 0.66 | 0.46 | Hertel (1999) |
| Germany | Götting. | 7.0 | 0–20 | 130 | 195 | 282 | 218 | 1.12 | 0.77 | 157 | 0.81 | 0.56 | Wu (2000) |
| Germany | Solling | 6.4 | 0–40 | 149 | 328 | 373 | 211 | 0.64 | 0.57 | 85 | 0.26 | 0.23 | Wu (2000) |
| France | Aubure | 6.0 | 0–30 | 161 | 83 | 120 | 165 | 2.00 | 1.38 | 77 | 0.93 | 0.64 | Stober et al. (2000) |
| *Picea abies:* | | | | | | | | | | | | | |
| Germany | Fichtel. | 5.3 | 0–60 | 140 | 175 | 224 | 304 | 1.74 | 1.36 | 104 | 0.60 | 0.47 | Gaul et al. (2009) |
| Germany | Barbis | 8.0 | 0–40 | 39 | 182 | 235 | 116 | 0.63 | 0.49 | 124 | 0.68 | 0.53 | Fritz (1999) |
| Germany | Eberg. | 7.8 | 0–40 | 34 | 150 | 188 | 83 | 0.56 | 0.44 | 90 | 0.60 | 0.48 | Fritz (1999) |
| Germany | Fichtel. | 5.5 | 0–40 | 40 | 245 | 340 | 156 | 0.64 | 0.46 | 160 | 0.65 | 0.47 | Fritz (1999) |
| Germany | Harz | 6.0 | 0–40 | 47 | 204 | 241 | 278 | 1.36 | 1.15 | 63 | 0.31 | 0.26 | Fritz (1999) |
| Estonia | Roela | 5.4 | 0–40 | 60 | 142 | 199 | 251 | 1.77 | 1.26 | 135 | 0.95 | 0.68 | Ostonen et al. (2005) |
| France | Aubure | 6.0 | 0–30 | 92 | 57 | 70 | 89 | 1.56 | 1.27 | 30 | 0.52 | 0.43 | Stober et al. (2000) |
| Norway | Nordm. | 3.8 | 0–40 | 50 | 462 | 603 | 298 | 0.65 | 0.49 | 282 | 0.61 | 0.47 | Eldhuset et al. (2006) |
| Norway | Nordm. | 3.8 | 0–60 | 60 | 56 | 62 | 63 | 1.13 | 1.02 | 17 | 0.31 | 0.27 | Børja et al. (2008) |
| Norway | Nordm. | 3.8 | 0–60 | 120 | 50 | 63 | 70 | 1.40 | 1.11 | 22 | 0.48 | 0.35 | Børja et al. (2008) |
| Sweden | Forsm. | 5.5 | 0–40 | 80 | 304 | 410 | 241 | 0.79 | 0.59 | 186 | 0.61 | 0.45 | Persson and Stadenberg (2010) |

**Table 3** (continued)

| Country | Site | Mean annual temp. (°C) | Depth (cm) | Stand age (yr) | Biomass (B) | | Decision Matrix | | | Maximum-Minimum | | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean ($g\ m^{-2}$) | Max. ($g\ m^{-2}$) | Production | Turnover rate | | Production | Turnover rate | | |
| | | | | | | | ($g\ m^{-2}\ yr^{-1}$) | $B_{mean}$ ($yr^{-1}$) | $B_{max}$ ($yr^{-1}$) | ($g\ m^{-2}\ yr^{-1}$) | $B_{mean}$ ($yr^{-1}$) | $B_{max}$ ($yr^{-1}$) | |
| *Pinus sylvestris:* | | | | | | | | | | | | | |
| Finland | Iloman. | 1.9 | 0–30 | 38 | 278 | 363 | 862 | 3.10 | 2.37 | 181 | 0.65 | 0.50 | Makkonen and Helmisaari (1999) |
| Sweden | Ivantj. | 5.2 | 0–30 | 120 | 120 | 153 | 242 | 2.03 | 1.58 | 69 | 0.58 | 0.45 | Persson (1980a) |
| *Populus spp.:* | | | | | | | | | | | | | |
| Italy | P. alba | 14.4 | 0–40 | 2 | 110 | 143 | 55 | 0.50 | 0.39 | 56 | 0.51 | 0.40 | Lukac et al. (2003) |
| Italy | P. nigra | 14.4 | 0–40 | 2 | 109 | 158 | 84 | 0.77 | 0.53 | 84 | 0.77 | 0.53 | Lukac et al. (2003) |
| Italy | P. eura. | 14.4 | 0–40 | 2 | 146 | 187 | 55 | 0.37 | 0.29 | 89 | 0.61 | 0.48 | Lukac et al. (2003) |
| *Quercus ilex/Q. cerrioides:* | | | | | | | | | | | | | |
| Spain | Bages | 14.4 | 0–50 | 10 | 858 | 1336 | – | – | – | 812 | 0.95 | 0.61 | Miguel Pérez (2010) |
| *Quercus petraea:* | | | | | | | | | | | | | |
| France | La Croix | 8.0 | 0–55 | 45 | 310 | 346 | 53 | 0.17 | 0.15 | 63 | 0.29 | 0.18 | Bakker (1999) |

**Table 4** Ingrowth cores: Mean and maximum biomass, annual production, and turnover rate of tree fine roots recorded with ingrowth cores. The annual production is calculated with the 'Decision Matrix' or the 'Maximum-Minimum' formula, and the turnover rate is calculated by dividing the annual production by the mean biomass ($B_{mean}$) or by the maximum biomass ($B_{max}$). (B=Biomass)

| Country | Site | Mean annual temp. (°C) | Depth (cm) | Year after install. (yr) | Stand age (yr) | Biomass (B) | | Decision Matrix | | | Maximum-Minimum | | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Mean ($g\ m^{-2}$) | Max. ($g\ m^{-2}$) | Production | Turnover rate | | Production | Turnover rate | | |
| | | | | | | | | ($g\ m^{-2}\ yr^{-1}$) | $B_{mean}$ ($yr^{-1}$) | $B_{max}$ ($yr^{-1}$) | ($g\ m^{-2}\ yr^{-1}$) | $B_{mean}$ ($yr^{-1}$) | $B_{max}$ ($yr^{-1}$) | |
| *Fagus sylvatica:* | | | | | | | | | | | | | | |
| Germany | Götting. | 7.0 | 0–20 | 2 | 130 | 42 | 107 | 107 | 2.58 | 1.00 | 107 | 2.58 | 1.00 | Wu (2000) |
| Germany | Solling | 6.4 | 0–20 | 2 | 149 | 48 | 123 | 123 | 2.57 | 1.00 | 123 | 2.57 | 1.00 | Wu (2000) |
| *Picea abies:* | | | | | | | | | | | | | | |
| Switzerl. | Schlad. | 9.6 | 0–10 | 2 | 70 | 80 | 106 | 65 | 0.81 | 0.62 | 65 | 0.81 | 0.62 | Genenger et al. (2003) |
| Estonia | Roela | 5.4 | 0–30 | 2 | 60 | 52 | 100 | 89 | 1.70 | 0.89 | 74 | 1.41 | 0.74 | Ostonen et al. (2005) |
| Estonia | Roela | 5.4 | 0–30 | 3 | 60 | 70 | 100 | 66 | 0.94 | 0.65 | 51 | 0.73 | 0.51 | Ostonen et al. (2005) |
| *Pinus sylvestris:* | | | | | | | | | | | | | | |
| Switzerl. | Pfynw. | 9.2 | 0–10 | 2 | 90 | 44 | 62 | 37 | 0.84 | 0.59 | 37 | 0.84 | 0.59 | Brunner et al. (2009) |
| Sweden | Ivantj. | 5.2 | – | 2 | 120 | 65 | 136 | 126 | 1.96 | 0.93 | 115 | 1.78 | 0.84 | Persson (1980a) |

### Soil stratification and root turnover rate

Our results show that a layer-per-layer approach yields a higher turnover rate than a 'one soil layer' approach (Fig. 2). Using average data for the whole of the soil profile, as opposed to using data for individual layers, does not capture all observed differences in root biomass and therefore results in a lower estimate of NPP and thus significantly lower turnover rate T ($T_{wholeprofile} = 0.88\ T_{layer-per-layer} - 0.17$; $r^2 = 0.91$, $p<0.001$; Fig. 2).

### Comparison between the Decision Matrix and the Maximum-Minimum method

Mean turnover rates calculated with the Decision Matrix were significantly higher than rates calculated with the Maximum-Minimum method ($p<0.001$, Fig. 3). The Decision Matrix methods yielded T approximately double the Maximum-Minimum method. The turnover rates were significantly different when using mean biomass data (1.14 $yr^{-1}$ from the Decision Matrix *versus* 0.57 $yr^{-1}$ from the Maximum-Minimum method) as well as when using maximum biomass data (0.88 $yr^{-1}$ *versus* 0.43 $yr^{-1}$). Using mean biomass data resulted in significantly higher turnover rates compared to the use of maximum biomass data ($p=0.006$, Fig. 3), with a mean difference of about 30 %.

## Discussion

### Decision Matrix *versus* Maximum-Minimum method

By analysing our European data set, we found about two times higher root turnover rates when using the Decision Matrix method compared to the Maximum-Minimum method. The observed discrepancy is best described by

**Table 5** Summary of biomass, annual production, and turnover rates (±SE) of fine roots of common European tree species. The annual production is calculated with the 'Decision Matrix' or the 'Maximum-Minimum' formula, and the turnover rate is calculated by dividing the annual production by the mean biomass ($B_{mean}$) or by the maximum biomass ($B_{max}$)

| Biomass (B) | | Decision Matrix | | | Maximum-Minimum | | |
|---|---|---|---|---|---|---|---|
| Mean ($g\ m^{-2}$) | Maximum ($g\ m^{-2}$) | Production ($g\ m^{-2}\ yr^{-1}$) | Turnover rate | | Production ($g\ m^{-2}\ yr^{-1}$) | Turnover rate | |
| | | | $B_{mean}$ ($yr^{-1}$) | $B_{max}$ ($yr^{-1}$) | | $B_{mean}$ ($yr^{-1}$) | $B_{max}$ ($yr^{-1}$) |
| Sequential coring method | | | | | | | |
| *Fagus sylvatica* ($n$=13): | | | | | | | |
| 327 | 411 | 278 | 1.11 | 0.86 | 163 | 0.53 | 0.41 |
| (±57) | (±71) | (±44) | (±0.21) | (±0.16) | (±31) | (±0.06) | (±0.03) |
| *Picea abies* ($n$=11): | | | | | | | |
| 184 | 240 | 177 | 1.11 | 0.88 | 110 | 0.57 | 0.44 |
| (±37) | (±49) | (±30) | (±0.14) | (±0.11) | (±24) | (±0.05) | (±0.04) |
| *Pinus sylvestris* ($n$=2): | | | | | | | |
| 199 | 258 | 552 | 2.57 | 1.98 | 125 | 0.62 | 0.48 |
| (±80) | (±105) | (±310) | (±0.54) | (±0.40) | (±56) | (±0.04) | (±0.02) |
| Ingrowth cores method | | | | | | | |
| *Fagus sylvatica* ($n$=2): | | | | | | | |
| 45 | 115 | 115 | 2.58 | 1.00 | 115 | 2.58 | 1.00 |
| (±3) | (±8) | (±8) | (±0.01) | (±0.00) | (±8) | (±0.01) | (±0.00) |
| *Picea abies* ($n$=3): | | | | | | | |
| 67 | 102 | 73 | 1.15 | 0.72 | 63 | 0.98 | 0.62 |
| (±8) | (±2) | (±8) | (±0.28) | (±0.09) | (±7) | (±0.21) | (±0.07) |
| *Pinus sylvestris* ($n$=2): | | | | | | | |
| 55 | 99 | 82 | 1.40 | 0.76 | 76 | 1.31 | 0.72 |
| (±11) | (±37) | (±45) | (±0.56) | (±0.17) | (±39) | (±0.47) | (±0.12) |

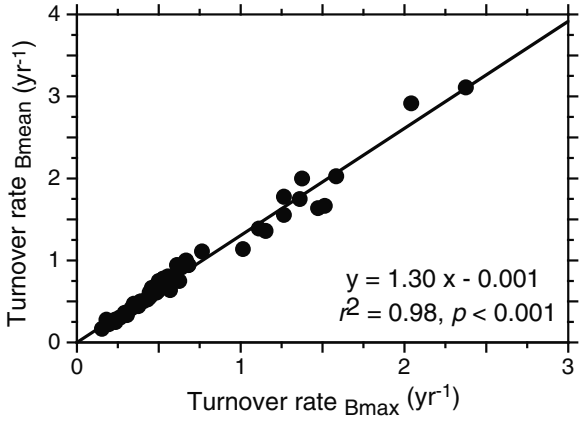


**Fig. 1** Relationship between turnover rates using mean biomass ($B_{mean}$) or maximum biomass data ($B_{max}$). Turnover rates were calculated from the whole data set of sequential coring and using the Decision Matrix and the Maximum-Minimum method

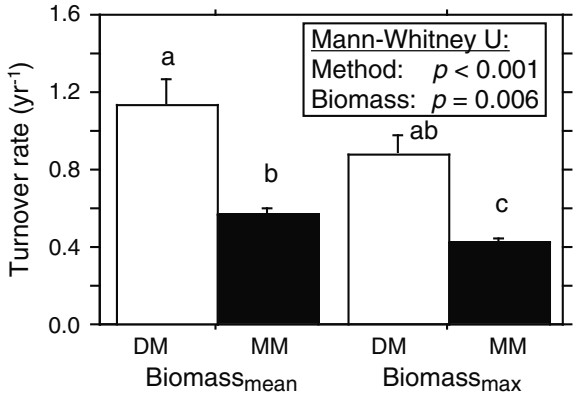


**Fig. 3** Mean turnover rates calculated from the whole data set of sequential coring and using the Decision Matrix (DM) or the Maximum-Minimum method (MM) and using mean biomass $B_{mean}$ or maximum biomass $B_{max}$ data

the fact that Decision Matrix accumulates differences between all observations—the larger the number of interim observations (e.g. monthly observations) the larger the potential for accounting all the peaks and troughs. The Maximum-Minimum method, on the other hand, makes use only of the annual net gain in biomass. On the basis of our comparison, we suggest that the Maximum-Minimum method should be used with caution; by definition, root turnover rates calculated by this method are bound between 0 and 1. Although this range

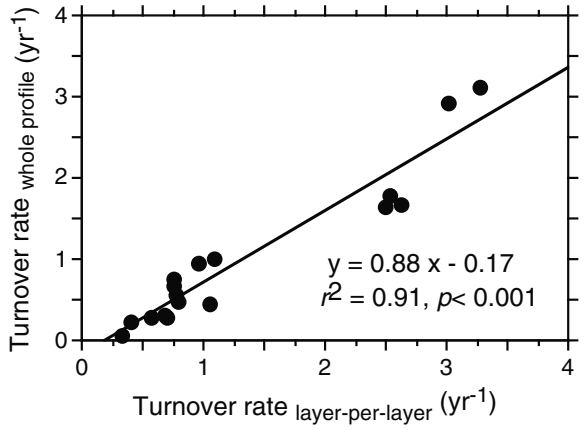


**Fig. 2** Relationship between turnover rates calculated per whole soils profiles or per individual soil layers (summed *versus* individual layers). Turnover rates were calculated the whole data set of sequential coring and using the Decision Matrix method and maximum biomass data (data from Hertel 1999; Richter 2007; Makkonen and Helmisaari 1999; Bakker 1999; Ostonen et al. 2005). Mean soil depth is 44 cm, and the average number of individual soil layers is four

may cover some ecosystems, it cannot correctly capture reality in systems where faster root turnover has been observed (e.g. Lukac et al. 2003) as it was shown for fast growing species (Jourdan et al. 2008). The Maximum-Minimum method is therefore only suitable for ecosystems with strong annual fluctuation of fine root biomass where turnover rate is not expected to exceed 1. In a forest ecosystem where root production and root death occur continuously and on a similar level all year round (e.g. 'steady state'), no differences between maximum and minimum biomass will be observed. Such an observation will result in a zero estimate of root production and subsequently a zero estimate of root turnover rate (see also Kurz and Kimmins 1987).

Moving on to the Decision Matrix method, the weak point of this method is—as with all methods using dead roots—the difficulty of quantifying root necromass. The potentially rapid disappearance of root necromass may lead to underestimates (Hendricks et al. 2006). One of possible reasons for the rapid disappearance is belowground herbivory (Stevens et al. 2002). Nevertheless, we propose that if necromass observations are available or can be obtained, the Decision Matrix should be favoured over the Minimum-Maximum formula. The former considers both living and dead fine roots, the calculation is thus based on more information, reducing the scope for significant errors. One potential source of error, however, is if all the differences in fine root biomass and necromass between sampling periods are subjected to the calculations regardless of their statistical significance. This may lead to overestimation.

Even though root coring methods—whether sequential or ingrowth—do deliver dependable and comparable measurements of fine root turnover, the application of the minirhizotron technique to estimate fine-root production and turnover is still favoured over the sequential coring or the ingrowth core method in certain situations (Hendricks et al. 2006). Turnover rate estimates obtained by minirhizotron studies can be higher than 1 and the method allows for repeated observation of the same roots. However, in some forest ecosystems, application of minirhizotron methods to measure fine-root production is hampered, e.g. in stony or shallow soils or on steep slopes. Sequential coring and ingrowth core methods are suitable even for these environments, giving them an advantage in terms of comparability of resulting data.

## Maximum biomass *versus* mean biomass

By definition, the denominator in the root turnover calculation equation is the representation of biomass present in the soil. An assumption inherent to all root turnover calculation method is that annual fine root production (obtained by whatever method) equals to fine root mortality and the system is at steady state on an annual basis. Over the course of a year, new growth replaces roots which have died. The proportion of roots which have been replaced can therefore be calculated as root production over biomass. At the present, both maximum and mean root biomass are used, with about two-thirds of studies using maximum biomass (Gill and Jackson 2000). They justified the use of the maximum biomass as "…because it is an extensively used model of root turnover and because of its heuristic value". When constructing models of root allocation in forests, a case can be made for maximum biomass to be the preferred parameter over mean or minimum values due to the importance of setting an upper limit for the allocation rate. Fine root allocation rate may depend on sink strength (C demand), but might ultimately be limited by the maximum fraction of GPP which trees can allocate to root systems (Astrid Meyer, personal communication; see also Farrar and Jones 2000; Gower et al. 1996; Poorter et al. 2012). Having said that, and bearing in mind that the root turnover calculation assumes an ecosystem at steady state, a mean value is indicative of the long-term average as it evens out seasonal variation in biomass. Maximum biomass, on the other hand, is substantially more susceptible to between-year fluctuations due to climatic variation, which occur even if a forest ecosystem is at a steady state. Thus, we propose that mean biomass rather than the maximum is more representative of the annual live biomass present in the soil. The use of mean biomass in our calculations increased the turnover rates by about 30 % compared to the use of the maximum biomass.

An additional factor significantly affecting the results of the turnover calculations is the use of summed up values of biomass, necromass, and productivity for the whole soil profile *versus* using these data for individual soil layers (horizons). We acknowledge that using individual horizons should be preferable as the root turnover rate may be affected by differing physical and chemical characteristics of individual horizons. We established that basing root turnover rate calculation on individual horizon data increases the overall turnover rate—probably because it allows for better capture of biomass and necromass variations over time. We are, however, aware that root biomass and production observation on a horizon basis constitutes a significant technical challenge and contend that using whole-soil data is acceptable. Further factors potentially influencing the turnover rate, e.g. soil depth, length of study, or root diameter class have also been tested in this study, however, the available European dataset for these parameters was limited and did not allow further deductions. Thus, besides the uncertainties due to climatic and calculation reasons, many other external factors may potentially affect the estimates of root turnover rates. At present, no available technique can solve this predicament and we put forward that our root turnover rates represent the best approximation obtained by using sequential soil or ingrowth cores.

## Turnover rates of European tree species

Our review of published studies from European forest stands revealed that most data for fine-root turnover rate originate from sequential coring, with the prevalence of *Fagus sylvatica* or *Picea abies* as the species of interest. Studies performed in forest stands with other dominating tree species such as *Quercus* spp., *Pinus* spp. were far less abundant. Similarly, turnover rate studies where ingrowth cores were used instead of employing the sequential coring method to measure fine-root production, were far less abundant. Whereas in our study the data sets of *F. sylvatica* derived mainly from Central Europe, the data sets of *P. abies* originated

from Central as well as from Northern Europe. Trees from Southern European countries were represented only by a few data sets, and no conclusive turnover rates can be suggested for this environment yet. Overall, we propose that only the fine root turnover rates in our study for the following species may be recommended for further use in biogeochemical models with a reasonable degree of accuracy: *F. sylvatica* and *P. abies*. We established a turnover rate of 1.11 $yr^{-1}$ for both *F. sylvatica* and *P. abies*, using the Decision Matrix formula and the mean biomass data from sequential coring.

## Turnover rates applied in biogeochemical models

One of the aims of the present study was to deliver suitable fine-root turnover data of European tree species, which may be used by modellers to construct ecosystem or biogeochemical models. Such models are applied in many European countries to report the change of belowground C in European forests as a reporting requirement for the Kyoto protocol signatories. A brief overview of the models applied so far shows that a wide variety of root turnover rates are used, some resembling measured values, others less so. In one of the first applications, the fine-root turnover rate was set to 1.0 $yr^{-1}$ for deciduous broad-leaf and deciduous needle-leaf trees and to 0.26 $yr^{-1}$ for evergreen needle-leaf trees (White et al. 2000, using the Biome-BGC model). The distinct difference between deciduous trees and evergreen needle-leaf trees mainly originated from the notion that fine-root turnover rate is equal to leaf turnover rate. A compilation of the various turnover rates applied in European modelling studies is shown in Table 6. Most recent studies applied a universal fine-root turnover rate of 0.7 $yr^{-1}$ to all forest tree species (Hickler et al. 2008, using the LPJ-GUESS model). This assumption is based on Vogt et al. (1996) and on Li et al. (2003) (Thomas Hickler, personal communication). Li et al. (2003) found a linear relationship between fine root production and fine root biomass, with the turnover rate 0.64 $yr^{-1}$ which was lower than the original estimate of 0.73 $y^{-1}$ from a previous analysis (Kurz et al. 1996). Using 'universal' turnover rates, however, should be discouraged if country-based C budgets have to be reported within the frame to the Kyoto

**Table 6** Fine-root turnover rates ($yr^{-1}$) of European trees used in biogeochemical models. (BGC = Biogeochemical cycles, GUESS = General ecosystem simulator, LPJ = Lund-Potsdam-Jena model)

| Tree type | Tree species | Turnover rate | Model | Reference |
|---|---|---|---|---|
| Broad-/Deciduous needle-leaved | | 1.0 | Biome-BGC | White et al. (2000) |
| Broad-leaved summergreen | | 1.0 | LPJ-GUESS | Smith et al. (2001) |
| Broad-leaved | | 1.0 | LPJ-GUESS | Hickler et al. (2004) |
| Broad-leaved | | 0.7 | LPJ-GUESS | Hickler et al. (2006, 2008) |
| | *Fagus sylvatica* | 1.023 | Biome-BGC | Cienciala and Tatarinov (2006)[a] |
| | *Fagus sylvatica* | 1.0 | Biome-BGC | Pietsch et al. (2005) |
| | *Quercus robur* | 1.023 | Biome-BGC | Cienciala and Tatarinov (2006)[a] |
| | *Quercus robur* | 1.0 | Biome-BGC | Pietsch et al. (2005) |
| | *Quercus petraea* | 1.023 | Biome-BGC | Cienciala and Tatarinov (2006)[a] |
| | *Quercus petraea* | 1.0 | Biome-BGC | Pietsch et al. (2005) |
| | *Larix decidua* | 1.0 | Biome-BGC | Pietsch et al. (2005) |
| Evergreen needle-leaved | | 0.26 | Biome-BGC | White et al. (2000) |
| Needle-/Broad-leaved evergreen | | 0.5 | LPJ-GUESS | Smith et al. (2001) |
| Needle-leaved | | 0.5 | LPJ-GUESS | Hickler et al. (2004) |
| Needle-leaved | | 0.7 | LPJ-GUESS | Hickler et al. (2006, 2008) |
| | *Picea abies* | 0.811 | Biome-BGC | Cienciala and Tatarinov (2006)[a] |
| | *Picea abies* | 0.195 | Biome-BGC | Pietsch et al. (2005) |
| | *Pinus sylvestris* | 0.18 | Biome-BGC | Pietsch et al. (2005) |
| | *Pinus cembra* | 0.18 | Biome-BGC | Pietsch et al. (2005) |

[a] and Tatarinov and Cienciala (2006)

protocol and species-specific and biome based values of root turnover rate are available.

## Conclusions

The present synthesis on fine-root turnover of European forests reveals that only *Fagus sylvatica* and *Picea abies* have sufficient data availability to suggest mean turnover rates obtained by soil coring to be used by National C reporters (0.86±0.16 $yr^{-1}$ for *F. sylvatica*, 0.88±0.11 $yr^{-1}$ for *P. abies*, when maximum biomass data are used; 1.11±0.21 $yr^{-1}$ for *F. sylvatica*, 1.11± 0.14 $yr^{-1}$ for *P. abies*, when mean biomass data are used). Data sets of other European forests or obtained by alternative methods such as ingrowth cores were too small to allow for distinct conclusions on the turnover rates. Based on our calculations, we put forward that usage of mean rather than maximum root biomass in turnover calculations is preferable as it better reflects long-term quantity of biomass.

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MARSCHNER REVIEW

# The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling

**A. Ekblad · H. Wallander · D. L. Godbold · C. Cruz · D. Johnson · P. Baldrian · R. G. Björk · D. Epron · B. Kieliszewska-Rokicka · R. Kjøller · H. Kraigher · E. Matzner · J. Neumann · C. Plassard**

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**Abstract** There is growing evidence of the importance of extramatrical mycelium (EMM) of mycorrhizal fungi in carbon (C) cycling in ecosystems. However, our understanding has until recently been mainly based on laboratory experiments, and knowledge of such basic parameters as variations in mycelial production, standing biomass and turnover as well as the regulatory mechanisms behind such variations in forest soils is limited. Presently, the production of EMM by ectomycorrhizal (EM) fungi has been

Responsible Editor: Philippe Hinsinger.

A. Ekblad (✉) · R. G. Björk
School of Science & Technology, Örebro University,
701 82 Örebro, Sweden
e-mail: alf.ekblad@oru.se

H. Wallander
Department of Biology, Microbial Ecology Group,
Ecology Building, Lund University, 223 62 Lund, Sweden

D. L. Godbold
Institute of Forest Ecology, Universität für Bodenkultur,
1190 Vienna, Austria

C. Cruz
Plant Biology, University of Lisbon, Lisbon, Portugal

D. Johnson
Institute of Biological and Environmental Sciences,
University of Aberdeen, Cruickshank Building,
St Machar Drive, Aberdeen AB24 3UU, UK

P. Baldrian
Laboratory of Environmental Microbiology,
Institute of Microbiology ASCR, 14220 Praha, Czech Republic

R. G. Björk
Department of Earth Sciences, University of Gothenburg,
P.O. Box 460, 405 30 Gothenburg, Sweden

D. Epron
UMR INRA-UL Forest Ecology and Ecophysiology,
Université de Lorraine, BP70239,
54506 Vandoeuvre-les-Nancy Cedex, France

B. Kieliszewska-Rokicka
Institute of Environmental Biology, Kazimierz Wielki
University, Al. Ossolinskich 12, 85-093 Bydgoszcz, Poland

R. Kjøller
Terrestrial Ecology, Biological Institute, University
of Copenhagen, Universitetsparken 15, bygning 1,
DK-2100 Copenhagen, Denmark

H. Kraigher
Slovenian Forestry Institute, Vecna pot 2,
1000 Ljubljana, Slovenia

E. Matzner · J. Neumann
Soil Ecology, University of Bayreuth, Dr. Hans Frisch Str. 1,
95440 Bayreuth, Germany

C. Plassard
INRA, UMR Eco & Sols,
34060 Montpellier Cedex 02, France

estimated at ~140 different forest sites to be up to several hundreds of kg per ha per year, but the published data are biased towards *Picea abies* in Scandinavia. Little is known about the standing biomass and turnover of EMM in other systems, and its influence on the C stored or lost from soils. Here, focussing on ectomycorrhizas, we discuss the factors that regulate the production and turnover of EMM and its role in soil C dynamics, identifying important gaps in this knowledge. C availability seems to be the key factor determining EMM production and possibly its standing biomass in forests but direct effects of mineral nutrient availability on the EMM can be important. There is great uncertainty about the rate of turnover of EMM. There is increasing evidence that residues of EM fungi play a major role in the formation of stable N and C in SOM, which highlights the need to include mycorrhizal effects in models of global soil C stores.

**Keywords** Decomposition · Exploration type · Extramatrical mycelium · In-growth bag · Minirhizotron · Soil organic matter · Rhizomorphs · Turnover rates

## Introduction

In forests, the total below-ground flux of carbon (C) represents between 25 and 63 % of gross primary production (Litton et al. 2007) and has a large influence on the physical, chemical and biological properties of the soil. While the flux of C into and out of the soil is relatively easy to estimate, little is known about the processes behind these fluxes. The production and turnover of the extramatrical mycelium (EMM) of mycorrhizal fungi is one of the least understood of these processes, which is an obstacle in modelling ecosystem C dynamics (Chapin et al. 2009; Meyer et al. 2010). In boreal and temperate forests, which is the main focus of the review, the EMM is mainly produced by ectomycorrhizal (EM) fungi associated with trees, but the amount of mycelium produced by arbuscular mycorrhizal (AM) fungi associated with herbs and some tree species can be large especially at high soil pH (Nilsson et al. 2005). The contribution of ericoid mycorrhizas to the soil mycelium remains largely unknown (Read and Perez-Moreno 2003). The EMM plays key roles in ecological processes such as plant nutrient uptake (Harley 1989), the nitrogen (N) cycling (Hodge and Fitter 2010), mineral weathering (Landeweert et al. 2001) and survival and establishment of seedlings (Smith and Read 2008) and in plant community composition (van der Heijden et al. 1998).

The EMM of mycorrhizal fungi likely has an important role in C cycling in ecosystems. Firstly, C flux through the EMM is probably large, secondly, it may be important for formation of soil organic matter (SOM) and thirdly, it may directly or indirectly affect decomposition of SOM. In this paper we discuss the factors that regulate the production, standing biomass and turnover of EMM, which are crucial parameters needed to assess the overall role of EMM in C cycling. The numbers of papers that present estimates of EMM production are increasing rapidly and we are for the first time putting all these data together to estimate typical mean values for different forest types. We give some attention to the importance of EMM for the formation of recalcitrant forms of C, its indirect and direct effects on decomposition of SOM and its contribution to fluxes of $CO_2$ in soil respiration. The interested reader may find additional information about the importance of the EMM in recent reviews of soil organic matter decomposition (Talbot et al. 2008), below ground litter quality (Langley and Hungate 2003), mineral weathering (van Schöll et al. 2008; Rosling 2009), soil aggregation (Rillig and Mummey 2006), mycelial networks (Simard 2009), C cycling (Jones et al. 2009; Cairney 2012), N cycling (Wu 2011), phosphorus (P) uptake (Cairney 2011) and broader ecological scopes (Read and Perez-Moreno 2003; Finlay 2008; Leake et al. 2004; Allen 2007; Courty et al. 2010; Hodge et al. 2010). In this review we focus on EM symbioses, these being the most important mycorrhizal type on trees in temperate and boreal forests (Read and Perez-Moreno 2003), but we make some comparisons with AM fungi. Much of the knowledge we have concerning the EMM is based on laboratory microcosm and pot studies, although an increasing number of studies are performed in situ, facilitated by techniques such as mycelium in-growth bags, chemical, molecular or isotopic markers and large scale manipulations such as trenching and girdling experiments (Nylund and Wallander 1992; Ekblad and Näsholm 1996; Ekblad et al. 1998; Wallander et al. 2001; Dickie et al. 2002; Johnson et al. 2002; Leake et al. 2006; Högberg et al. 2010;

Heinemeyer et al. 2007, 2011 and see Wallander et al. 2013 for a discussion of advantages and disadvantages of these methods).

## Assessing mycelial growth: which structures to look at and where?

### Morphological heterogeneity: fine hyphae and rhizomorphs

Understanding the importance of the EMM of EM fungi in C cycling requires accurate predictions of mycelial growth. Detailed studies of soil microcosms in laboratory conditions show wide variation in growth rates and morphology between mycorrhizal mycelial systems of EM fungi (e.g. Duddridge et al. 1980; Finlay and Read 1986; Bending and Read 1995; Donnelly et al. 2004; Rosling et al. 2004). In many EM fungi, hyphae progressively aggregate behind the growing front to form rhizomorphs that are typically hydrophobic and long-lived (e.g. Unestam 1991; Unestam and Sun 1995; Agerer 2001). All mycelium types explore the soil via fine hydrophilic hyphae, often with substrate particles adhering to the surface, so-called 'substrate adhesion hyphae' or 'exploiting hyphae'. Few quantitative data on the relative proportion of rhizomorphs versus single hyphae of a mycelium are available. In a laboratory study of *Pisolithus tinctorius* in symbiosis with *Pinus taeda* seedlings, the rhizomorphs contributed to only 7 % of the length of the mycelium but their dry matter was twice that of the diffuse mycelium (Rousseau et al. 1994). The rhizomorph proportion of the EMM probably has a large impact on its standing biomass and turnover rate (see section on EMM standing biomass and turnover below). Rhizomorphs may be a more energetically efficient means of supporting an increasingly extended mycelium over large areas (Donnelly et al. 2004).

### Exploration types

Based on the amounts of emanating hyphae and the presence and differentiation of rhizomorphs, Agerer (2001) defined five main exploration types, ranging from contact exploration types with smooth mycorrhizal tips having only a few short emanating hyphae, via short and medium exploration types to long distance exploration types with highly differentiated rhizomorphs. Exploration types have been differentiated based on about 400 different morphotypes of ectomycorrhizas (www.deemy.de; Agerer and Rambold 2004–2011), representing about 5 % of known fungi that can form EM (Taylor and Alexander 2005). From this limited database, it appears that in many genera all known species produce only one exploration type, e.g. species in most of the investigated genera of the Boletales belong to the long-distance exploration type that has hydrophobic rhizomorphs, while in other genera, e.g. *Russula* and *Lactarius*, the exploration type varies between different species and can range from contact, to medium distance or even long distance exploration types (Agerer 2001; Kraigher et al. 2008; Hobbie and Agerer 2010). An EM community's species composition is made up of a range of exploration types, suggesting a degree of separation of function between them.

### Where do EMM develop (organic vs mineral soil)?

The spatial heterogeneity in EMM production and standing biomass is high and laboratory soil microcosm experiments have shown that local 'hot-spots' of various inorganic and organic materials stimulate the growth of EM mycelium (e.g. Finlay and Read 1986; Unestam 1991; Bending and Read 1995; Perez-Moreno and Read 2000; Jentschke et al. 2001; Rosling et al. 2004). Field demonstration of such effects comes from the observation of the stimulation of mycelial in-growth into bags spiked with inorganic P sources (Hagerberg et al. 2003; Nilsson and Wallander 2003; Potila et al. 2009) or wood ash (Hagerberg and Wallander 2002) placed in conifer forest soils, and from the formation of hyphal mats in some forests (Cromack et al. 1979; Unestam 1991; Ingham et al. 1991). The higher accumulation of hyphal biomass in these patches is supported by studies of $^{14}C$ allocation (Finlay and Read 1986; Bending and Read 1995; Leake et al. 2001; Rosling et al. 2004).

Although EM fungi can proliferate into leaf litter in laboratory microcosms (Unestam 1991), the few studies from the field suggest that they do not grow on or utilize young litter material in the forest floor (Treseder et al. 2006; Lindahl et al. 2007). In one of the few studies carried out in forests, new litter was dominated by saprotrophs while EM fungi dominated in old litter, the underlying mor layer and in mineral soil (Lindahl et al. 2007), suggesting that saprotrophs are more competitive in the litter layer. There might be

a niche differentiation not only between EM fungi and saprotrophs but also between exploration types, species and genotypes of mycorrhizal fungi. In support of this, the EM community structure was shown to differ between soil layers estimated both as mycorrhizal root tips (Dickie et al. 2002; Landeweert et al. 2003; Rosling et al. 2003; Tedersoo et al. 2003; Genney et al. 2006; Lindahl et al. 2007) and the EMM (Landeweert et al. 2003). Based on analyses of mycorrhizal root tips, half of the fungal taxa were restricted to the mineral soil in a podzol of a 60–80 year old *Picea abies* forest (Rosling et al. 2003).

## Estimation of mycelial growth rates and production in forest ecosystem

### Measurement of hyphal length and growth rates using microcosms (in the lab) or minirhizotrons (in the field)

Growth rates of EM hyphae in laboratory microcosm are typically 2–4 mm $day^{-1}$ (Read 1992), with maximal rates of up to 8 mm $day^{-1}$ (Donnelly et al. 2004). Similar growth rates were recorded in an outdoor experiment using 2 m tall mesocosms filled with peat. In this work, a maximum growth rate of 2 mm $day^{-1}$ for *Laccaria proxima*, which does not form rhizomorphs, and of 3 mm $day^{-1}$ for *Thelephora terrestris*, which forms rhizomorphs, was recorded in July (Coutts and Nicholl 1990). An indirect way to estimate the mycelial growth rate in the field may be to measure the size of genets formed by mycorrhizal fungi on trees planted on areas that have not been covered by plants previously, e.g. large sand pits. A genet size of up to 5 m was found for *Suillus bovinus* (long distance exploration type) in a sand pit with 20-years-old *Pinus sylvestris* (Dahlberg and Stenlid 1994). This would imply a genet growth rate of 25 cm $yr^{-1}$ over the 20 years, equivalent to an increase of the genet radius of 0.7 mm $day^{-1}$ over the growing season, assuming that the mycelium growth period is similar to that of the vegetation, which is about 180 days at this site. This rate, which is somewhat lower than the rates recorded in microcosms, is without doubt lower in some periods of the season and significantly higher in others (see further on seasonal variations below).

Some rhizomorph-forming fungi produce dense mycelial mats, in which the rhizomorphs can represent 30–50 % of soil dry matter (Ingham et al. 1991). The hyphal length varies greatly from 2 – 600 km $g^{-1}$ soil in the mats to only 0.3–0.8 km $g^{-1}$ in nearby non-mat soil (Ingham et al. 1991), although some mycelial necromass might also have been included in this standing biomass measurement. The mycelial length varies not only spatially but also seasonally; the total mycelial length varied seasonally from 100 to 800 m $g^{-1}$ soil in the organic mor layer and from 50 and 150 m $g^{-1}$ in the upper 10 cm of the mineral soil of a boreal *Pinus sylvestris* forest (Söderström 1979).

Minirhizotrons have been used in a few studies of rhizomorph growth (Treseder et al. 2005; Vargas and Allen 2008; Pritchard et al. 2008). However, growth in such studies is recorded as rhizomorph length per photographed frame area, making comparisons with the measurements of expansion of the mycelial front difficult. Nevertheless, yearly growth rates of 0.1–0.6 mm per frame were recorded in a *Pinus taeda* forest, suggesting growth rates of <1 cm $m^{-2}$ of frame surface $day^{-1}$, while in a mixed conifer/oak forest, maximum rates of 100 cm $m^{-2}$ of frame surface $day^{-1}$ were observed (Vargas and Allen 2008), suggesting that the importance of rhizomorph forming fungi can differ very much between different forest sites.

### The use of in-growth bags, a method that targets ECM (compared to saprotrophs) and enables us to estimate the production of EMM

One difficulty when making measurements of EMM production in the field is to separate the mycorrhizal mycelium from that of saprotrophs. This step has been facilitated by the use of mycelial in-growth bags (Wallander et al. 2001) or in-growth cores (Godbold et al. 2006; Hendricks et al. 2006). The bags, usually filled with sand, are made of nylon with a typical mesh size of 50 μm allowing the ingrowth of hyphae but not of roots. Saprotrophs can grow into these bags but the fungal biomass within them seems to be dominated by mycorrhizal fungi as judged from trenched controls as well as DNA analyses (Wallander et al. 2001; Kjöller 2006). Using this technique EMM production rates have been estimated at ~140 different forest sites (Table 1). The majority of these sites (107) are located in Sweden, and *Picea abies* is the dominating tree species. Data have also been reported from Denmark (15 sites), Finland (13 sites), North America (2 sites) and France (1 site). These studies indicate an average

production rate in the upper 10 cm of a forest soil of 160 kg dry matter $ha^{-1}$ $year^{-1}$ (Table 1). However, this rate varies tremendously between sites, e.g. from 20 kg $ha^{-1}$ over 12 months in some *Quercus robur* sites in southern Sweden (Nilsson et al. 2007) to 980 kg dry matter $ha^{-1}$ over 4 months in a *Pinus taeda* plantation at low elevation in North Carolina (Parrent and Vilgalys 2007). It can also vary greatly from year to year at the same site, e.g. in a *P. abies* plantation on a peat soil south west of Sweden, it was close to zero 1 year, but found to be 100 kg dry matter $ha^{-1}$ the year after (R. G. Björk and A. Ekblad, unpublished). This large variation may derive from the factors regulating EMM production as well as from differences in the various methods used to assess mycelial biomass (ergosterol, phospholipid fatty acids, dry matter etc.; see Wallander et al. (2013)). Although EMM production data exist from a number of sites, there is a strong bias towards Norway spruce (*P. abies*) and southern Scandinavia and data from other areas and other forest types are needed.

Most published data reflect the production of EMM in the upper 10 cm of the soil (which includes the organic layer). However, EMM production can also be high in deeper soil layers as shown in the few studies which report values from more than one soil depth (Table 1). Thus, of the 590 kg $ha^{-1}$ $year^{-1}$ of EMM biomass produced down to 30 cm depth in a *Picea abies* forest, half was found in the upper 10 cm and half in the 10–30 cm depth (Wallander et al. 2004), a distribution pattern similar to that of fine roots in this forest (Thelin et al. 2002). Other studies have also shown that the distribution of EMM generally follows that of tree fine roots (Korkama et al. 2007; Pritchard et al. 2008).

The production rates estimated by in-growth bags can be compared to the very few estimates of C allocation to EMM in forests. Recently, Hobbie (2006) surveyed the C allocation patterns of EM plants in 14 culture (laboratory) studies and five field studies. Using the data in Hobbie (2006), we estimate that on average 4.7 % of total NPP (9 % of below ground NPP) in the culture studies and 7.2 % of total NPP (13 % of below ground NPP) in the field studies was allocated to the EMM. If we combine these values together with NPP estimates ranging from 333 to 590 g C $m^{-2}$ $year^{-1}$ in three 40-year-old Swedish *P. abies* forests (Berggren Kleja et al. 2008), we estimate a NPP of the EMM of 16 – 42 g C $m^{-2}$ $year^{-1}$or 350 – 940 kg dry matter $ha^{-1}$ $year^{-1}$ (assuming a C content of 45 % of dry matter). These numbers, which estimate the mycelium production in the whole soil profile, are comparable with the estimates of EMM production in *P. abies* forest soils using ingrowth bags. From the data available in Table 1 we estimate an EMM production in the upper 10 cm of soil in a 40-year-old Swedish *P. abies* forests to be around 200 kg dry matter $ha^{-1}$ $year^{-1}$ and for the whole soil profile this value should probably be at least doubled.

## Factors regulating the carbon supply for EMM production in forest soils

The EMM is fuelled by C from the host and any factors regulating C availability from the host-plant such as global change, weather conditions, forestry management and plant properties as well as intrinsic properties of fungal C use can potentially cause large variations in EMM production of EM fungi (Fig. 1) that will further sustain differences between sites, seasons and years.

### Seasonal effects and forest aging

Seasonal variations in EMM production may be driven by abiotic variables notably light, temperature and moisture but also by phenological phenomenon, both in the hosts and symbionts (for moisture effects see further down).

The growth of EM fungi is mainly dependent on newly produced photosynthates (Söderström and Read 1987; Högberg et al. 2001; Johnson et al. 2002; Högberg et al. 2010; Steinman et al. 2004). The major growth of EMM is therefore expected to occur when below-ground allocation of carbohydrates is relatively large, shortly after fine root production has peaked. In a cool temperate climate this is late summer to early autumn (July–October), while in a temperate planted spruce-beech forest in Bavaria the peak in beech fine root production was in June (Grebenc and Kraigher 2007). Indeed, in a northern boreal *Pinus sylvestris* forest, below-ground C allocation in late August can be 5 times that in mid June (Högberg et al. 2010). While in a temperate forest in France, the below-ground $^{13}C$ allocation after pulse labelling of beech trees was much higher in July than in May and late August (Epron et al. 2011). The few published studies on temporal variations in the production of EMM of EM fungi fit with this view (Lussenhop and Fogel 1999; Wallander et al. 2001; Nilsson et al. 2007). In

**Table 1** The production of extramatrical mycelia (EMM) of ectomycorrhizal fungi in various forests. Estimations were made based on sand filled mesh bags or cores that were incubated in the soil. Soil was used as a substrate in a few cases (Hendricks et al. 2006 and Sims et al. 2007). Mesh bags were placed in the soil 1) vertically (covering a range of soil depths), 2) horizontally (at a specific depth) or 3) in the interface between organic and mineral layer. Fungal biomass produced in the mesh bags have been estimated by 1) loss of ignition (LOI), 2) elemental carbon analysis of extracted mycelium (EA), 3) dry matter of harvested mycelium (Dry matter), 4) ergosterol content (Ergo) or 5) phospholipid fatty acid 18:2ω6,9 (PLFA). Incubation time varies between sites, but usually a complete growth season is covered in the measurements. For comparisons between sites and tree species, the amount of EMM produced per hectare in the top 10 cm of the soils has been calculated. The average EMM production per site using all 137 sites in the table was 170 kg EMM per hectare. If different methods were used to estimate biomass in one site, the average value was used. The following conversion factors were used: 3 μg ergosterol $mg^{-1}$ fungal biomass; 2 nmol PLFA 18:2ω6,9 per $mg^{-1}$ fungal biomass (Wallander et al. 2001). To convert the biomass values found per gram sand to kg $ha^{-1}$ we used the density of sand (1.56 g $cm^3$) to calculate the EMM biomass per $cm^3$

| Forest type | Sites (located in Sweden, otherwise country indicated) | Age (years) | Soil type | Soil depth (cm) | Incub. time (months) | Method used for analysis of EMM biomass (concentr. $g^{-1}$ sand) | EMM production in the upper 10 cm (kg $ha^{-1}$ per growing season) | Reference |
|---|---|---|---|---|---|---|---|---|
| Boreal forests | | | | | | | | |
| *Picea abies* | Betsele | ~130 | Haplic podsol | Interface | 4 | PLFA (0.1 nmol) | 80 | Nilsson et al. 2005 |
| *P. abies* | Flakastugan | ~120 | podsol | Interface | 4 | PLFA (0.2 nmol) | 160 | Nilsson et al. 2005 |
| *P. abies* | Kryddgrovan | ~120 | podsol | Interface | 4 | PLFA (0.1 nmol) | 80 | Nilsson et al. 2005 |
| *P. abies* | Varjisån | ~125 | podsol | Interface | 4 | PLFA (0.25 nmol) | 200 | Nilsson et al. 2005 |
| *P. abies* | Flakaliden | 35 | Podsol | Interface | 12 | PLFA<br>Ergo | 170<br>150 | Leppälammi et al. unpublished |
| *Pinus sylvestris* | Varjisån | ~125 | podsol | Interface | 4 | PLFA (0.35 nmol) | 280 | Nilsson et al. 2005 |
| *P. sylvestris* | Betsele | ~130 | Haplic podsol | Interface | 4 | PLFA (0.12 nmol) | 100 | Nilsson et al. 2005 |
| Average | | ~125 | | | | | 151±28 | |
| Boreonemoral forests | | | | | | | | |
| *P. abies* | Grängshammar | 19 | Podsol | Interface | 12 (mean 3 y) | Ergo (0.3 μg) | 380 | Wallander et al. 2011 |
| *P. abies* | Hällefors | 16 | Podsol | Interface | 12 (mean 3 y) | Ergo (0.35 μg) | 440 | Wallander et al. 2011 |
| *P. abies* | (62°10′N, 27°16′E) Finland | 10 | podsol | 0-10 cm | 4 | LOI (0.025–0.15 mg)<br>PLFA (0.1-0.34 nmol) | 40–230<br>80-270 | Korkama et al. 2007 |
| *P. abies* | Släne | 55 | Podsol | Interface | 8 | PLFA (0.12 nmol) | 114 | Wallander and Thelin 2008 |
| *P. abies* | Torpa | 65 | Podsol | Interface | 8 | PLFA (0.20 nmol) | 190 | Wallander and Thelin 2008 |
| *P. abies* | Vrå 72 | 60 | Podsol | Interface | 8 | PLFA (0.13 nmol) | 124 | Wallander and Thelin 2008 |
| *P. abies* | Vrå 180 | 60 | Podsol | Interface | 8 | PLFA (0.12 nmol) | 114 | Wallander and Thelin 2008 |

**Table 1** (continued)

| Forest type | Sites (located in Sweden, otherwise country indicated) | Age (years) | Soil type | Soil depth (cm) | Incub. time (months) | Method used for analysis of EMM biomass (concentr. $g^{-1}$ sand) | EMM production in the upper 10 cm (kg $ha^{-1}$ per growing season) | Reference |
|---|---|---|---|---|---|---|---|---|
| *P. abies* | Ebbegärde | 16 | Podsol | Interface | 12 (mean 3 y) | Ergo (0.4 μg) | 500 | Wallander et al. 2011 |
| *P. abies* | Toftaholm | 16 | Podsol | Interface | 12 (mean 3 y) | Ergo (0.25 μg) | 310 | Wallander et al. 2011 unpublished |
| *P. abies* | Tönnersjöheden (56°41′N, 4°57′E) | 37 | Podsol | Interface | 13 | PLFA (0.4 nmol) | 320 | Hagerberg and Wallander 2002 |
| *P. abies* | Tönnersjöheden (5 sites) | 5–10 | Podsol | Interface | 12 | Ergo (0.10 μg) | 130 | Wallander et al. 2010 |
| *P. abies* | Tönnersjöheden (5 sites) | 10–20 | Podsol | Interface | 12 | Ergo (0.21 μg) | 270 | Wallander et al. 2010 |
| *P. abies* | Tönnersjöheden (5 sites) | 20–30 | Podsol | Interface | 12 | Ergo (0.1 μg) | 130 | Wallander et al. 2010 |
| *P. abies* | Tönnersjöheden (5 sites) | 30–40 | Podsol | Interface | 12 | Ergo (0.17 μg) | 220 | Wallander et al. 2010 |
| *P. abies* | Tönnersjöheden (5 sites) | 40–50 | Podsol | Interface | 12 | Ergo (0.05 μg) | 65 | Wallander et al. 2010 |
| *P. abies* | Tönnersjöheden (5 sites) | 50–90 | Podsol | Interface | 12 | Ergo (0.11 μg) | 140 | Wallander et al. 2010 |
| *P. abies* | Tönnersjöheden (5 sites) | 90–130 | Podsol | Interface | 12 | Ergo (0.07 μg) | 90 | Wallander et al. 2010 |
| *P. abies* | Brevens bruk | 68 | Sandy | 0-10<br>10-20 | 12 | EA (138 μg)<br>EA (31 μg) | 215 | Boström et al. 2007 |
| *P. sylvestris* | Liesineva Finland (12 sites) | 80 | Peat | Interface | 4<br>16<br>4<br>16 | PLFA (0.2 nmol)<br>PLFA (0.2 nmol)<br>Ergo (0.15 μg)<br>Ergo (0.35 μg) | 160<br>160<br>190<br>440 | Potila et al. 2009 |
| Average | | ~50 | | | | | 188±12 | |
| Nemoral forests | | | | | | | | |
| *P. abies* | Skogaby (56°33N, 13°13′E) | 45 | Haplic podsol | 5, 10, 20 cm | 12 | PLFA (5 cm 0.12 nmol)<br>PLFA (10 cm 0.15 nmol)<br>PLFA (20 cm 0.15 nmol) | 100 | Majdi et al. 2008 |
| *P. abies* | *Björstorp* | 60 | Podsol | Interface | 13 | | 182 | Hagerberg et al. 2003 |

**Table 1** (continued)

| Forest type | Sites (located in Sweden, otherwise country indicated) | Age (years) | Soil type | Soil depth (cm) | Incub. time (months) | Method used for analysis of EMM biomass (concentr. $g^{-1}$ sand) | EMM production in the upper 10 cm (kg $ha^{-1}$ per growing season) | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | LOI (0.18 mg), Ergo (0.14 μg) | | |
| *P. abies* | *Dyneboda* | 65 | Podsol | Interface | 13 | LOI (0.17 mg), Ergo (0.14 μg) | 182 | Hagerberg et al. 2003 |
| *P. abies* | *Ignaberga* | 50 | Podsol | Interface | 13 | LOI (0.19 mg), Ergo (0.18 μg) | 156 | Hagerberg et al. 2003 |
| *P. abies* | *Västra Torup* | 55 | Podsol | Interface | 13 | LOI (0.04 mg), Ergo (0.3 μg) | 390 | Hagerberg et al. 2003 |
| *P. abies* | Jämjö (56°53′N, 15°16,5′E) (4 sites) | 60 | Dystric cambisol | 5, 10, 20 cm | 12 | LOI (5 cm 0.19 mg) LOI (10 cm 0.12 mg) LOI (20 cm 0.07 mg) | 300 | Wallander et al. 2004 |
| *P. abies* | Thyregod, W Denmark | 25 | Inceptisol (FAO) | 0–8 cm | 8 | Dry matter | 54 | Kjøller et al. Unpublished |
| *P. abies* | Klosterhede, NW Denmark | 91 | Haplic podsol | 0–8 cm | 12 | Dry matter | 47 | Kjøller et al. Unpublished |
| *P. abies* | 19 sites in Scania 14 sites in Denmark along a C/N ratio gradient | 18–85 | Acidic pH (KCl): 2.7–4.9 | 5 cm | 6 | PLFA (0.12–0.72 nmol) | 40–240 | Nilsson et al. 2012 |
| *P. abies/ Quercus robur* | Jämjö (56°53′N, 15°16,5′E) (4 sites) | 40–80 | Dystric cambisol | 0–30 cm | 12 | LOI (5 cm 0.19 mg) LOI (10 cm 0.05 mg) LOI (20 cm 0.04 mg) | 300 | Wallander et al. 2004 |
| *P. sylvestris* | Silvåkra | ~30 | Sandy | Interface | 12 | PLFA (0.4 nmol) Ergo (0.23 μg) | 320 390 | Wallander et al. 2001 |
| *Q. robur* | Halland (5 sites) | > 80 | | 5 cm | 12 | PLFA (0.03 nmol) | 20 | Nilsson et al. 2007 |
| *Q. robur* | Småland (6 sites) | > 80 | | 5 cm | 12 | PLFA (0.15 nmol) | 120 | Nilsson et al. 2007 |
| *Q. robur* | Skåne (4 sites) | > 80 | | 5 cm | 12 | PLFA (0.14 nmol) | 110 | Nilsson et al. 2007 |
| *Q. robur* | Öland (4 sites) | > 80 | | 5 cm | 12 | PLFA (0.06 nmol) | 50 | Nilsson et al. 2007 |
| *P. pinaster* | The Landes Forest France (44°42′N, 0°46′W) | 13 | Podsol | 0–10 | 12 | LOI | 60 | Bakker et al. 2009 |
| Average | | ~60 | | | | | 138±9 | |
| Warm temperate | | | | | | | | |
| *Pinus. palustris* | Georgia USA | 21 | | 0–30 cm | 2 | Ergo (0.05 μg -sand) | 65 (2 month) | Hendricks et al. 2006 |

**Table 1** (continued)

| Forest type | Sites (located in Sweden, otherwise country indicated) | Age (years) | Soil type | Soil depth (cm) | Incub. time (months) | Method used for analysis of EMM biomass (concentr. $g^{-1}$ sand) | EMM production in the upper 10 cm (kg $ha^{-1}$ per growing season) | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | Loamy Arenicpaleudult | | | Ergo (0.2 μg - soil) | 260 (2 month) | |
| *P. palustris* | Georgia USA | 22 | Loamy Arenicpaleudult | 0–30 cm | 12 | Ergo (soil) | 280 | Sims et al. 2007 |
| *Pinus taeda* | Duke forest NC USA | 20 | Clay loam | Interface | 4 | PLFA (1.25 nmol) | 1,000 | Parrent and Vilgalys 2007 |
| Average | | ~20 | | | | | 611 | |
| Total average of all sites | | | | | | | 160±7 | |

a detailed phenological study in a *Pinus strobus* forest in northern, Lower Michigan the EMM growth of *Cenococcum geophilum* peaked in mid July, three weeks after the peak in fine root growth (Lussenhop and Fogel 1999). In contrast, in a warm temperate *Pinus palustris* plantation, EMM production was high all year around (Sims et al. 2007). Even in a cooler temperate forest, the EMM can grow at a low rate during winter months if air temperatures remain above zero (Coutts and Nicholl 1990). *Thelephora terrestris*, producing rhizomorph, grew at a rate of 0.4 mm $day^{-1}$ in winter, while *Laccaria proxima*, that produced only diffuse mycelium, grew from June to October and the mycelium disappeared after this (Coutts and Nicholl 1990), suggesting that differences in phenology among the symbionts can be of importance.

In contrast to the view that maximum EMM production in temperate and boreal forests occurs from late summer to autumn, a detailed study of total mycelium production over 27 months in a *P. sylvestris* forest in mid Sweden, showed two peaks of similar amplitude, one in April-May and one in August-October (Söderström 1979). That study did not distinguish between mycorrhizal and saprotrophic mycelium. Other studies suggest the main EMM growth period to occur in the second half of the growing season (Wallander et al. 2001; Boström et al. 2007; Nilsson et al. 2007) so the spring peak observed by Söderström (1979) may have been dominated by saprotrophs. In a more recent study, spatial separation of EM fungi and saprotrophs, with the saprotrophs dominating in the litter and mycorrhizal fungi dominating in the organic layer and mineral soil, has been suggested (Lindahl et al. 2007). The soil sampling in the latter study was performed in September at the same *P. sylvestris* site studied by Söderström (1979). The question is if this mycorrhizal versus saprotroph dominance is constant or if the two fungal groups have different seasonal dynamics? To answer this question we need further studies on seasonal variations in mycelium production by both saprotrophs and mycorrhizal fungi among EM exploration-types and throughout soil profiles. One problem in such investigations is that the ecological role of a large number of fungal taxa that can be identified by molecular methods in a soil sample is unknown (Lindahl et al. 2007). Increased knowledge in this aspect will therefore increase our ability to draw sound conclusions about temporal or spatial changes in EM/saprotroph ratios or exploration types.

In addition to the yearly effect of season, a multitude of changes take place in an ecosystem over a forest cycle. The most dramatic changes in plant cover, species composition, soil chemistry, hydrology, climate etc. occur directly after tree harvest and then up to canopy closure after which the changes are slower. There are therefore many factors that may directly or indirectly affect EMM production and its standing biomass. Many of these are probably connected to successional changes in species composition above and below ground as well as changes in below ground C allocation, but EMM production has not been studied greatly in this context (Last et al. 1987). Tree growth varies over a rotation period, usually with a peak around canopy closure when nutrient demand also reaches a maximum (Kimmins 2004). This occurs between 25 to 40 years of age in *P. abies* forests in central-southern Sweden (Schmalholz and Hylander 2009). The production of EMM seems to peak around the time when tree growth is highest (Wallander et al. 2010; Kalliokoski et al. 2010).

### Effect of elevated atmospheric $CO_2$

In agreement with the fact that EM fungi rely on C supplied by the host, several studies have shown a stimulation of EMM production under elevated atmospheric $CO_2$ concentrations (e.g. Godbold et al. 1997; Treseder 2004; Alberton et al. 2005; Fransson et al. 2005; Alberton and Kuyper 2009). However there are exceptions, for example Weigt et al. (2011) found no increase or only a slight increase in EMM length using seedlings of *Picea abies* inoculated with *Piloderma croceum* and exposed to double or ambient $CO_2$ concentration alone or in combination with addition of ammonium nitrate solution. The effect of elevated $CO_2$ on EMM production has mostly been studied in laboratory grown seedlings. The few results available from field studies fail to show a $CO_2$ effect on EMM production (Kasurinen et al. 2005; Godbold et al. 2006; Parrent and Vilgalys 2007). A response shown in many laboratory and some field experiments is that changes in C availability causes an increase in the degree of mycorrhization (Godbold et al. 1997; Garcia et al. 2008). But in forests types, such as Boreal forest where the tree root tips are close to 100 % colonized by EM fungi (Taylor and Alexander 2005), a response to $CO_2$ is unlikely to be of great significance. More generally the EM-fungal community has been shown to change both in experiments with elevated $CO_2$ (e.g.; Godbold et al. 1997; Fransson et al. 2001; Parrent et al. 2006; Parrent and Vilgalys 2007) and in defoliation experiments (Saikkonen et al. 1999; Cullings et al. 2001; Markkola et al. 2004; Saravesi et al. 2008). The change in EM-fungal community has often manifested itself in a shift between morphotypes differing in mantle thickness. A reduction in C availability, by e.g. defoliation, seems to favour smooth mycorrhizal types and disfavour types that produce thick mantles and rhizomorphs (Saikkonen et al. 1999; Cullings et al. 2001; Markkola et al. 2004; Saravesi et al. 2008). So far one laboratory study has reported an increased proportion of mycorrhizas producing thick mantles and abundant rhizomorphs in response to elevated $CO_2$ (Godbold et al. 1997), and only one of the few field studies showed that rhizomorph production was almost doubled by elevated $CO_2$ in deeper soil layers in a *Pinus taeda* forest (Pritchard et al. 2008). The production of EMM varies greatly between different exploration types (Weigt et al. 2011) and it seems reasonable to find increased abundance of high C demanding exploration types when C availability is increased by elevated $CO_2$. Clearly further field studies on the effects of elevated $CO_2$ on mycelium production are needed.

### Effect of soil fertility and potential use of a stoichiometric C:N:P model for understanding fungal C allocation in response to N and P fertilization

Among the factors that can affect the C availability for mycelium production, site fertility – and thus fertilization practices, may strongly regulate belowground C allocation (Fig. 1). Trees allocate proportionally more C to shoots and less to roots at sites with high productivity while at sites of low productivity proportionally more C is allocated belowground to enhance nutrient uptake by roots and EM fungi (Högberg et al. 2003). However, since high fertility also results in high photosynthesis, the total amount of C allocated below ground may sometimes be larger at a more productive site than at a less productive site. Indeed, a positive correlation between EMM biomass and site fertility was found in mixed boreal forests in Finland (Kalliokoski et al. 2010) and fast-growing *P. abies* clones produced more EMM than slow growing clones (Korkama et al. 2007). It was shown that the fast growing clones hosted EM fungi that belong to the types that produce extensive mycelia with rhizomorphs, e.g. *Piloderma*, while the slower growing clones had more fungi that produce less mycelium such as the Ascomycete *Wilcoxina* (Korkama et al. 2007).

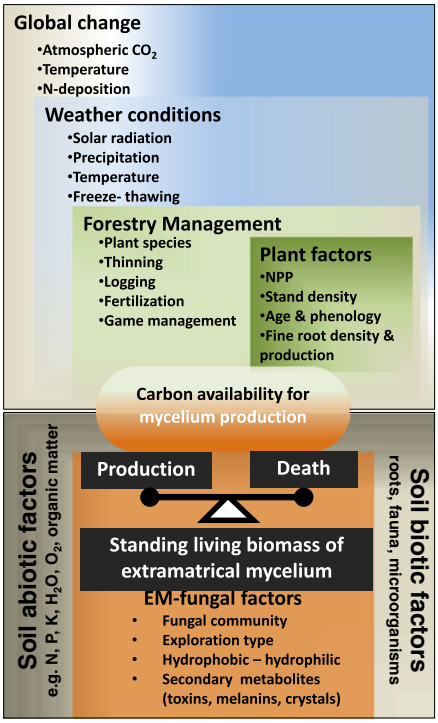


**Fig. 1** Overview of the factors that directly or indirectly may affect the production, standing biomass and death of the extramatrical mycelium of ectomycorrhizal fungi

However, when site fertility was increased by high N fertilization of forests, it resulted in reduced production of EMM by the EM fungi (Kårén and Nylund 1997; Nilsson and Wallander 2003; Sims et al. 2007; Högberg et al. 2011), while the effect on mycorrhizal colonization on roots is usually much smaller (Kårén and Nylund 1997; Treseder 2004). This reduction in EMM production may be caused both by a lower standing fine root biomass at high N (Nadelhoffer 2000) as well as that large amount of C is needed to take up and assimilate the excessive N in the fertilized plots (Bidartondo et al. 2001; Ek 1997). This C consumption may result in C limitation of EMM production (Wallander 1995). Under unbalanced nutrient conditions, much of the excess N is transported to the shoot and is stored in the vacuoles in the leaf in the form of amino acids (Näsholm et al. 1997). In laboratory microcosms, a cessation of EMM growth was noted when the mycelial front of certain species reached peat amended with inorganic N (Arnebrant 1994). Different species seems to be more or less sensitive to high inorganic N concentrations and high N fertilization typically causes changes in the species composition of EM fungi making the smooth mycorrhizal types more common (e.g. Kårén and Nylund 1997; Parrent and Vilgalys 2007). Accordingly, Gorissen and Kuyper (2000) applied the terms nitrophilic and nitrophobic species based on their tolerance of inorganic N. *Laccaria bicolor*, a nitrophilic species, retained more N in the fungal biomass while the N sensitive (nitrophobic) *Suillus bovinus* delivered more N to the host plant when studied in a pot experiment (Gorissen and Kuyper 2000). This would imply that nitrophobic species spend more C on N assimilation and amino acid transfer to their host plant while nitrophilic species can tolerate N by spending less C on N assimilation, which would allow them to spend more C on EMM growth under excess N. Difference in C demand and tolerance to specific elements by individual EM species in forest soils may be one explanation for the high diversity usually found in such communities.

In contrast to the negative effect of high doses of N on EMM production, intensive fertilization with a balanced nutrient mix, including all elements needed, resulted in no change in EMM production in two sites but a reduction in a third site (Wallander et al. 2011). This suggests that the balance between the availability of C and N and possibly other nutrients is of importance. Recently, Johnson (2010) recommended a stoichiometric C:N:P perspective to provide the basis for a more predictive understanding of fertilization responses of AM symbioses to N and P fertilization. It was predicted that the function of the AM symbiosis is dependent on the availability of N and P such that the mutualistic benefit is greatest at the combined condition of high N and low P, which would give high photosynthesis rates when the symbiont is efficient in P uptake. Furthermore, the study also predicted the response of plant and fungal morphology to a change in resource availability, e.g. N fertilization can induce P-limitation, which would result in more C allocation to production of roots and AM fungi. Johnson (2010) brings up several field and laboratory experiments supporting these models for AM symbioses. In EM symbioses, localized additions of inorganic N can stimulate the proliferation of mycelium production, at least of some fungi (Jentschke et al. 2001; Clemmensen et al. 2006). However, as pointed out above, large scale N fertilization in temperate and boreal forests is known to result in reduced production of EMM (Kårén and Nylund 1997; Nilsson and Wallander 2003). The reason for this discrepancy between AM and EM systems is unknown but it may be that P availability is not low enough in many temperate and boreal forests to allow N–induced P limitation to develop over the experimental

period. It has been shown that N fertilization can give rise to P limitation of forest production in boreal *P. abies* forests in long-term factorial fertilizer experiments (Tamm 1991), but it remains to be shown what happens to the EMM production in such experiments. Indeed, laboratory experiments on *Pinus sylvestris* seedlings with EM showed very high EMM production at the combination of high N, low P conditions (Wallander and Nylund 1992; Ekblad et al. 1995). It should be noted that in the paper by Wallander and Nylund (1992), there were similar EMM production responses to the N and P conditions in both the nitrophilic *Laccaria bicolor* as well as in the nitrophobic *Suillus bovinus*. This suggest that a C:N:P perspective may be valid for a nitrophobic as well as a nitrophilic species when studied separately. However, in the soil with many different species competing for a living space on the same tree root system, species differences in the C and N use (see above) could potentially have large impact on the competition between species. Phosphorus fertilization of naturally P–limited soils would be an alternative way of testing the validity of the C:N:P model for EM symbioses. Peat soils are naturally low in P and K and recent results from a long lasting PK-fertilizer experiment on a drained peatland show that the production of EMM, as well as the colonization of roots, was stimulated by low P availability, and the EMM production was also stimulated by low K conditions (Potila et al. 2009). These results also support the applicability of a stoichiometric C:N:P model for EM symbioses. The availability of different forms of N and P, and the ability of different species and genotypes of EM fungi to use them may also be important factors in regulating tree growth and C allocation feedbacks. We identify the need for studies of EMM production in long-term factorial N, P fertilizer experiments in forest ecosystems to further test the C:N:P model for EM symbioses.

## Abiotic and biotic factors regulating mycelial growth

### Soil moisture

Extramatrical mycelium production can be sensitive to soil moisture, for example it can be reduced by 50 % in a dry year compared to a wet year in a well-drained *P. abies* forest (Majdi et al. 2008). However, it appears that mycelial production, at least of some fungal species, is not as sensitive to drought as sporocarp production, which responds strongly to soil moisture conditions (Wiklund et al. 1995). Indeed, despite a very dry year with very few fruiting bodies produced, high mycelial in-growth in the upper 6 cm of the soil was found in a *P. taeda* forest (A. Ekblad et al. unpublished). Production of EMM can be extensive in the deeper mineral soil (Wallander et al. 2004; Boström et al. 2007; Majdi et al. 2008) and so potentially a reduced production of mycelium in the surface could be compensated for by an increase in production or a slower turnover rate further down in the soil (Pritchard et al. 2008). The survival and growth of mycelia during drought conditions may be enabled by the passive movement of water from deeper moist soils to dryer surface soils via roots by so called nocturnal hydraulic lift (Caldwell et al. 1998; Querejeta et al. 2003, 2007). Indeed, $^{18}O$ tracer experiments indicate that sporocarps of fungal species formed during very dry conditions derived 30–80 % of their water from hydraulically-lifted or deep water (Lilleskov et al. 2009). Recently, an experiment using deuterium labelled water presented strong evidence for hydraulic redistribution of soil water by a common mycorrhizal network from mature trees to seedlings under field conditions (Warren et al. 2008).

Periodically dry habitats seem to be dominated by rhizomorph-forming fungi, many of them hydrophobic (Unestam 1991). Wet conditions may instead be detrimental to rhizomorph-formers since laboratory studies show that mycorrhizal colonization of hydrophobic but not hydrophilic fungi may be hampered by wet conditions (Stenström 1991). In fact, recent mini-rhizotron data show that rhizomorph length was negatively correlated with soil water content in a mixed conifer and oak forest and daily recordings show that the rhizomorphs grew rapidly at very low soil water content, so it was hypothesised that plants invest in C for rhizomorphs in exchange for water during harsh conditions (Vargas and Allen 2008).

### Grazing effects

Grazing of above ground plant parts normally consumes a minor part of net primary production in forests and usually has minor effects on the standing plant biomass in such ecosystems (Kimmins 2004). However, grazing is selective and can have significant impact on plant species composition in a community (Pastor and Naiman 1992; Persson et al. 2000) and may therefore indirectly affect species composition of mycorrhizal fungi (Gehring and Whitham 2002), and consequently also

have effects on EMM production. Severe grazing of leaves can result in drastically reduced photosynthesis, reduced C allocation below ground and reduced mycelium production, similar to that of experimental defoliation (see above).

The presence of fungivores as well as of other soil organisms could potentially affect growth, standing biomass and turnover of the EMM in the soil. Laboratory microcosm experiments suggest that the growth of EMM may be reduced, unaffected or stimulated by the presence of grazing invertebrates such as collembola. The direction of this change may be determined by the species composition and population density of the fungivores (Fitter and Sanders 1992; Ek et al. 1994; Setälä 1995; Setälä et al. 1999). However, it is not clear to what extent changes in EMM biomass are a direct effect of animal grazing or the result of other processes acting indirectly on the EMM (Setälä et al. 1999) involving e.g. selective grazing of competing saprotroph fungi, recycling of minerals locked up in senescing tissues or removal of growth inhibitors (Fitter and Sanders 1992). Indeed, soil arthropods significantly affect the rate of N mineralization in forest soils (Persson 1989).

As with grazing above ground, below ground grazing is probably selective. This selection may be directed by the fungal odour (Bengtsson et al. 1988; Bengtsson et al. 1991) together with contents of defence substances (e.g. crystals on the surface and content of repellents) rather than its C and N content (Taylor and Alexander 2005; Böllmann et al. 2010). The vitality of the mycelium may also be important because severed mycelium, and mycelium of *Pisolithus tinctorius* grown on agar was grazed more by the collembolan *Folsomia candida* than mycelium connected to a host plant (Kaneda and Kaneko 2004). Many fungi produce bioactive secondary metabolites that have been shown to be nematicidal (Stadler and Sterner 1998), e.g. many *Lactarius* and *Russula* species produce the biologically inactive precursor stearoylvelutinal that after a wound is rapidly converted to strongly antibiotic and pungent sesquiterpenoids (Stadler and Sterner 1998; Spiteller 2008). The EM fungus *Laccaria bicolor* was even shown to paralyse, probably by a toxin, and then invade and kill the springtail *F. candida* (Klironomos and Hart 2001). The N in the springtail was found to be beneficial for growth of the host plant, which is a demonstration of a dramatic shortcut of the N-cycle. It is unknown if other EM fungi have this striking capacity.

In accordance with optimal foraging theory, animals will feed on the food source yielding the greatest reproductive success (MacArthur and Pianka 1966). Laboratory experiments have shown that soil fauna can graze on EM fungi grown in vitro (e.g. Shaw 1988). In grassland, in situ $^{13}C$ labelling has unequivocally demonstrated that collembola can significantly affect release of recent assimilate by external arbuscular mycorrhizal mycelium (Johnson et al. 2005). In a field $^{13}C$ pulse-chase experiment in a young *Pinus sylvestris* forest some Collembola were $^{13}C$-labelled within days, which was interpreted as evidence for grazing of active hyphal tips of EMM by these animals (Högberg et al. 2010). However, in this experiment, it cannot be excluded that the $^{13}C$ label was derived from grazing of algae or lichens on the soil surface, or from grazing of microbes in the rhizosphere, since it is known that many Collembola can feed on several different substrates (Hopkins 1997). In fact, some other recent studies suggest that the EMM of EM fungi is the optimal food for relatively few soil animals in situ. Indeed, tree girdling experiments in Sweden of two *Picea abies* forests and one *P. sylvestris* forest reduced the population of Protura and only one species of orbatid mite, *Oppiella nova*, but the latter was only reduced in the *P. abies* forests not in the *P. sylvestris* forest (Remén et al. 2008; Malmström and Persson 2011). The collembolans were either not affected or stimulated by the girdling (Malmström and Persson 2011). Furthermore, in a windfall area of a *P. abies* forest, very high densities of Protura were found in the vicinity of small *P. abies* plants, while in areas without surviving *P. abies*, the proturan density was low, supporting the view that EM fungi is an important food source for this animal group (Krauss and Funke 1999). In a microcosm experiment, it was found that *O. nova* could grow and increase its population on some EM fungi in symbiosis but not on others, while none of the other common soil animals tested succeeded to reproduce when feeding on EM fungi (Remén et al. 2010). Furthermore, in laboratory microcosm the presence of four different EM fungi grown in symbiosis with *P. sylvestris* had no effect on soil animal populations (Setälä et al. 1999; Setälä 2000). It seems that the importance of EMM as an easily available food source for the detritus soil food web could be smaller than previously believed (Setälä 2000), although more targeted experimental work needs to be undertaken under field conditions. It is

possible that EMM should be considered a large C store in the soil rather than a C source (see further below and Setälä et al. 1999), and that grazing of saprotrophic microorganisms is relatively more important than grazing of EM fungi. If so, this may have major implications for plant-microbe interactions and the cycling of limiting mineral nutrients, such as N and P. For example, the positive effect of bacterial and fungal feeding nematodes on the biomass production of non-mycorrhizal *P. sylvestris* was of equivalent magnitude to the positive effects of formation of mycorrhizas, suggesting that the grazing by the nematodes released N that otherwise was locked into saprotroph biomass (Setälä et al. 1999).

## Estimation of standing biomass and turnover of EMM

The data discussed above suggest that there is substantial amount of C invested in the production of EMM. However, in order to fully assess its importance in the forest C cycle, data on its standing biomass and turnover are required. In this section we will present the few data available, and briefly discuss the factors that may affect EMM turnover. A large standing biomass can be the result of a high production or a slow turnover or a combination of both.

### The standing biomass and turnover of EMM

Laboratory studies show that mycelial fans of EM fungi, consisting of thousands of single hyphae, can develop and disappear in a few weeks (e.g. Finlay and Read 1986; Bending and Read 1995). These studies have led to the general view that EMM turnover is very rapid perhaps occurring once per week during the growing season (Finlay and Söderström 1992; Smith and Read 2008). However, it is unknown if these results of laboratory studies, typically using monocultures of EM fungi living in symbiosis with small seedlings under low light conditions, are directly applicable in the field. For field studies, quite a large number of EMM production estimates have been published (Table 1), but to calculate the turnover rate we need both production and standing biomass estimates. This is problematic due to the difficulty to distinguish mycorrhizal from saprotrophic mycelium. We know of only one study in which estimates of both standing biomass and production of EMM have been made. Using a soil-incubation technique, it was estimated that EM fungi contributed to approximately half of the standing mycelial biomass in coniferous forests soils in southern Sweden (Bååth et al. 2004). Based on these results, Wallander et al. (2004) calculated total EMM standing biomasses in the upper 70 cm of the soil of $4.8\times10^3$ kg ha$^{-1}$ in a *P. abies* forest and $5.8\times10^3$ kg ha$^{-1}$ in a mixed *P. abies*/*Quercus robur* forest. This is an order of magnitude higher than the production rates determined from in-growth bags, suggesting a mean residence time of 10 years (Wallander et al. 2004), or a turnover rate of about 0.1 year$^{-1}$, which is considerably lower than those of fine roots in boreal and temperate forests which have been estimated to be between 0.4 –1.3 year$^{-1}$ (Gill and Jackson 2000; Finér et al. 2011; Brunner et al. 2012). A mean residence time of the whole mycelium of 10 years is surprisingly high as shown above, and suggests a large contribution of long-lived rhizomorphs (see below) to the standing biomass in these forests. Alternatively, this dichotomy is simply an illustration of the difficulty of estimating EMM standing biomass and production accurately. For example, one problem may be a possible underestimation of EMM production rates with the sand bags (Hendricks et al. 2006) as well as the imprecise conversion factors between fungal biomarkers and biomass. An underestimate of production combined with an overestimate of standing biomass would result in an underestimate of the rate of turnover. A solution to this problem may be to combine sequential harvesting of in-growth bags with a $^{13}CO_2$ pulse labelling of the mycelium via the plant and analyses of $^{13}C$ in structural components of the mycelium such as glucosamine (for further technical discussions, see Wallander et al. 2013).

### Rhizomorph longevity

Different parts of the mycelium definitely turn over at different rates and it is likely that single hyphae of many fungi turn over much more rapidly than rhizomorphs. Recent minirhizotron studies show that mean life-span of rhizomorphs can range from 7 to 22 months and some can survive several growing seasons (Treseder et al. 2005; Pritchard et al. 2008; Vargas and Allen 2008). In a *Pinus taeda* forest exposed to elevated $CO_2$, the average life-span of rhizomorphs was dependent on rhizomorph diameter, soil depth and the $CO_2$ treatment (Pritchard et al. 2008). The longest average life-span was found for thick, rhizomorphs,

at greater soil depth and under high $CO_2$-conditions. These findings suggest that the turnover of the complete EMM is probably highly dependent on the relative contribution of rhizomorphs to the standing biomass and possibly their average diameter and soil depth distribution. Knowing that a forest's EM community is typically dominated by a few fungal species, with a large number of other species that are rare (Dahlberg 2001), even a minor shift in species composition may therefore have a profound effect on the standing biomass and turnover of the EMM. It should be noted that most rhizomorphs are hydrophobic, but some fungi, e.g. *Thelephora terrestris*, produce hydrophilic rhizomorphs (Unestam 1991). It is unknown if hydrophobicity affects the turnover rates, but a hydrophobic surface is probably less easily attacked by extracellular enzymes which could result in suppressed microbial degradation rates.

Rhizomorphs can be much more long-lived than roots, as demonstrated in the *P. taeda* forest mentioned above. In this forest, the mean life-span of rhizomorphs was 2 to 9 times longer those of the mycorrhizal tips (Pritchard et al. 2008). This difference has several important ecological implications. For instance, new roots can, at relatively low C and N costs, connect to and take advantage of all the benefits of an established extensive mycelial network. A long life-span is advantageous to the fungus which is more likely to cover a large area of the forest floor. In addition, a large mycelial network will immobilize N, reducing the N leakage from the forest. Indeed, leakage of N after heavy N-fertilization is suggested to be intensified due to the reduction of EMM (Högberg et al. 2011). However, the mean life-span of rhizomorphs is not always longer than that of the root tips, as was shown in a mixed conifer oak forest (Vargas and Allen 2008). Differences in estimates of longevity may reflect the species composition of fungal communities and illustrates the need for further studies comparing the longevity of rhizomorphs and root tips.

## Variation in EMM biomass and turnover

Large seasonal and year-to-year variations in standing biomass and turnover are likely due to environmental factors that directly affect the mycelium, such as winter soil freezing, but also indirect effects via the host, such as seasonal changes in C availability or more catastrophic events such as drastic declines in leaf area, and thus reductions in the C supply to the mycorrhizas. Thus, a summer drought combined with an ice storm in December of the same year resulted in reduced leaf area index and in high rhizomorph mortality, reduced production and standing biomass of mycorrhizas and rhizomorphs the following year (Pritchard et al. 2008). Since the EMM biomass contains a large pool of N, reductions in its standing biomass are likely to cause an increase in easily available N, as indicated by the increased N concentration and increased $\delta^{15}N$ of dwarf shrubs the year after tree girdling in a boreal forest (Bhupinderpal-Singh et al. 2003).

Changes with soil depth in disturbances such as drying-wetting cycles are likely to result in faster turnover of mycelium in the upper soil horizons, which may at least partly explain the depth differences seen for rhizomorphs. It is not known whether there are also substrate-characteristic differences in turnover rates. Laboratory studies show that the intensive colonization of organic patches with EM mycelium is of short duration and recedes after a few weeks (e.g. Finlay and Read 1986; Bending and Read 1995; Donnelly et al. 2004). In contrast, when mineral material from the E-horizon (60 % sand and 40 % silt) of a podzol was used, the EMM grew vigorously throughout the experiments (14 to 19 weeks; Rosling et al. 2004). However, since different fungi were used in these experiments, we cannot exclude species differences as a possible source of variation rather than substrate effects. On the other hand, a substrate dependent difference in longevity was indicated when the EMM of *Rhizopogon* colonized either small patches with organic materials or acid washed silica sand; the mycelium disintegrated within a few weeks after colonizing the organic patch while it remained vital in the mineral patch throughout the experiment (Wallander and Pallon 2005). We propose that a substrate dependent difference in turnover would be a logical consequence of the different functions that the mycelium may fulfil. Thus, in the mineral soil the main activity of EMM is to take up minerals like P and K, and additionally aid their release by the weathering of primary and secondary minerals. Weathering is a very slow process and therefore the mycelium is more persistent in these environments. In contrast, in the organic horizons, the availability of nutrients varies both temporally and spatially and the strategy is to rapidly colonize short-lived patches of labile organic matter. When the first patch is depleted, the mycelium autolyses and some of the material in the old

mycelium is translocated to other hotspots. However, further studies are needed to test if there are substrate differences in the growth habits of EM fungi. It is also unknown how much of the fine mycelium that is autolysed and reused and how much that is decomposed by other organisms.

The direct effect of grazing by soil microarthropods on EMM production may be small (see above) but indirect effects of fauna on EMM turnover and standing biomass of mycelial materials could still be of importance, e.g. grazing or disturbance caused by activities of mesofauna and larger animals may be of importance but this is unknown. Such faunal effects on EMM turnover may vary between ecosystems, along with differences in faunal and fungal communities. For example, in soils with high activity of earthworms, such as in many broad leaved forests in Europe, the physical disturbance to the mycelium is likely to be large, which would increase the turnover of EMM and reduce its standing biomass. In areas with high density of wild boar the disturbance caused by their rooting can be tremendous (Massei and Genov 2004). Hypogeous sporocarps can contribute significantly to the wild boar diet which can stimulate the production and spread of these sporocarps (Lawrynowicz et al. 2006).

## Importance of mycelial C cycle for SOM formation and cycling

The amount of C invested into EMM is large and this important component of the soil biomass may potentially affect the amount of C stored in SOM in several ways. Firstly, residues of the EMM may contribute to the formation of stable SOM (Godbold et al. 2006). Secondly, the activity of the mycorrhizal roots and EMM may indirectly or directly affect the decomposition of organic materials.

### Soil organic matter formation

There is increasing evidence that microbial residues play an important role as precursors for stable SOM (Ehleringer et al. 2000; Godbold et al. 2006; Wallander et al. 2011), however which residues are involved remains unclear (Koide and Malcolm 2009). The precursors may both come directly from mycorrhizal tissues (Godbold et al. 2006) or as a result of microbial turnover during the degradation of plant necromass (Ehleringer et al. 2000). Much of the early evidence for the importance of microbial residues in formation of SOM is based on measurements of changes in isotopic ratios. In forest soils, there is an enrichment of $^{13}C$ and $^{15}N$ and a decrease in C/N ratio of the SOM with soil depth, which approaches that in the fungal biomass (Gebauer and Schulze 1991; Högberg et al. 1996; Wallander et al. 2003; Gleixner 2005; Boström et al. 2007). The enrichment of $^{13}C$ and $^{15}N$ has been used to suggest increased importance of microbial input to SOM with increasing soil depth. In fact, the $\delta^{15}N$ signature of SOM in the mineral soil approaches that of EM fungi (Boström et al. 2007), suggesting that EMM is the main precursor of this material. In support of the idea that the EMM may be important in formation of SOM, analyses of the $\delta^{13}C$ of mycelial and root in-growth cores suggested that EMM was the dominant pathway through which C entered the SOM pool (Godbold et al. 2006), contributing up to 60 % of newly formed SOM. In this work, by using in-growth cores with different mesh sizes the input from the EMM could be distinguished. A greater recalcitrance of fungal substances such as chitin compared to plant residues from cellulose and lignin has been used as an explanation for the apparent accumulation of microbial residues (Gleixner et al. 1999; Gleixner et al. 2002; Godbold et al. 2006). Other potentially important fungal substances include melanin, hydrophobins, and in AM fungi, glomalin (Treseder and Allen 2000). The assumption that fungal cell walls (chitin) may be more resistant to degradation than plant cell walls (cellulose and lignin) is based on comparison of decomposition rates of whole tissues. The decomposition rate of EM root tips in the field in a study by Langley et al. (2006) was 65 % slower than non-mycorrhizal root tips despite having a lower C:N ratio which would, in plant material, be expected to increase the decomposition rate. However, recent data question whether EM roots tips have slower rates of decomposition than non-mycorrhizal root tips (Koide et al. 2011), and the higher recalcitrance of chitin than lignin (Koide and Malcolm 2009). The higher recalcitrance of chitin than other fungal compounds has also been questioned (Fernandez and Koide 2012). Estimates of the rates of decomposition of EMM are few (Wilkinson et al. 2011; Fernandez and Koide 2012). Söderström (1979) showed that only 2–4 % of hyphae isolated from a *P. sylvestris* forest were metabolically active, suggesting that the degradation of

inactive hyphae is slow. However, EM mycelium grown in the laboratory have been shown to decompose within several weeks (Fernandez and Koide 2012), with a relationship between the C:N of the mycelium and rates of decomposition (Koide and Malcolm 2009). But in contrast, Wilkinson et al. (2011) could find no relationship between the efflux of $CO_2$ of decomposing hyphal material and the C:N ratio of the added necromass. They did, however, find that $CO_2$ efflux was dependent on the species richness of the necromass added. Clearly our current understanding of decomposition of fungal tissues is poor. A recent analysis (Schmidt et al. 2011) has questioned the importance of both composition and chemical recalcitrance of litter in the formation of SOM. These authors suggest that rather the persistence of organic matter is due to complex interactions with the soil environment such as sorption onto clay minerals and isolation in aggregates. Thus close contact of the fine hyphae of the EMM with soil mineral surfaces could explain the apparent persistence of mycorrhizal inputs in some forest soils but not in all. For example this mechanism is not applicable in most forest soils in Sweden which typically have very low clay content, without aggregate formation in the mineral soil and an organic layer on top.

We suggest that the decomposition of EMM could, in principle, follow three initial pathways: firstly, by autolysis, where by much of the hyphal material may be reused, secondly, through the activity of saprotrophic fungi and bacteria, and thirdly by grazing soil animals (Fig. 2). The relative importance of these mechanisms is unknown. Physical disturbances that disrupt the mycelium contact with the C supply of the host can cause rapid growth of saprotrophic fungi that use the dying mycorrhizal mycelium as a substrate (Lindahl et al. 2010). Such functional shifts in fungal communities, induced by disturbance, may be highly important for the nutrient release from EMM mycelia in boreal forests (Lindahl et al. 2010). The residual materials produced in the above three principal pathways may have different quality. In the first two cases, i.e. internal cycling (autolysis) or saprotrophic microorganisms, the residues are probably N poor and further decomposition may be relatively slow. Grazing, on the other hand, could leave a residue that is fragmented and relatively nutrient rich making further decomposition faster. Not all animals that feed on mycelium are grazers; Protura, one of the few groups of microarthropods that might be specialized on EM fungi, seem to have adapted to suck on hyphae (Pass and Szucsich 2011). Whether they suck out a minor part of the cytoplasm and leave living hyphae behind, or if they leave dead membranes is unknown. Currently, we can say no more than that microbial precursors appear to be very important in formation of SOM, and that the nature of these precursors and the pathways involved are still inadequately investigated.

## Heterotrophic activity

The role of EM fungi in the decomposition of organic matter is currently a subject of much debate. Laboratory experiments have shown that many ericoid and EM fungi can decompose complex organic compounds (Read and Perez-Moreno 2003) and culture studies showed that EM can mineralize cellulose and lignin, but typically only at one tenth the rate of saprotrophic fungi (Trojanowski et al. 1984).

Several recent studies have tried to identify the factors triggering saprotrophy in EM fungi, and to assess its ecological significance (Courty et al. 2007; Cullings et al. 2008; Talbot et al. 2008; Cullings and Courty 2009). They hypothesise that saprotrophic C acquisition by EM fungi may be an alternative strategy: (1) during periods with low photosynthate supply from the host, (2) during periods of high photosynthate supply from the host, but when a supplementary resource for massive mycelial production is required, or (3) during decomposition of dying tree roots (Talbot et al. 2008; Baldrian 2009). However, there is presently little evidence for any of these hypotheses. In one of the few field studies testing the saprotrophic activity of EM fungi, addition of $^{14}C$-labelled litter to an oak forest floor showed that the EM fungi did not utilize the litter C and were totally dependent on host C (Treseder et al. 2006). While generalizations are impossible due to the missing experimental data, the ecological relevance of saprotrophic behaviour of EM fungi should be placed in the context of the large sustained supply of C derived from the autotrophic plant.

While the importance of the saprotrophic capacity of EM fungi to the C cycle is unknown, there is good evidence for their involvement in degrading N and P containing organic compounds. For instance, protease and chitinase production in EM fungi has

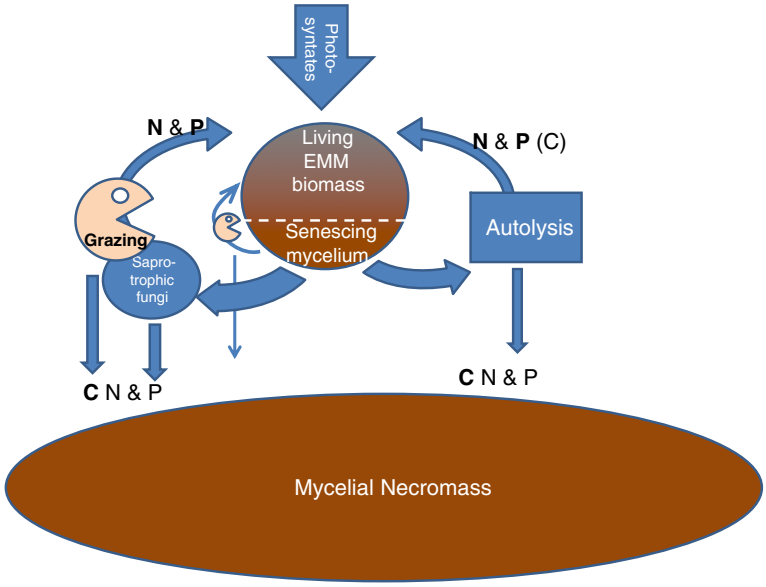


**Fig. 2** Simplified scheme showing the different routes for turnover of extramatrical mycelium (EMM). Carbon for growth of the fungal biomass is supplied by plant photosynthates. The fungus is present as living hyphae (living EMM), senescing mycelium and mycelial necromass. The decomposition of the EMM could in principle follow three initial pathways: firstly, by autolysis, where by much of the N and P may be reused, secondly, through the activity of saprotrophic fungi and bacteria, and thirdly by a direct grazing by soil animals. The relative importance of these pathways is unknown but probably of great importance for the further decomposition of the materials. In this figure the direct grazing of the EMM is smaller than grazing of saprotrophic microorganisms, since this is suggested by recent studies (see section on grazing of mycelium) and the necromass is much larger than the standing biomass

been reported in several species (Hodge et al. 1995; Lindahl and Taylor 2004; Nygren et al. 2007), which will release amino sugars and amino acids from the degradation of chitin or polypeptides. The genome of *L. bicolor* contains >100 putative extracellular proteases and several chitinases and testifies to the ability to use a variety of N-containing compounds (Martin and Selosse 2008) but the ability to use proteins and peptides varies greatly among EM fungi (Abuzinadah and Read 1986). The acquisition of organic P by EM fungi is mediated by the widespread activity of surface-bound phosphatases (Alvarez et al. 2006), although these activities appear to be largely species-dependent (Plassard et al. 2011).

Ectomycorrhizal fungi can also affect decomposition indirectly. Litter decomposition is less in plots with mycorrhizal roots than in plots without these roots (Gadgil and Gadgil 1975; Berg and Lindberg 1980). This so called 'Gadgil effect' is suggested to be caused by the efficient uptake of N and P by EM fungi reducing the availability of these nutrients for other microorganisms. Such a nutrient limitation may be strengthened further by the immobilisation of N and P into a large biomass and possibly necromass of EMM (see above). Indeed, molecular methods show that forests dominated by EM trees have low abundance of bacteria (de Boer et al. 2005) and saprotrophic fungi that are primarily found in the surface litter (Lindahl et al. 2007). An alternative explanation of the 'Gadgil effect' is that water uptake by mycorrhizal roots and EMM reduce the soil water content causing a water limitation of decomposition (Koide and Wu 2003). The opposite effect, a stimulation of decomposition as a result of water uptake by mycorrhizal roots and EMM was found in soils with high groundwater levels (Jaatinen et al. 2008). The presence of EM hyphae can theoretically increase microbial decomposition of complex organic compounds by priming the co-metabolism of recalcitrant substrates by saprotrophic microorganisms, e.g. by the production of low molecular mass organic acids like oxalate (Kuzyakov et al. 2000; Fontaine et al. 2003) or specifically affect (inhibit) the activity of certain groups of decomposers by antibiosis (Tsantrizos et al. 1991; Frey-Klett et al. 2005). Because these indirect and

direct effects of EM fungi on decomposition may act simultaneously, the net effect is difficult to calculate.

## Importance for soil $CO_2$ fluxes and Dissolved Organic Carbon (DOC)

Soil $CO_2$ and dissolved organic carbon (DOC) efflux are the major pathways for C loss from soils. Partitioning of soil $CO_2$ efflux into autotrophic respiration (from roots, mycorrhizal fungi and rhizosphere organisms, driven by photosynthates) and heterotrophic respiration from decomposition of SOM is recognized as critical for further improvement of models of ecosystem C budgets (Hughes et al. 2008; Chapin et al. 2009). Among the autotrophic components, mycorrhizal roots often exhibit higher specific respiration rates than non-mycorrhizal roots (Colpaert et al. 1996; De Grandcourt et al. 2004). This has been ascribed to higher construction costs that lead to a higher growth respiration coefficient and cost of nutrient absorption. The maintenance cost per unit biomass is indeed higher for hyphae than for roots (Fitter 1991).

While recent studies have documented the substantial amount of C translocated to the production of EMM, the contribution of the EMM to the soil $CO_2$ efflux has rarely been studied under field conditions. This has been estimated using in-growth cores partly or wholly covered by fine mesh of different size, allowing or restricting the EMM growth into the core. Thus, it was estimated that the EMM contributed up to 60 % of the autotrophic soil $CO_2$ efflux and 25 % of the total soil $CO_2$ efflux in a *Pinus contorta* forest (Heinemeyer et al. 2007). A four–year study in an oak forest, attributed 18 % of the annual soil $CO_2$ efflux to EMM respiration (Heinemeyer et al. 2011), also showing the large seasonal and annual variability of the EMM contribution that may explain diverging findings; Moyano et al. (2008) reported lower contributions of EMM to the total soil $CO_2$ efflux (8 % in a spruce forest and only 3 % in a beech forest).

Dissolved organic C in forest soils is a complex mixture; most is humic substances and a small proportion, often <10 %, is compounds such as organic acids, amino acids, sugars and phenols (Kalbitz et al. 2000; Jones et al. 2004). The processes leading to its formation are still poorly understood and the contribution of roots and especially of EMM has only recently been addressed. The amount of water extractable organic C in the mor layer of a *Pinus sylvestris* forest decreased by 45 % 1 month after tree girdling compared to the control (Högberg and Högberg 2002). This points to a direct link between assimilate transport to roots and soil solution chemistry (Giesler et al. 2007). Similarly, the water extractable organic C was several times higher in mycelial mats than in soils outside mats (Griffiths et al. 1994) and in the mineral soil, oxalate concentration was 40 times higher in mats than in non-mat soil (Kluber et al. 2010), suggesting a large contribution of EMM to DOC production. In a laboratory experiment, the DOC produced by *P. sylvestris* seedlings with EM was 50 % larger than the controls without EM (Johansson et al. 2009).

Both laboratory and field studies confirm the potential of EM to contribute to DOC in forest soil solutions, although there is a need for more detailed investigations which can be extrapolated to field conditions.

## Conclusion and future research

Until a decade ago our knowledge about EMM production and its importance in C cycling was mainly based on laboratory experiments. Recent research using in-growth cores and mesh bags has demonstrated that the production of EMM is up to several hundreds of kilograms per ha per year in forests ecosystems, but the production seems to vary greatly between different forests and between years. We conclude that much of the recorded variation in EMM production can be explained by variations in the availability of C and other factors, such as N and P availability, may act mainly indirectly via the plant. Whether a change in EMM production is preceded by a change in the EM community is unknown, but perturbations that decrease the C availability seem to favour contact and smooth exploration types.

The EMM may not be an easily available food source for the decomposer community. Relatively few species of soil animals exhibit feeding preferences towards EM fungi and it is possible that animal grazing of saprotrophs is quantitatively more important than that of EM fungi, but this is an open area of research.

The lack of data on mycelial turnover rates is an obstacle to development of models of forest C cycling.

We suggest that the slowest turnover rates of the total EMM are to be found in forests where long distance types are common in the fungal community. In such forests, both the standing EMM biomass as well as its N and P retention capacity may be large.

The classification of EM taxa into exploration groups based on morphological growth characteristics may be one way to describe the complex EM communities from a functional perspective. However, the ecological role is poorly known for many of the fungal taxa in a soil sample, and future research aiming at better characterization of these taxa is needed.

We hypothesise that the turnover of EM hyphae is more rapid in organic materials than in the mineral soil, which is motivated by the different functions the mycelium probably has in the two substrates. These two functions might be equally important as indicated from the few studies reporting EMM production rates also in the mineral soil. But this topic needs further studies.

**Table 2** Critical questions for future research on the extramatrical mycelium (EMM) of ectomycorrhizal (EM) fungi

| Question | Comment |
|---|---|
| Are there geographical and/or tree species differences in EMM production, standing biomass and turnover? | Much of the current knowledge comes from *Picea abies* and Scandinavia. |
| How much of the variation in this respect is explained by variations in the EM community? | There are likely to be large differences between different exploration types but also within a single type. |
| How much of the variation in this respect is explained by variations in the C:N:P stoichiometry? | Long term factorial fertilizer experiments are needed perhaps combined with defoliation experiments. |
| Why is not increased C availability, by elevated $CO_2$ causing an increase in the occurrence of long distance types? | Perturbations that decrease the C availability for EMM growth seems to make contact and smooth exploration types more competitive than long distance types. Why not the opposite? |
| Are there soil depth differences in mycelium production, standing biomass and turnover? | Most studies have focussed on the upper organic layer. |
| Ditto for substrate differences? | We need to test our hypothesis that mycelium turnover is slower in mineral soil than in organic layers. A difference in turnover is motivated by the different possible functions of the EMM in the two substrates. |
| How large proportion of the production and standing biomass is rhizomorphs? | We know of only one laboratory study. This is very important to know for C-turnover models. |
| How large are the seasonal and between year variation in production and standing biomass? | We need studies with higher time resolution, one harvest of ingrowth bags per year is not enough. |
| Is the EM fungal versus saprotroph niche separation as found in a boreal forest (dominance of EM in the organic layer and mineral soil and saprotrophs in the litter) stable or has the two fungal groups different seasonal dynamics? | Only one study at one time of the year so far. |
| Is this niche separation the rule also in other forest ecosystems? | In many broad leaved forests, the soil mixing by earthworms may make this depth separation of fungal functional groups less clear, but this needs to be studied. |
| How fast is the decomposition of the mycelium? | There are probably large species differences. Mycelium grown in pure culture may differ significantly from that of a natural mycelium. |
| How important are soil animals for the turnover of EMM? | The picture we get from the few field studies is that the grazing of EMM may be limited but that conclusion is based on few studies. In many broad leaved forests the disturbance from earthworms may cause the mycelium to turnover more rapidly than in coniferous forests. |
| How important are mammals for the EMM standing biomass and turnover of EMM? | Selective grazing above as well as below ground may have great importance, e.g. the rooting of wild boar can be important. |
| Is EMM an important precursor for stable soil organic matter? | There is some evidence supporting this but much more work is needed on this tricky question! |
| What is the relation between the biomass of EMM, environmental factors and the production of $CO_2$ and DOC? | So far, no study has analysed $CO_2$ efflux in combination with EMM biomass. |

Although the number of papers on EMM in forest soils has increased dramatically over the last decade, there are still very big gaps in our knowledge in most of the topics brought up in this review. As a guide for future research, the most important of these gaps are formulated as questions in Table 2. The EMM of EM fungi has several important roles in ecosystems. In this paper we have focused on its role in the C-cycle. There is increasing evidence that residues of EM fungi play a major role in the formation of stable N and C in SOM, calling for a greater inclusion of EM inputs into models of soil C stores in forests.

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Review

# Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils – A review

H. Wallander [a,*], A. Ekblad [b], D.L. Godbold [c], D. Johnson [d], A. Bahr [a], P. Baldrian [e], R.G. Björk [f], B. Kieliszewska-Rokicka [g], R. Kjøller [h], H. Kraigher [i], C. Plassard [j], M. Rudawska [k]

[a] Department of Biology, Microbial Ecology Group, Ecology Building, Lund University, SE-223 62 Lund, Sweden
[b] School of Science & Technology, Örebro University, SE-701 82 Örebro, Sweden
[c] Institute of Forest Ecology, Universität für Bodenkultur, 1190 Vienna, Austria
[d] Institute of Biological and Environmental Sciences, University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen AB24 3UU, UK
[e] Laboratory of Environmental Microbiology, Institute of Microbiology ASCR, CZ-14220 Praha, Czech Republic
[f] Department of Biological and Environmental Sciences, University of Gothenburg, P.O. Box 461, SE-405 30 Gothenburg, Sweden
[g] Institute of Environmental Biology, Kazimierz Wielki University, Al. Ossolinskich 12, PL-85-093 Bydgoszcz, Poland
[h] Terrestrial Ecology, Biological Institute, University of Copenhagen, Øster Farimagsgade 2D, DK-1353 Copenhagen, Denmark
[i] Slovenian Forestry Institute, Vecna pot 2, 1000 Ljubljana, Slovenia
[j] INRA, UMR Eco & Sols, 34060 Montpellier Cedex 02, France
[k] Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland

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ABSTRACT

Mycorrhizal fungi constitute a considerable sink for carbon in most ecosystems. This carbon is used for building extensive mycelial networks in the soil as well as for metabolic activity related to nutrient uptake. A number of methods have been developed recently to quantify production, standing biomass and turnover of extramatrical mycorrhizal mycelia (EMM) in the field. These methods include mini-rhizotrons, in-growth mesh bags and cores, and indirect measurements of EMM based on classification of ectomycorrhizal fungi into exploration types. Here we review the state of the art of this methodology and discuss how it can be developed and applied most effectively in the field. Furthermore, we also discuss different ways to quantify fungal biomass based on biomarkers such as chitin, ergosterol and PLFAs, as well as molecular methods, such as qPCR. The evidence thus far indicates that mycorrhizal fungi are key components of microbial biomass in many ecosystems. We highlight the need to extend the application of current methods to focus on a greater range of habitats and mycorrhizal types enabling incorporation of mycorrhizal fungal biomass and turnover into biogeochemical cycling models.



## 1. Introduction

A better understanding of below ground carbon (C) flux is of fundamental importance to predict how changing climate will influence the C balance of forest (and other) ecosystems (Litton and Giardina, 2008). Litton et al. (2007) reported below ground C allocation in forest ecosystems can represent 25–63% of GPP on a global scale, and this C has a large influence on the physical, chemical and biological properties of soils. The below ground allocation of C links activity in the forest canopy to the activity in the soil, and provides a flow of organic C from shoots to soil via fine roots and mycorrhizal hyphae. The pathways by which this organic C can enter soils are complex, involving both biomass turnover (Godbold et al., 2003), biomass grazing (Setälä et al., 1999) and turnover of low molecular weight exudates from roots and fungal hyphae (van Hees et al., 2005). The fate of C entering soil systems is also complex. Much of this C is lost as respiration (Janssens et al., 2001) and a small but significant fraction enters the soil organic matter (SOM) pool. Determination of the pools and fluxes of biomass inputs in isolation from fine roots and mycorrhiza provides a major scientific challenge. Some studies (e.g. Wallander et al., 2004) suggest that biomass pools and inputs from fine roots and mycorrhizal hyphae are in the same order of magnitude. However, estimates of fungal inputs rely on

* Corresponding author. MEMEG, Department of Biology, Ecology Building, Lund University, SE-223 62 Lund, Sweden. Tel.: +46 46 222 4247; fax: +46 46 222 4158.
*E-mail address:* hakan.wallander@biol.lu.se (H. Wallander).

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methods and conversion factors that contain a certain degree of inaccuracy that needs to be considered.

Precise measurements of production, standing biomass and turnover of extramatrical mycelium (EMM) of mycorrhizal fungi are essential in order to accurately describe the C cycle of terrestrial ecosystems. Although several techniques are available for this, they all have limitations that need to be taken into consideration.

Existing biogeochemical models often treat the uptake apparatus as a single organ, meaning that there is no distinction between roots and mycorrhizal hyphae. It is possible, and probably necessary, to amend this by allocating carbon and nutrients specifically for the fine roots and mycorrhizal hyphae respectively. This would require the development of dynamic allocation routines responsive to carbon, nutrients and water availability (Jönsson, 2006), and would allow the models to simulate nutrient uptake and carbon flux dynamically. In this review, we will discuss and compare available methods to estimate production, standing biomass and turnover of mycorrhizal mycelia (summarized in Tables 1–3). We focus on temperate and boreal forests, in which the dominant plants associate with ectomycorrhizal (ECM) fungi. From a methodological perspective, greatest progress has been made in quantification of production, biomass and turnover of ECM fungi compared to the other main mycorrhizal types (arbuscular and ericoid mycorrhizas). This progress has been driven partly by technical reasons but more importantly because of the recognition of the key roles boreal and temperate forests play in the global C cycle. However, we emphasise from the outset that greater effort must be applied to other ecosystems in which plants are primarily colonised by arbuscular and ericoid mycorrhizal fungi, which also have important roles in regulating biogeochemical cycles. We will also highlight knowledge gaps that need to be filled in order to incorporate mycorrhizal mycelia in models of biogeochemical cycles, which will enable us to better describe the C cycle in forests. Firstly methods to estimate EMM production are described and discussed, since the methodology in this field has developed rapidly over the last decades. We then discuss the advantages and disadvantages of different methods to estimate fungal biomass. Finally we discuss how we can assess the turnover of fungal hyphae. This area needs clearly to be developed in future research as it is a key process in C sequestration of forest soils. We also include aspects of sampling strategies and indirect estimates of EMM production that have great potential for the future. The mechanisms through which EMM regulate C cycling in terrestrial ecosystems have been considered recently in another review (Cairney, 2012).

## 2. Measurements of mycorrhizal hyphal production

A key problem in the determination of mycorrhizal hyphal production is lack of methods to distinguish growth of mycorrhizal hyphae from that of saprotrophic fungi. As ECM fungi do not form a monophyletic clade (Hibbett et al., 2000; Tedersoo et al., 2010) no single biochemical or DNA based marker can be found to quantify this group from the complex soil environment. Therefore various methods are needed to distinguish the biomass of EMM from that of other fungal mycelia. Mycelial growth can be estimated by direct observation in minirhizotrons (Treseder et al., 2005; Pritchard et al., 2008; Vargas and Allen, 2008a) and by the use of root free in-growth bags or cores, which is the most commonly applied method to measure EMM production in forests (Wallander et al., 2001; Godbold et al., 2006; Hendricks et al., 2006; Kjøller, 2006; Korkama et al., 2007; Parrent and Vilgalys, 2007; Hedh et al., 2008; Majdi et al., 2008).

### 2.1. Observational methods

The first observational studies used plastic sheets placed at the litter/soil interface above root clusters where individual ECM tips were observed by pulling back and replacing the litter at different times (Orlov, 1957, 1960). Lussenhop and Fogel (1999) used a method developed by Waid and Woodman (1957) to estimate hyphal production of the ECM fungus *Cenococcum geophilum* by burying nylon mesh in the soil and harvesting them at two week intervals. Rygiewicz et al. (1997) introduced the minirhizotron technique, commonly used to study fine roots, to measure temporal occurrence and lifetime of mycorrhizal root tips. However, the use

**Table 1**
Strengths and weaknesses of currently used methods to estimate production of ECM extramatrical mycelium (EMM).

| Methods | | Strengths | Weaknesses | Comments |
|---|---|---|---|---|
| Production of ECM mycelium | Direct minirhizotron observation | • Repeated non-destructive sampling possible.<br>• Not dependent on conversion factors. | • Cannot differentiate between saprotophic or mycorrhizal hyphae.<br>• High resolution needed to observe individual hyphae.<br>• Growth might be different in observation chamber compared to soil.<br>• Difficult to transfer to biomass per land area. | • Changes in rhizomorph production, which are easier to observe, does not automatically imply similar changes in total EMM production. |
| | Root free in-growth mesh-bags or cores | • Easy and relatively cheap method that can be applied in large scales.<br>• Substrates that have no background of old mycelium, chemical markers, DNA etc. can be used.<br>• Substrates can be 'spiked' with isotopic labelled materials, minerals etc.<br>• Relative comparisons may be more reliable than estimates of absolute amounts. | • Growth, standing biomass and turnover may be different in mesh bags compared to soil, and this needs to be further studied.<br>• May select for early colonizers of fungus free space.<br>• Disturbance at installation & harvest. Interactions with soil animals are restricted.<br>• The way the mycelial biomass is assessed may give different results. | • When bags are left in the soil over years or more, the mycelial mass is possibly a reflection of the standing biomass rather than production?<br>• Disturbance is probably larger for larger bags or cores.<br>• Mycelial biomass can be assessed with: dry weight, loss on ignition or with chemical markers. |
| | Assessment of exploration types | • Definition of exploration types is based on EMM production.<br>• ECM communities have been studied in a number of forest ecosystems.<br>• Possible to combine with molecular methods to indirectly non-destructively estimate EMM production. | • Estimation of EMM production is based on observations from (simplified) laboratory conditions – growth might be different in soil due to nutrient conditions and season etc. | • Only 5–10% of all ECM fungi have been characterized and are assigned into exploration types. |

**Table 2**
Strengths and weaknesses of currently used methods to estimate the biomass of ECM extramatrical mycelium (EMM).

| Methods | | Strengths | Weaknesses | Comments |
|---|---|---|---|---|
| Biomass of ECM mycelium | Direct measurement of mycelium length in the soil | • Not dependent on chemical conversion factors. | • Difficult to separate mycelium of mycorrhizal and decomposing fungi and living biomass from necromass. | • Dependent on correct conversion factors from length to biomass. |
| | Root free in-growth mesh-bags or cores | • See above for mycelium production using bags. | • See above for mycelium production using bags. | • See comments on production estimates using in-growth bags above. |
| | Chemical markers (chitin, ergosterol, PLFAs) combined with incubation | • Highly sensitive, small amounts can be estimated. | • Dependent on conversion factors which can vary between species and growth conditions. | • Field studies on variation in conversion factors are lacking. |
| | Molecular DNA and RNA methods | • Possible to estimate biomass of individual species.<br>• Targeted especially to dominant species in ECM communities.<br>• Techniques under fast development. | • Suitable primers depend on fungal species, a number yet to be developed.<br>• High costs of next generation sequencing. | • Techniques are under development |
| | Assessment of exploration types | • Data from ECM Communities on root tips can be extrapolated to EMM.<br>• Non-destructive estimation of EMM production possible based on ECM community composition. | • EMM biomass of individual exploration types is based on a combination of previously defined estimations.<br>• Few ECM types have been grown in cultures, therefore species-specific fungal diameter and conversion of volume into biomass needs further studies. | |

of observational methods to estimate production, biomass and turnover of EMM in the field has been limited. It has mostly been used to study mycorrhizal roots tips (e.g. Rygiewicz et al., 1997; Majdi et al., 2001; Tingey et al., 2005), but few attempts have been made to estimate the length and longevity of rhizomorphs and hyphae (Treseder et al., 2005; Pritchard et al., 2008; Vargas and Allen, 2008a,b). Similar observations may also be possible using root observation windows (Stober et al., 2000). However, none of the direct techniques can distinguish between the mycelium of ECM and saprotroph mycelia. Two types of minirhizotron cameras are commonly used, which also give different image sizes; BTC 100× microvideo camera (Bartz Technologies, Santa Barbara, CA, USA) that provides image sizes of 1.9 × 1.3 cm, and a CI-600 (CID Bio-Science Inc., Camas, WA, USA) that provides a 360-degree image (21.59 × 19.56 cm). The advantage of the minirhizotron techniques, unlike other methods that rely on excavation which can disrupt extraradical hyphae, is the potential to make repeated, non-destructive observations *in situ* of the same specimen. This allows the specimen to be followed from its emergence (birth) to its disappearance (death). Although the technique has been found useful to monitor the formation and death of mycorrhizal root tips as well as rhizomorphs, several shortcomings exist. For instance, the minirhizotron technique is limited by the resolution and quality of the images (although the technology in this area is progressing rapidly, see for instance Rundel et al., 2009) and the time required for processing (which also restricts sampling intensity, depth and the number of tubes used). Since the technique cannot yet capture the production and turnover of diffuse mycelium it does not enable calculation of overall mycelium production and turnover rates. Furthermore, there is uncertainty in determining when a rhizomorph is dead, leading to the use of different criteria. For instance, Treseder et al. (2005) classified the time of death as the first visual

**Table 3**
Strengths and weaknesses of currently used methods to estimate the turnover of ECM extramatrical mycelium (EMM).

| Methods | | Strengths | Weaknesses | Comments |
|---|---|---|---|---|
| Turnover of ECM mycelium | Direct minirhizotron | • Birth and death of individual hyphae can be followed. | • Risk of missing the exact birth or death of the hyphae (recording frequency dependent).<br>• May target the fast turnover pool since the length of the study period is limited. | • The problem with lag-time can possibly be solved if small vertically installed bags are used. But this needs to be evaluated. |
| | Direct measurements in growth mesh-bags | • In areas with rapid EMM growth and insignificant lag times for mesh bag colonization, sequential harvests at different incubation times could be a way to estimate turnover times. | • Lag-times to colonize the mesh bags may be too high for this method to give reliable results (see Fig. 1).<br>• Turnover may be different in sand than in soil. | • The problem with lag-time can possibly be solved if small vertically installed bags are used. But this needs to be evaluated. |
| | Isotopic techniques | • Pulse labelling via the plant is possible.<br>• Mesh bags amended with $C_4$ substrates can be used to continuously measure C input. | • Analyses of bulk mycelial materials may give false impression of a fast turnover. Analyses of isotopes in structural components would solve that problem.<br>• The method to use $C_4$ materials is not very sensitive, large fluxes are needed for reliable results. | |

appearance of fragmentation of the rhizomorph, whereas some authors also used the disappearance from the image for determining the death of a rhizomorph (e.g. Pritchard et al., 2008). If a rhizomorph disappears, a judgment had to be made as to whether the rhizomorph has truly died or has become obscured from view due to soil or tube movement. Both criteria are often used for estimating turnover, but may give highly variable results when compared (Børja et al., unpublished). Furthermore, it is not possible to know exactly when a rhizomorph may form or disappear from the camera's visual field between any two subsequent recording events (typically a month, but new automated minirhizotrons for recording images at multiple times per day are in progress (Rundel et al., 2009)). The long lifetime of some rhizomorphs makes it difficult to estimate turnover rate since most minirhizotron studies are conducted over a one (or two) year period. Thus, when using minirhizotrons to estimate production and turnover of rhizomorphs, it is important to consider the recording frequency and study length because both of these affect the accuracy of the estimations.

One method, which was not applied in a forest, but is worthy of mentioning is the 'root box' method of Coutts and Nicoll (1990), as it allows detailed investigation of the growth and survival of diffuse mycelium as well as of rhizomorphs over the year. These authors planted pine seedlings in peat in 2 m tall transparent acrylic tubes, placed the tubes outside and followed the growth of mycelia and rhizomorphs in detail daily from March 1987 to April 1988. This technique may be ideal for detailed studies of various ECM symbioses, for example studies of the different exploration types as defined by Agerer (see below Section 6). Although observational methods have limitations, they also have many advantages, which can substantially increase our understanding of mycelia production and turnover.

### 2.2. In-growth mesh bags and cores

Mesh bags (e.g. Wallander et al., 2001) are typically made from nylon mesh fine enough to prevent in-growth of roots, but large enough to allow in-growth of fungal hyphae. The fungal communities that colonize the mesh bags are usually dominated by mycorrhizal hyphae as has been verified by trenching experiments (Wallander et al., 2001) and with DNA analyses (Kjøller, 2006; Korkama et al., 2007; Parrent and Vilgalys, 2007; Hedh et al., 2008; Wallander et al., 2010). Mesh sizes between 25 and 50 μm are commonly used. In forest soils with little understorey vegetation, 50 μm prevents in-growth of tree roots, but if understorey Ericaceae or herbs are present, care should be taken so that the fine roots of these do not penetrate the mesh. For example, the fine "hair roots" of ericaceous plants can have diameters of just 20 μm (Bonfante-Fasolo and Gianinazzi-Pearson, 1979). The bags can have different forms and the sides of the nylon mesh can be sealed by sewing, heating and gluing.

The mesh bags are usually placed at the interface between mineral and organic horizons. This will maximize fungal in-growth since mycorrhizal fungi are most abundant in this region (Lindahl et al., 2007). However, when the main aim is to estimate EMM production on an area basis, tubular bags that are placed vertically to a desired soil depth have been used (e.g. Kjøller, 2006). This design also allows the comparison of adjacent soil and root samples taken with the same volume, and it is suitable for sequential harvests since the mesh bags can be replaced with minimal disturbance. In addition to bags, cores can be made of plastic tubes with windows made of mesh to allow fungal in-growth. One advantage with such cores is that they can be rotated regularly to detach fungal in-growth in order to function as controls with similar soil physical conditions but no, or little, fungal in-growth (Johnson et al., 2001, 2002a,b). This is a considerable advantage when the cores are filled with a natural substrate such as soil (see below). Keeping the volume of the in-growth bags (or cores) as small as possible is important when quantifying EMM production because this helps to ensure that soil physical and chemical conditions inside mesh bags are similar to those outside. In addition, small bags may be colonized more rapidly than larger ones.

Mesh bags are usually incubated in the soil during one growing season because this will give the net production for that year. In some cases a prolonged incubation time (two growing seasons) is necessary in order to detect EMM stimulation by specific substrates such as apatite or other minerals (e.g. Hagerberg et al., 2003; Potila et al., 2009). Berner et al. (2012) suggested that this may be an effect of early colonization by fast-growing ECM species, while species stimulated by minerals are more slowly growing. It has been shown that the stimulation of EMM by apatite was dependent on the P status of the forest (Wallander and Thelin, 2008), while other studies showed that large differences in EMM growth occurred after 5 months along a nitrogen deposition gradient (Kjøller et al., 2012) and in a nitrogen fertilized forest (Nilsson and Wallander, 2003). These findings show that effects of forest management on EMM growth can sometimes be detected with shorter incubation periods. The length of the incubation period thus depends on the purpose of the study. If the main goal is to test for differences between treatments (e.g. forest management or effects of substrates amended to the mesh bags), a longer incubation time can be used. But if the main goal is to estimate net annual production from a specific site, one growing season should be used. On the other hand, if quantifying temporal variation in fungal production is the goal, shorter incubation times than one growing season are used (e.g. Nilsson et al., 2005). Regardless of the approach, it should be noted that a lag time exists before EMM enter bags after they have been inserted into the soil. As an illustration of this, twice as much fungal biomass was found in mesh

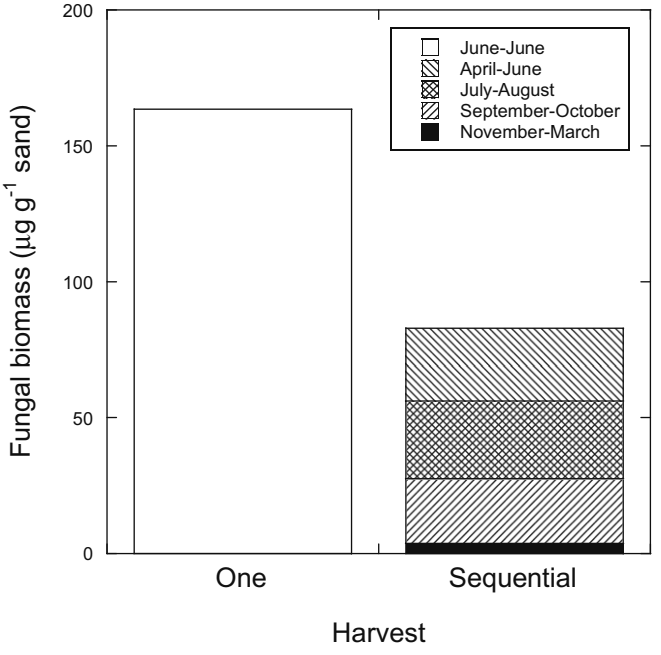


**Fig. 1.** Fungal in-growth into mesh bags buried in young (10–20 years) Norway spruce forests at Tönnersjöhedens experimental park. Bags were either incubated for 12 months (one harvest) or for 2 (July–August, September–October), 3 (April–June) or 5 (November–march) months periods (sequential harvests). SE for the mean EMM production after one harvest was 37.5, and the SE for the added sequential harvests was 9.3.

bags that were incubated for 12 months, compared to the added amounts in mesh bags that were incubated for 2–5 month periods in 10 young Norway spruce sites in southern Sweden (Fig. 1). Another aspect that complicates the estimate of production is the turnover of the fungal biomass in the mesh bags. A longer incubation period allows more necromass to form and decompose, which results in underestimation of the total production.

There has been concerns raised that the use of pure quartz sand in mesh bags may affect growth of EMM, which can lead to inaccuracies in production rates and biomass estimates (Hendricks et al., 2006). Hendricks et al. (2006) used 10 cm wide cores placed *in situ* for 1 month to demonstrate that mycelial in-growth was greater when natural soil was used as the in-growth substrate rather than pure sand. Whilst for many habitats the use of natural soils as a substrate is desirable, subsequent measurements can be confounded because of the large and variable amounts of background fungal biomass. If more specific methods to quantify ECM fungal biomass are developed (see below Section 3.6), natural soil could be used more reliably. Indeed, growth of arbuscular mycorrhizal fungi has been quantified in mesh bags amended with natural SOM using fatty acids (Labidi et al., 2007; Hammer et al., 2011), which are available for this mycorrhizal group (NLFA 16.1ω5, Section 3.5). Another uncertainty with the mesh bag method is that some ECM fungi appear to show preferences towards certain types of resource. In addition, some species avoid growing in mineral substrates (*Cortinarius*) probably because they are adapted to an environment where they utilize organic nutrients from SOM (Read and Perez-Moreno, 2003). Therefore, despite being abundant on root tips, species within the genera *Cortinarius* may avoid sand filled mesh bags even when they are common on the root tips while the opposite situation is the case for other species (e.g. *Xerocomus*; Kjøller, 2006; Kjøller et al., 2012). The EMM community in mesh bags may thus not represent the community that prevails in the soil, which may be a problem in some studies. An important advantage with the mesh bag method is that the fungi studied are recently formed, while fungi that we can detect in the soil can be old and inactive (see below Section 2.2).

Another important aspect that needs to be considered is that newly placed mesh bags provide a non-exploited area in the soil. Such spaces are probably rare in established forests but may be common in newly planted forests where the EMM from the previous forest can be expected to die back. In a tree age chronosequence, the EMM production was 3 times greater in young forest (10–20 y) compared to older forests (30–130 y) suggesting that young trees are investing more C to establish a mycorrhizal network, while less C is needed to sustain this network in older forests (Wallander et al., 2010). It is possible that mesh bags select for fast-growing species adapted to newly planted forests. For this reason, EMM production may be overestimated when incubating mesh bags over one growing season. As noted previously, such effects may be minimized by reducing as far as possible the volume of mesh bags and cores.

From the discussion above it seems that some factors result in overestimation while other results in underestimation of EMM production using the mesh bag method. As methods to measure fungal biomass and necromass improve (see e.g. Section 3.6), it might be possible to follow the fungal community in mesh bags over several years and quantify the yearly production after the initial empty space has been colonized. A combination of chitin and ergosterol analysis (see below) may give an indication of the ratio between biomass and necromass. Another way to quantify annual production of EMM, including necromass, is to analyse the isotopic change in $^{13}C/^{12}C$ in mesh bags that have been amended with organic material from $C_4$ plants, and follow this change through time (Wallander et al., 2011). A similar approach was used by Godbold et al. (2006) who filled cores with $C_4$ soil to estimate the contribution of fungal hyphae to new soil C over a 2.5 year period. Amendment of organic matter in the mesh bags would make the substrate more natural for growth of ECM fungi and probably produce communities more similar to those of the surrounding soil but brings with it greater abundance of saprotrophic fungi. An interesting approach to reduce in-growth by saprotrophic fungi but still use more natural soil was reported by Melanie Jones and co-workers in Canada who used an outer mesh bag with sand, which functioned as a barrier for saprotrophs, and an inner mesh bag with sterilized soil where EMM of ECM fungi proliferated (Lori Phillips and Melanie Jones, pers. comm.).

It is clear that the fungal community colonizing mesh bags may not accurately mirror the mycelial community in natural soil i.e. some species or clades may be over represented and some are underrepresented or even missing in the mesh bags. On the other hand, when working with natural soil it is also difficult to claim that only EMM are in the extracted DNA pool. One needs to be very careful in removing all roots and in reality it will be difficult to state that a soil sample is indeed completely free of ectomycorrhizal root tips or small detached pieces of ectomycorrhizal mantle. Furthermore, extraction of fungal spores in the soil may lead to false positives in the community profile. Extracting DNA or RNA from sand-filled mesh-bags at least ensures that only nucleic acid from actively (or recently active) mycelia is amplified. Another benefit is that the hyphae from the mesh bags are easily extracted from the sand and simple and cheap nucleic acid extraction methods can be applied to produce good quality templates for PCR. Whether extracting nucleic acid from sand-filled mesh bags or directly from soil, primer bias is a confounding factor preventing an accurate description of the fungal community. For each specific primer combination chosen, some groups will be over, and some groups under expressed or even completely missed (Bellemain et al., 2010). As an example of the latter, *Tulasnella* sp. are often completely missed with the standard ITS1-F and ITS4 primer combination (Taylor and McCormick, 2008). In general, careful consideration of primers combinations for the specific study system in question should be made, and the results obtained treated with sound caution.

## 3. Quantification of fungal biomass in mesh bags and soil

The examination of mycelia in mesh bags should start with a visual classification under a dissecting microscope. This allows a check for the presence of mycelial strands, whether or not they are hydrophilic, and gives insights in exploration types of mycorrhizal fungi (see below Section 5). The amounts of total hyphae can be estimated either by extracting fungal hyphae and converting estimates of hyphal length to biomass, or by using different chemical markers (chitin, ergosterol, phospholipid fatty acid 18:2ω6,9) as proxies for biomass. These methods are described below and the benefits and disadvantages are discussed (Tables 1–3).

### 3.1. Direct measurements of fungal weight and hyphal length

One approach to estimate fungal biomass that can be used in mesh bags only, is to extract the mycelium from the sand substrate and determine its weight. In this way conversion factors between biomass and a chemical marker can be avoided, but it assumes that all extractable matter is of fungal origin. This is not the case because bacteria and precipitated SOM can be present in the mesh bags, but they probably contribute very little to the weight of putative fungal material extracted. Since it is difficult to remove all sand grains, it is usually necessary to burn the extracted mycelia and use the loss on

ignition as an estimate of the biomass (Hagerberg et al., 2003; Korkama et al., 2007). The C concentration of fungal material is approximately constant (around 45%; Taylor et al., 2003) and C content can be used as a proxy for biomass in the mesh bags. When analysed on a mass spectrometer, both the content and isotopic signature of C can be obtained, which makes it possible to calculate the proportion of ECM and saprotrophic mycelia in the mesh bags because these two groups have different isotopic signatures (Wallander et al., 2001). The recovery of mycelium using this method can be tested by analysing the ergosterol content of both the sand (before and after extraction) and the extracted mycelia.

Fungal hyphae can be extracted from the mesh bags and separated from sand particles by centrifugation and collected on a filter paper for estimates of hyphal length. This approach produced similar results as direct estimates of EMM weight as described above (Wallander et al., 2004). Estimates of hyphal lengths can be converted to biomass using conversion factors from Fogel and Hunt (1979). However, a possible problem with this method is to fully account for rhizomorphs, which are multi-hyphal organs produced by many ECM fungi during growth through soil. The rhizomorphs facilitate efficient transport of carbon towards the mycelia front and mineral nutrients towards to mycorrhizal roots (Cairney, 1992). Separate counts must be carried out for rhizomorphs and hyphae, as they differ greatly in weight per unit length.

### 3.2. Chemical markers; chitin

Among the three possible fungal biomarkers, chitin seems the most stable parameter to assess the total fungal contribution to microbial tissue in soil (Joergensen and Wichern, 2008). Recent results (Drigo et al., 2012; Koide et al., 2011) from additions of laboratory cultivated mycelial necromass suggest rapid decomposition of chitin in soil. Indeed, fungal cell walls of all true fungi contain chitin, a structural compound with a similar role as cellulose in higher plants. In soil, other organisms may contribute to chitin contents such as microarthropods that contain chitin in their exoskeleton. However, this contribution is probably minimal as their biomass is typically below 0.5% of the fungal biomass (Beare et al., 1997; Simpson et al., 2004). An average chitin concentration of 5% of dry matter was found in a review of various species of fungi mainly grown *in vitro* and belonging mainly to Basidiomycetes, Ascomycetes and Zygomycetes (Appuhn and Joergensen, 2006). No statistically significant difference between the mean values from the three fungal orders was found, and a conversion factor from glucosamine to fungal C of 9 was proposed (Appuhn and Joergensen, 2006).

Using data from Joergensen and Wichern (2008), we estimate that ±one standard deviation around the mean gives a span of around 6–50 of the glucosamine to C conversion factor, which suggests a rather low precision in the conversion. However, it is unknown if the variation in glucosamine content is smaller or larger when in symbiosis. In the one published study known to us, extramatrical mycelium of *Paxillus involutus* in symbiosis with *Pinus sylvestris* had a glucosamine content of 4.5% (Ekblad et al., 1998). A similar value was found in mycelium extracted from mycelial ingrowth bags that were installed in the upper-most soil horizon at the tree line in a *Larix decidua* and *Pinus uncinata* stand near Davos. These two values are close to the average for the pure cultures of the Joergensen and Wichern (2008) review.

It is also possible to measure the chitin content from the pellet left after protein, lipid or DNA extraction (Kjøller and Rosendahl, 1996; Kjøller et al., 2012). This then allows measurements of enzyme activities or molecular identity in the exact same samples that are quantified for chitin. Further studies are needed on chitin concentrations in EMM of mycorrhizal fungi when in association with roots in forest soil. However, this fungal biomarker does not enable us to distinguish between saprotrophic and ECM fungi in soil samples or to separate living and dead mycelium, although this may be possible by combining ergosterol and chitin analysis (see below).

Chitin assay is easily performed using one of two steps; (i) hydrolysis either with KOH (e.g. Frey et al., 1994) that produces deacylated chitin (chitosan), or with HCl (Appuhn et al., 2004), $H_2SO_4$ (e.g. Zamani et al., 2008) or methanesulfonic acid (Olk et al., 2008) that produces glucosamine, and (ii) measurement of the concentrations of hydrolysis products. Chitosan and glucosamine contents can be measured with colorimetric procedures specifically assaying amino sugars (Plassard et al., 1982). Free glucosamine can also be measured with chromatographic techniques (Ekblad et al., 1998).

### 3.3. Chemical markers; ergosterol

The second chemical marker that has been used to estimate fungal biomass is ergosterol (22E)-Ergosta-5,7,22-trien-3β-ol ($C_{28}H_{44}O$). This compound is a membrane lipid, found almost exclusively in membranes of living fungal cells, and is the commonest sterol of Ascomycota and Basidiomycota. As ergosterol is generally not synthesized by plants and animals, and only present in low amounts in some microalgae (Grant and West, 1986; Newell et al., 1987; Weete, 1989), it has been frequently used as fungal biomarker in soils (Djajakirana et al., 1996; Möttönen et al., 1999; Bååth, 2001; Wallander et al., 2001; Hagerberg et al., 2003; Zhao et al., 2005; Högberg, 2006; Karliński et al., 2010) and correlations with other methods are usually good (Bermingham et al., 1995; Stahl and Parkin, 1996; Montgomery et al., 2000; Ruzicka et al., 2000; Högberg, 2006). Assay of ergosterol was first employed by Seitz et al. (1977) to quantify fungal infections in stored grain. In mycorrhizal fungi, the analysis of ergosterol was first applied by Salmanowicz and Nylund (1988), but has been used frequently since then (e.g. Nylund and Wallander, 1992; Ekblad et al., 1995, 1998; Laczko et al., 2004; Olsrud et al., 2007). Total ergosterol contents in mycorrhizal roots of *P. sylvestris* plants was correlated to visual estimates of root colonization (Ekblad et al., 1995) as well as to the chitin contents (Ekblad et al., 1998). In contrast, total ergosterol concentration of ericoid hair roots of dwarf shrubs from northern subarctic mires did not correlate with visual estimates of colonization but was instead positively correlated with the colonization of dark septate endophytes, which makes it questionable as a marker for ericoid mycorrhizal fungal colonization (Olsrud et al., 2007). Some studies suggest that ergosterol is a good proxy for active fungal biomass because it was found to degrade shortly after the cells death (Nylund and Wallander, 1992), and ageing mycorrhizal root tips contain low ergosterol concentrations (Ekblad et al., 1998). However, other studies suggest a slow metabolism of ergosterol under certain circumstances, such as disruption of below ground C allocation, increased N loads, addition of toxic compounds like pesticides, or existence of substantial amounts of free ergosterol in soil for considerable periods with little mineralization (Zhao et al., 2005). Soil perturbations, that may negatively influence vitality and growth of soil fungi, resulted in disruption of the proportion between soil ergosterol concentration and soil fungal biomass C (Zhao et al., 2005) and between ergosterol and phospholipid fatty acid (PLFA) 18:2ω6,9 (Högberg, 2006). These contradictory results were further criticized and discussed by Young et al. (2006) and Zhao et al. (2006). Mille-Lindblom et al. (2004) reported very slow degradation of free ergosterol in environmental samples without living mycelium when protected from sunlight and suggested that ergosterol may be stable when connected to dead fungal mycelium.

However, significant degradation of ergosterol was observed by the authors under influence of light.

Calculations of conversion factors from ergosterol to fungal biomass have been derived from various fungi and considerable variations in ergosterol concentration in fungal mycelium were reported (Lösel, 1988; Weete, 1989; Nylund and Wallander, 1992; Djajakirana et al., 1996; Montgomery et al., 2000). The average concentration of ergosterol reported thus far for different soil, aquatic and plant inhabiting fungi is 4.5 μg $mg^{-1}$ dry mass of mycelia, and this is used to determine fungal biomass in soil. However, the ergosterol concentration in fungal mycelium extracted from mesh bags is less (1.2 μg $mg^{-1}$; Hagerberg et al., 2003). This may indicate that laboratory-grown mycelia contain more ergosterol than field grown mycelia, or that mycelia from mesh bags are contaminated with non-fungal material. The average relative recovery of ergosterol from soil samples was 62%, ranging from 58 to 88% (Montgomery et al., 2000), and the recovery factor value was 1.61 (1/0.62). The authors concluded that determination of fungal biomass (*FB*) on the basis of ergosterol analysis requires correcting ergosterol concentrations by the proportion of unextracted mycelial ergosterol according to the following calculation:

$$FB\left(\mu g\ g^{-1}\ soil\right) = \text{Ergosterol}\left(\mu g\ g^{-1}\ soil\right) \times f \times Rf,$$

where $f = 250$ (1/4 × 1000, mg biomass $\mu g^{-1}$ ergosterol), and $Rf = 1.61$ (correction factor for average percent recovery, 1/0.62) (Montgomery et al., 2000).

Separation of ergosterol into free and esterified forms might give some additional information of the vitality of the fungal mycelium. Usually total ergosterol is quantified (Nylund and Wallander, 1992), in other cases the free form is used as a biomass marker (Martin et al., 1990). Free ergosterol is a component of the cell membranes, while the esters are found in cytosolic lipid particles. A $^{14}C$-labelling study of *Saccharomyces cerevisiae* indicated that the free sterols and esters are freely inter-changeable and that relatively more esters are formed when the fungus is going into a stationary phase (Taylor and Parks, 1978). Analysis of dried fungal material suggests that the free form can also be converted into the esterified form in this material and that the esterified is more stable than the free form (Yuan et al., 2008). The majority of ergosterol from in-growth bags was found in the free form (90%), while the free ergosterol was below 20% in the mineral soil, supporting the view of increasing proportion of esterified ergosterol in older SOM (Wallander et al., 2010). The relation between free and esterified ergosterol and ergosterol and chitin (Ekblad et al., 1998) could potentially be used as markers for the ratio of active and inactive fungi in soil. This possibility would be very useful but needs to be evaluated further. One problem with analysing free ergosterol in certain soils is to get the extracts clean for chromatographic analysis (Adam Bahr, pers. comm.). Ergosterol can be easily extracted from variable materials and is detectable in low concentrations. The assay comprises of extraction, purification and quantification of the molecule using high-performance liquid chromatography with a UV detector. Young (1995) developed an efficient microwave-assisted method (MAE) to extract ergosterol from a variety of matrices, which has since been applied to soil samples (Montgomery et al., 2000).

### 3.4. Chemical markers; PLFAs

PLFAs are essential components of cell membranes and they decompose quickly after cell death (White et al., 1979) and are commonly used as chemical markers of soil fungi. As eukaryotes and different groups of prokaryotes contain more or less specific ester-linked lipid fatty acids (Lechevalier and Lechevalier, 1988; Zelles, 1997, 1999), the analysis of PLFA composition and concentrations are useful as a tool for quantitative and qualitative examination of microbial communities in soil (fungi, bacteria, protozoa; e.g. Tunlid and White, 1992; Cavigelli et al., 1995). However, use of PLFAs for biomass estimation has recently been questioned, because the same PLFAs are stated to indicate very different groups of organism (Frostegård et al., 2011). For instance, the PLFAs cy17:0 and cy19:0, usually considered to be indicators of Gram-negative bacteria are also found in large amounts in some Gram-positive bacteria (Schoug et al., 2008). The PLFA 16:1ω5, common in arbuscular mycorrhizal fungi (Graham et al., 1995; Olsson et al., 1995), and sometimes used as a marker of Glomeromycota fungi in soil, plant roots and external mycelium (e.g. Gryndler et al., 2006), is also found in bacteria (Nichols et al., 1986). Moreover, some environmental conditions, such as temperature or toxic soil contaminants may influence the rate of PLFA degradation, independently with the turnover of soil microorganisms (Frostegård et al., 2011).

The PLFA 18:2ω6,9 is the most commonly used PLFA to estimate fungal biomass (Wassef, 1977; Lechevalier and Lechevalier, 1988; Dembitsky et al., 1992). It occurs in all eukaryotes, and is only found in low amounts in bacteria. This PLFA is a dominating fatty acid of fungal fruit bodies (e.g. Dembitsky et al., 1992; Olsson, 1999; Karliński et al., 2007) and spores (Brondz et al., 2004). A strong positive correlation was found between PLFA 18:2ω6,9 and the fungal marker ergosterol in soils from cultivated fields, gardens, grasslands and forests (Frostegård and Bååth, 1996; Kaiser et al., 2010). The PLFA 18:2ω6,9 has been used as a bioindicator of EMM in soil (Högberg et al., 2010), but it is particularly useful in experiments where other soil fungi can be eliminated or reduced, such as when using in-grow mesh bags where ECM mycelium is preferentially trapped (e.g. Wallander et al., 2001; Hagerberg and Wallander, 2002). To convert PLFA 18:2ω6,9 to microbial carbon content, Joergensen and Wichern (2008) reported a weighted conversion factor of 107 μg C nmol $PLFA^{-1}$, but values between different species grown in culture could vary 17-fold (Klamer and Bååth, 2004). Another PLFA that is common in fungi, especially Zygomycota, is 18:1ω9 (Dembitsky et al., 1992; Ruess et al., 2002; Brondz et al., 2004). The concentration of 18:1ω9 is usually closely correlated to 18:2ω6,9 (Frostegård et al., 2011). This PLFA is, however, also present in some bacteria (Schoug et al., 2008) and has not proven useful as a fungal indicator in agricultural soils (Frostegård et al., 2011).

A faster way to analyse fatty acids in soil samples is to analyse the whole cell fatty acids (WCFAs) without separation of neutral lipid fatty acids (NLFAs) and PLFAs. WCFAs reflect both microbial biomass and energy reserves of eukaryotes and are a relatively reliable method of studying fungi (Larsen et al., 2000; Thygesen et al., 2004; Karliński et al., 2007) and mycorrhiza-associated microorganisms in the field (Brondz et al., 2004; Ruess et al., 2005; Karliński et al., 2007). The analysis of WCFA composition requires 10 times less soil material than the PLFA analysis (Drenovsky et al., 2004). Since much of the WCFA is in the form of neutral lipid fatty acids (NLFAs) in triacylglycerols, a storage compound in eukaryotes, a recorded change in WCFA of NLFAs may be a result of changes in the amount of storage C rather than a change in size of the microbial population in a soil. Incorporation of glucose into fatty acids can be used to demonstrate the high microbial activity in soils. Lundberg et al. (2001) used 'solution state' $^{13}C$ NMR and found that the amount of $^{13}C$ in fatty acids peaked 3–13 days after glucose addition to a forest soil, and that it had declined by 60% 28 days after the glucose addition. A similar result was found after extraction and analyses of NLFAs and PLFAs at different time intervals after glucose additions to various soils

(Bååth, 2003). Due to the potential for large temporal variation in storage triacylglycerols, NLFAs and WCFAs are probably less suitable than PLFAs as relative measures of the microbial biomass in soils. However, the ratio of neutral lipid fatty acids (NLFAs) and PLFAs was proposed as a method to study the physiological state of the microbial population in the soil (Bååth, 2003).

The analytical procedure for PLFAs and NLFAs comprises four steps: (i) extraction of lipids, (ii) lipid fractionation, (iii) mild alkaline methanolysis, and (iv) GC analyses (White et al., 1979; Frostegård et al., 1991). Recently, the lipid fractionation was modified slightly by Dickson et al. (2009), who reported that the replacement of pure chloroform by the mixture chloroform: acetic acid (100:1, v/v) increased the effectiveness of NLFAs elution from the silica columns and eliminated an interference of NLFAs with glycolipid and phospholipid fractions. Following hydrolysis, their fatty acids (FA) are released and detected using gas chromatography (GC). PLFA analyses should be done as soon as possible after sampling since the composition may change even when stored at low temperatures (Wu et al., 2009). The best strategy is to shock-freeze the samples with liquid nitrogen and further storage at −18 °C until analysis. Homogenization of soil samples using a ball mill to a particle size less than 10 μm prior to analysis has been recommended to achieve the most reliable results (Wilkinson et al., 2002).

### 3.5. Comparison of chemical markers

It is clear that each of the chemical markers described will bring different information about the fungal biomass, whether total or active. Each of them has advantages and limitations (Tables 1–3). Chitin and ergosterol assays are easier to carry-out than fatty acid (PLFAs or WCFAs) extraction, but fatty acid profiles will bring more information about microbial communities than chitin and ergosterol. On the other hand, the PLFA method is more rapid and less expensive than methods based on nucleic acids (Ramsey et al., 2006; Frostegård et al., 2011). However, none of these chemical markers will enable us to distinguish between fungal types (ECM versus non-mycorrhizal fungi) that can be present in forest soil samples. To distinguish between these "functional" types, molecular analysis (see below) should be used. Biomass estimates when using biomarkers, such as ergosterol, chitin and PLFAs, are highly dependent on the use of conversion factors. Different fungal species vary in concentrations of such biomarkers, but the biomarker to biomass ratio is probably more stable in a more complex community. The concentration of ergosterol in pure cultures of ECM fungi ranged between 1.8 and 17.6 mg $g^{-1}$ d.wt. (Nylund and Wallander, 1992; Olsson et al., 1995) and concentration of PLFA 18:2ω6,9 ranged from 0.45 to 12 μmol $g^{-1}$ d.wt. (Olsson et al., 2003). The content of the WCFA 18:2ω6,9 was reported as 17–75% of total WCFAs in fruit bodies and as 53–71% of total WCFAs in axenic cultures of ECM fungi (Karliński et al., 2007). Biomarker concentrations may reflect both the biomass and community composition of fungi. In addition, concentrations in a single species can change due to different environmental conditions as were reported for wood-rotting basidiomycete isolates grown in different soils (Tornberg et al., 2003) and ageing, as shown for ergosterol concentrations in the basidiomycete *Hebeloma cylindrosporum* (Plassard et al., 2000), and for ergosterol and fatty acids in pure culture of ECM fungus *Pisolithus tinctorius* (Laczko et al., 2004).

### 3.6. Potential of qPCR for the quantification of EMM biomass

In addition to the lipidic or polysaccharidic markers to quantify the biomass of fungi, the developments of quantitative PCR (qPCR) seem to offer a possible taxon-based alternative. The strength of DNA (or RNA) based methods is that potentially any phylogenetic level from genotypes to large groups or even total (true) fungi can be targeted (Fierer et al., 2005; Šnajdr et al., 2011). Indeed, methods to quantify general fungi or basidiomycetes have been proposed and tested (Fierer et al., 2005; Manter and Vivanco, 2007; Feinstein et al., 2009). A single species laboratory study comparing quantification of *Trametes versicolor* in wood based on chitin content, ergosterol, wood mass loss, and qPCR, showed reasonable correlations with more discrepancies occurring only with older cultures (Eikenes et al., 2005). There are currently two main limitations of the methodology: nucleic acid extraction bias and the differences in target occurrences per unit DNA or biomass. Different methods of nucleic acid extraction yield not only different quality of DNA and RNA but also different proportions of microbial taxa in the extracts (Feinstein et al., 2009). The success of qPCR rapidly decreases with fragmentation of nucleic acids, resulting in lower counts of target sequences per unit DNA. If a treatment is imposed that alters the extractability of nucleic acid or if different soil types are to be compared, this may influence the qPCR success. For the most frequently used target sequence of fungi-specific qPCR – the rDNA cassette – significant differences in copy number per genome were recorded, ranging from 10 to 200 in different species (Garber et al., 1988; Maleszka and Clark-Walker, 1990; Corradi et al., 2007; Amend et al., 2010), which adds another important source of bias. With the advance of fungal population genomics (five ECM species sequenced to date; see the website of JGI (http://genome.jgi-psf.org/) and Martin et al., 2008, 2010) in the future it may be possible to identify a universal single copy gene with adequate sequence variation for counting fungal genomes rather than rDNA copies or for delimitation of certain fungal taxa. Population genomics also brings even greater potential to test hypotheses concerning the contribution of particular genotypes to ECM fungal biomass and turnover (Johnson et al., 2012).

When qPCR specifically targets individual species of fungi, PCR-based abundance estimates represent a plausible proxy of fungal biomass content because the numbers of rDNA copies do not show high variation within a species (Amend et al., 2010). Analyses of individual fungi including *Suillus bovinus*, *P. involutus* and *Hypholoma fasciculare* in the DNA from complex samples showed that it is possible to use qPCR to specifically quantify the biomass of fungi at the species level within a community. Such data are comparable to the much more laborious or expensive approaches like cloning, pyrosequencing or DGGE approaches (Landeweert et al., 2003; Parladé et al., 2007; Šnajdr et al., 2011). Competitive PCR (a variant of qPCR) was used to demonstrate that *Hebeloma cylindrosporium* biomass in bulk soil is greatest near fruit bodies (Guidot et al., 2002). A conversion factor between qPCR-based copy number and fungal biomass and hyphal length was obtained for laboratory cultures of the ECM fungus *Piloderma croceum* showing its potential to quantify the biomass of particular species (Raidl et al., 2005). Unfortunately, due to the appearance of ECM fungi in multiple phylogenetic lineages, the finding of suitable primers to specifically amplify ECM fungal DNA and to distinguish it from non-ECM fungi is highly improbable. However, if combined with the cloning approaches or next generation sequencing, qPCR may provide estimates of ECM fungal biomass in soils. Contemporary next generation sequencing results showed that, at least in certain forest soils, fungal communities are dominated by relatively few species (Buée et al., 2009; Baldrian et al., 2012). These findings suggest that qPCR can be used to target specifically the identified dominant members of the community as an estimate of ECM fungal biomass. Recently, qPCR used for analysis of environmental samples has been expanded from the quantification of DNA towards the quantification of RNA, typically the rRNA, representing microbial ribosomes or ITS sequences in unspliced transcripts of the rDNA

operon. Although it is unknown whether the DNA or the RNA content better corresponds with the quantity of fungal biomass, it is clear that the analysis of ITS sequences in the non-spliced rDNA transcripts (indicating fungal taxa synthesizing their ribosomes) is more suitable to quantify the active part of the fungal community (Anderson and Parkin, 2007). Indeed, decomposers in spruce logs or fungi active in soil in winter with limited photosynthate allocation have been specifically identified by combining DNA and RNA analysis (Rajala et al., 2011; Baldrian et al., 2012).

## 4. Indirect estimation of length, space occupation and biomass of extramatrical mycelium of ectomycorrhizal fungi

Agerer (2001) proposed a classification of ECM mycelial systems into five exploration types. Accurate determination of EMM production and abundance of different exploration types within ECM communities may be used to estimate the overall production of EMM. The exploration types are described according to their pattern of differentiation, indicating their different ecology: contact type (CT), short distance (SD), medium distance (MD), long distance (LD) and pick-a-back (PB) exploration type. The exploration types have been differentiated based on about 400 different morphotypes of ectomycorrhiza, which have been identified as belonging to different fungal species on several host plant roots based on their morphological and anatomical characteristics (Agerer and Rambold, 2004–2011). The characterized ECM morphotypes represent about 5% of known fungi that can form ectomycorrhiza (Taylor and Alexander, 2005), the number of which is estimated to be 5000–6000 fungal species (Agerer, 2006). From this limited database, it appears that in many genera all known species produce only one exploration type (Agerer, 2001; Hobbie and Agerer, 2010), although some genera (i.e. *Russula* spp.) need species-based classification into an exploration type (Table 4).

An estimation of EMM of ECM fungi in natural soils could be deduced from semi-quantitative estimations of the EMM formed by SD and MD exploration types grown in rhizotrons in symbiosis with Norway spruce (Agerer and Raidl, 2004). The observations in rhizotrons have lately included other MD subtypes and LD exploration types (Weigt et al., 2011), and indices of specific space occupation, mycelial length and biomass were proposed for each exploration type. Mycelial biomass was estimated based on length measurements using calculations described in Weigt et al. (2011); the standard values for the selected exploration types are presented in Table 5. These standards for the most frequent exploration types, expressed as biomass and occupied space of EMM per unit of ECM system, are suggested as basic factors for characterizing mycelial production costs and space occupation in ecological field studies without any extraction of mycelium and for fungal communities in the soils. Since different exploration types show not only differences in distance of EMM from the root tip, space occupation, biomass and energy (C) inputs, but also in other functional relationships within the ecosystem, the ECM fungal community structure and function can be extrapolated. The differentiation into exploration types can be extrapolated from morphotype characterization based on outer morphology of ectomycorrhiza, rhizotron photographs, and fungal species identifications, using molecular based methods of fungal community composition (Grebenc and Kraigher, 2009), in which fungal species identity is linked to growth characteristics and assigned to a certain exploration type.

Indices, such as specific potential mycelial space occupation ($mm^2\ cm^{-1}$ ECM $tip^{-1}$), specific EMM length (m $cm^{-1}$ ECM $tip^{-1}$), specific EMM biomass (μg $cm^{-1}$ ECM $tip^{-1}$) can be developed for each exploration type. The specific contribution to EMM by exploration types can be achieved for cultivable and non-cultivable species, and up-scaling of cost–benefit relations is possible (Weigt et al., 2011). The method provides an estimation based on ECM fungi synthesized in experimental laboratory conditions, i.e. on prepared soil substrates, which can influence EMM growth in different exploration types. Therefore, for the calculations presented in Table 5 a number of assumptions had to be made, including i) that growth conditions concerning mycelial growth and space occupation in experimental substrates was similar to

**Table 4**
Representative fungal genera belonging to different exploration types (summarized from Agerer, 2001; Agerer and Rambold, 2004–2011; Agerer, 2006).

| Exploration type | Morphology/anatomy | Fungal genus[a] |
|---|---|---|
| Contact | Smooth mantle, only few emanating hyphae, ECM tips in close contact with substrates | *Arcangeliella*, *Balsamia*, *Chroogomphus*, *Craterellus*,[b] *Lactarius*,[c] *Leucangium*, *Russula*, *Tomentella* |
| Short distance | Voluminous envelope of emanating hyphae, no rhizomorphs | *Acephala*, *Byssocorticium*, *Cenococcum*, *Coltricia*, *Coltriciella*, *Craterellus*,[b] *Descolea*, *Descomycetes*, *Elaphomyces*, *Genea*, *Hebeloma*, *Humaria*, *Hygrophorus*, *Inocybe*, *Pseudotomentella*, *Rhodocollybia*, *Rozites*, *Russula*, *Sebacina*, *Sphaerosporella*, *Sphaerozone*, *Tomentella*, *Tricharina*, *Tuber*, *Tylospora* |
| Medium distance: fringe subtype | Fans of emanating hyphae and rhizomorphs, frequent ramifications and anastomoses, rhizomorph surfaces hairy, extended contact to the soil; rhizomorphs type A,[e] exceptionally C,[e] D[e] | *Amphinema*, *Cortinarius*, *Dermocybe*, *Hydnum*, *Lyophyllum*, *Piloderma*, *Sistotrema*, *Stephanopus*, *Thaxterogaster*, *Tricholoma* |
| Medium distance: mat subtype | Limited range, rhizomorphs undifferentiated or slightly differentiated type A,[e] C,[e] exceptionally D[e] | *Bankera*, *Boletopsis*, *Clavariadelphus*, *Cortinarius*, *Gautieria*, *Geastrum*, *Gomphus*, *Hydnellum*, *Hysterangium*, *Phellodon*, *Ramaria*, *Sarcodon* |
| Medium distance: smooth subtype | Rhizomorphs internally undifferentiated, slightly differentiated or with a central core of thick hyphae. Mantles smooth with no or only a few emanating hyphae. Rhizomorphs type B,[e] C[e] and D,[e] exceptionally E[e] | *Albatrellus*, *Amanita*,[d] *Byssoporia*, *Cantharellus*, *Entoloma*, *Gomphidius*, *Hygrophorus*, *Laccaria*, *Lactarius*, *Naucoria*, *Polyporoletus*, *Pseudotomentella*, *Russula*, *Thelephora*, *Tomentella*, *Tomentellopsis* |
| Long distance | Smooth mantle with few but highly differentiated rhizomorphs type F.[e] ECM sparsely monopodially branched, coralloid and tuberculate. | *Alpova*, *Amanita*,[d] *Austropaxillus*, *Boletinus*, *Boletus*, *Chamonixia*, *Gyrodon*, *Gyroporus*, *Leccinum*, *Melanogaster*, *Paxillus*, *Pisolithus*, *Porphyrellus*, *Rhizopogon*, *Scleroderma*, *Suillus*, *Truncocolumella*, *Tricholoma*, *Tylopilus*, *Xerocomus* |
| Pick-a-back | Grow within F[e]-type rhizomorphs or mantels, can produce haustoria, can become ectendomycorrhizal. Can form contact, or smooth medium distance type. | *Gomphidiaceae* (*Gomphidius*, *Chroogomphus*) growing within *Suillus* or *Rhizopogon*; *Boletopsis leucomelaena* within unknown ECM; *Xerocomus parasiticus* within *Scleroderma citrinum* |

[a] In case of controversial issues genus was categorized to exploration types according to Agerer and Rambold (2004–2011).
[b] *Craterellus tubaeformis* forms contact exploration types on *Quercus* but short distance exploration types on *Pinus*.
[c] Underlined genera have representatives in more than one exploration types.
[d] *Amanita citrina* on *Pinus* can form medium distance and long distance exploration types.
[e] The type of rhizomorphs according to Agerer (1987–1998).

**Table 5**
Characteristics of EMM length, space occupation and biomass for different exploration types (modified after Weigt et al., 2012a,b).

| Exploration type | No. of analysed mycelia | Max. distance from root tip (cm) | Projected area per mycelial system ($mm^2$) | Mycelial coverage per occupied space ($mm^2\ mm^{-2}$) | Specific EMM length ($m\ cm^{-1}$ ECM $tip^{-1}$) | Specific EMM biomass[a] ($\mu g\ cm^{-1}$ ECM $tip^{-1}$) |
|---|---|---|---|---|---|---|
| Short distance | 7 | 1.2 | 33 ± 9 | 0.39 ± 0.07 | 3.72 ± 1.19 | 3.24 ± 1.03 |
| Medium distance | 14 | 1.9 | 84 ± 5 | 0.58 ± 0.05 | 6.91 ± 0.54 | 6.02 ± 0.47 |
| Long distance | 3 | 9.6 | 630 ± 181 | 0.28 ± 0.03 | 55.91 ± 20.25 | 48.67 ± 17.62 |

[a] Mycelial biomass was estimated based on length measurements using calculations described in Weigt et al. (2011) combining the formula $B = r^2\pi^*L^*D^*M$ (Frankland et al., 1978), where $B$ = fungal biomass, $r$ = hyphal radius, $L$ = hyphal length, $D$ = relative hyphal density, $M$ = % dry mass = (100 − mycelial moisture content as % of fresh weight)/100. $r^2\pi^*L$ = hyphal biovolume (assuming hyphae to be perfect cylinders) with $r$ based on the species-specific hyphal diameter (in their study it was 2.2 μm for *Piloderma croceum*, deduced from Brand, 1991; Raidl, 1997). $L$ was measured using WinRhizo. $D$ = 1.09 $g/cm^{-3}$ and $M$ = 21% following (Bakken and Olsen, 1983) conversion of hyphal volume into biomass with $D^*M$ = 0.2289 g dry mass $cm^{-3}$.

natural soils, ii) no competition or facilitation among mycelia of different fungi has been included, and iii) no site-related growth conditions have been addressed, and several calculation-based assumptions had to be defined (see the explanation at Table 5). However, the proposed exploration type specific standard values may provide a suitable tool for quantification of space occupation, biomass and energy trade-offs of EMM in natural soils. A combination of a further development of the database with descriptions of ECM fungi (Agerer and Rambold, 2004–2011) and functional relationships of different exploration types, grown, observed and assessed in different growth conditions, will contribute to an increasing understanding of the complex belowground mycelial interactions, cost–benefit relations and trade-offs in belowground competition or facilitation.

## 5. Assessment of turnover rates

Accurate estimates of the turnover of EMM are essential in order to evaluate the role of mycorrhizal fungi in the C cycle. This requires understanding of both the rate of production and decomposition of mycorrhizal mycelium. Sequential harvesting of EMM in mesh bags may be one way to estimate turnover rates, but there seems to be a lag-phase before EMM enter the mesh bags (Fig. 1). However, the lag-phase is probably dependent on the level of disturbance caused by the installation and further tests with bags of different sizes and sampling frequency are needed to evaluate the applicability of this method. Pulse labelling with $^{13}C$ or $^{14}C$ has been applied to estimate turnover in arbuscular mycorrhizal mycelium (Staddon et al., 2003; Olsson and Johnson, 2005) but not for estimates of ECM mycelium. One of the problems with isotope techniques is the risk of differences in labelling of different chemical components of the fungal biomass, some of them having a high turnover rate (respiratory substrates), while the heavy isotope will have longer residence time in structural cell materials (Dawson et al., 2002). Analyses of the turnover rate of specific components, such as chitin, may be a way to overcome this. Another issue concerns the collection of sufficient and representative amounts of ECM mycelium for isotopic analysis.

The difference in natural abundance $\delta^{13}C$ between $C_3$ and $C_4$ organic matter has been used in studies of the turnover of plant and microbial substances in soils. In these analyses a combination of pyrolysis–gas chromatography and isotope ratio mass spectrometry was used (Gleixner et al., 1999, 2002). The combination of ingrowth cores with a mesh allowing only hyphae or allowing both roots and hyphae filled with $C_4$ dominated soils have been used to estimate the contribution of mycelia and roots to the formation of stable SOM (Godbold et al., 2006; Wallander et al., 2011). A critical factor when using differences in natural abundance of $^{13}C$ is to have reliable $\delta^{13}C$ values of the end-members, e.g. mycelial and plant residues. Similar to pulse labelling techniques, differences in isotopic signature between various components within the plant and fungal materials may be a potential problem that should be considered. For example, chitin is depleted in both $^{13}C$ and $^{15}N$ compared to the total fungal biomass (Dijkstra et al., 2006). The EMM in the top soil was depleted in $^{15}N$ with around 5‰ compared to mycorrhizal fruit bodies in a Norway spruce site (Wallander et al., 2004), possibly reflecting lower chitin and higher protein contents in fruit bodies compared to mycelia.

We are not aware of any study that has exploited the possibility to estimate the production and turnover of mycelium in FACE-experiments (Free Air Carbon dioxide Enrichment). Sequential installation and harvest of in-growth bags in connection with the initiation or termination of $CO_2$ treatments should offer ideal periods to estimate the production and turnover of mycelia biomass. During these periods there are drastic shifts in the $^{13}C$ signal of the photosynthates (given that the $CO_2$ that is used to treat the plants has a different $\delta^{13}C$ than the atmosphere, which is the case if fossil C has been used to produce the $CO_2$). However, these experiments do not have corresponding plots that are isotopically-enriched at ambient $CO_2$ concentrations, and so exploiting FACE facilities would only be useful to estimate turnover under elevated $CO_2$ conditions.

In contrast to other types of organic inputs to soils, surprisingly little is known about the decay rate of mycorrhizal mycelium. Mesh bags of the type normally used to assess leaf litter decomposition have been used recently to demonstrate that the N concentration of hyphae explained a large part of the mass loss during the initial 4 weeks of decay (Koide and Malcolm, 2009). An alternative method is to capture and quantify $CO_2$ produced when hyphae are added to micro-respirometers. This approach was used to show that ECM fungal hyphae rapidly stimulated $CO_2$ efflux but that the effect was dependent on the species richness of the hyphae entering soil (Wilkinson et al., 2011a). Thus species richness of ECM fungi can be important both for maintaining productivity (Wilkinson et al., 2011b) and in regulating their own decomposition. These findings indicate that decomposition of ECM hyphae may be a key pathway by which C rapidly enters the saprotrophical microbial biomass in soil. The application of stable isotope probing (Radajewski et al., 2000) in which ECM fungal hyphae is enriched in $^{13}C$ has recently been used to demonstrate the rapidity of C incorporation into free-living soil fungi via this pathway (Drigo et al., 2012). Despite these recent advances, there is scope for considerably more research quantifying the rate of decay of different genotypes, species and morphologies of ECM fungal hyphae under a range of environmental conditions.

## 6. Importance of sampling design

Regardless of the effort placed in developing reliable methods to quantify production, biomass and turnover of ECM fungi, the utility of the resulting data is often dependent on the sampling design used to obtain the data in the first place. Moreover, it is often desirable to obtain similar datasets from a wide-range of different

ecosystems and habitats, particularly from a modelling perspective. This requires sampling approaches that have similar ability to quantify spatial variation in EMM abundance and biomass. Yet very few investigations employ spatially-explicit sampling strategies designed to deal with the often vast heterogeneity of EMM production in forest systems. This is in part because variation is likely to occur at a wide range of spatial scales; recent work in Douglas fir stands have demonstrated that genets of *Rhizopogon* spp. could form common mycelial networks connecting individual trees within a 30 × 30 m area (Beiler et al., 2010). In contrast, there is also clear evidence that ectomycorrhizas and their associated mycelium can form patchy clusters at scales of just a few cm (Guidot et al., 2002), perhaps due to their plasticity in responding to inputs of nutrient-rich substrates (Bending and Read, 1995). Moreover, spatial variation occurs in three dimensions. Only rarely is quantification of abundance and biomass of either ectomycorrhizal roots or EMM undertaken at multiple depths. Among surface soil horizons in a Swedish boreal forest, ECM fungi tended to be associated with slightly older partially-decomposed organic matter (Lindahl et al., 2007). In the UK, detailed analyses of the vertical distribution of 7 species of ectomycorrhizas and their EMM in a Scots pine stand showed contrasting vertical distribution patterns from 0 to 20 cm (Genney et al., 2006). The EMM of some species like *Cadophora finlandia* was distributed quite evenly with depth while the EMM of *Cortinarius* spp. was concentrated in the upper 10 cm (Genney et al., 2006). This study also demonstrated unequal distribution of the EMM of many species at 2 cm intervals. Geostatistical techniques (Legendre and Legendre, 1998) have recently been applied to provide rigorous analysis of the temporal and spatial patterns of ectomycorrhizas (Lilleskov et al., 2004; Pickles, 2007). For example, Pickles (2007) sampled 48 cores at increasing distances in a 20 × 20 m area to determine when the abundance of key common species showed spatial autocorrelation. Subsequent more intense sampling events (217 cores) in the same location exploited this information and used regular distances of either 1 or 2 m as the primary separation distance to avoid issues with spatial autocorrelation, and to provide detailed interpolated maps of species' abundance (Pickles et al., 2010). The use of geostatistical tools therefore requires an initial high investment in sampling units, but can reap benefits later once optimum sampling distances are identified. Moreover, obtaining data on spatial autocorrelation enables more meaningful inter-site comparisons and so this is an approach we advocate in future studies.

## 7. Conclusions

Although significant progress has been made over the last ten years in our understanding of the importance of the ECM fungal mycelium in C cycling in ecosystems, our understanding is still highly fragmented. In this paper we have summarized the state of the art in this subject as well as the strengths and weaknesses in the methods and techniques applied. Our aim is that this information will ultimately enable researchers to obtain valuable data on the production, biomass and turnover of mycorrhizal mycelium in all biomes, and modify the approaches outlined here for arbuscular and ericoid mycorrhizal systems. Such data are likely to be essential for improving process-based models of terrestrial biogeochemical cycles that currently ignore the distinct role played by mycorrhizal fungi. This may improve their potential to predict nutrient leaching and carbon sequestration. Moreover, these data could also be incorporated into spatially-explicit modelling frameworks of population dynamics.

All of the applied methods and techniques have their own sets of limitations which the users of these methods should consider before applying them (Tables 1–3). To combine several techniques in the same study, e.g. chemical markers and isotope labelling, may be a way to overcome some of these limitations. An issue that needs more attention is the turnover of EMM, especially the turnover of diffuse mycelium versus rhizomorphs. The ratio between free and total ergosterol, and the ratio between chitin and ergosterol as an indicator of the necromass/biomass ratio may be useful in such experiments and deserves further studies. Also, it could be useful to develop methods enabling us to quantify specifically the level of $^{13}C$ enrichment of C in glucosamine residues. Combined to environmental variation of carbon sources available to the ECM fungi (e.g. in FACE experiment using enriched or depleted $^{13}C–CO_2$ sources), such a method could fill the gap regarding the actual rate the turnover of ECM fungi in forest soils.

Indices, such as specific EMM length or specific EMM biomass, developed for different exploration types, can be used for indirect estimations of the C costs of growth and storage in ECM fungal mycelium. The utility of such indirect measures are greatest providing the ECM fungal community structure is known, that the identified species belong to different exploration types, and these show different space occupation, mycelial length and biomass.

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