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Fungal and bacterial contributions to decomposition in terrestrial and aquatic ecosystems

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Fungal and bacterial contributions to decomposition in terrestrial and aquatic ecosystems

Fungal and bacterial contributions to decomposition in terrestrial and aquatic ecosystems

Margarida Soares



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DOCTORAL DISSERTATION

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the 24th May 2019 at 09.30.

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Abstract Microbial decomposers process the majority of net primary production in the biosphere and thereby regulate carbon (C) and nutrient cycling. Microbial communities are extremely diverse and often not explicitly represented in global C-cycling models, but one strategy to overcome this challenge is to focus on the major decomposer groups: fungi and bacteria. These groups have distinct life strategies and are differently affected by environmental conditions. During microbial decomposition of organic matter (OM), the fraction of C assimilated into microbial growth or used in cell maintenance and respiration is defined as microbial carbon-use efficiency (CUE). Since soils represent a large C pool with a critical role in regulating atmospheric carbon dioxide concentrations, CUE is a key parameter central to understanding the soil C-cycling and its feedback to environmental change. In this thesis I compared the bacterial and fungal contributions to decomposition by developing conversion factors to measure microbial growth in units of C. I estimated CUE and studied the influence of environmental factors on the fungal-to-bacterial ratio (F:B) and how that affected CUE. These approaches were applied to a survey of field sites and verified in laboratory microcosms. When all sites were compared high CUE was associated with low F:B ratios in high C:N ratio soils which could be an index of low fertility. However, within each field site higher CUE was associated with soils with higher fertility (high N). Using microcosms, higher CUE resulted from low F:B ratios in treatments with low mineral N and high pH, with no effect of OM quality. This indicated that CUE in our field survey was also regulated by a component of soil fertility other than mineral N, and I suggest that plant community traits such as litter and rhizosphere inputs may influence F:B ratio and CUE. I also investigated the effect of long and short term-stress on CUE in a subarctic region. CUE was unaffected by increasing heavy metal concentrations along a gradient of long-term contamination. Fungi were less affected by heavy metal pollution than bacteria but F:B and CUE were unrelated. In experimental microcosms I tested the effect of short-term stress with heavy metal additions both in soils previously exposed to stress and unexposed soils. CUE decreased only in unexposed soils, showing that in soils previously exposed to stress microbial decomposers had grown tolerant to heavy metals and CUE was unaffected. Differences between fungal and bacterial decomposition of plant litter in aquatic and terrestrial systems were explored in a boreal catchment forest site, where litter bags were installed in soils and adjacent streams along a pH gradient and resolved during 1.5 years. Fungal growth and litter leaching were higher in streams than in soils but overall mass loss was higher in soils. Litter decomposition was explored with IR spectroscopy and litter transformations in terms of chemical functional groups (carbohydrate and aromatic compound loss) were fundamentally different between systems. I investigated the priming effect - increased mineralization of native OM in response to an external labile C source- on biofilms associated with plant litter. In these systems the spatial proximity between photosynthetic algae and microbial decomposers allows for products of metabolisms to be exchanged. I found that labile C of photosynthetic algae origin did not affect the decomposition of plant litter in terms of mass loss, but increased the fungal removal of N from plant litter. In conclusion, microbial growth rates in C units and CUE can now be estimated in natural environments. This thesis provided a deeper understanding of the fungal and bacterial contributions to decomposition in different systems, and how F:B and CUE are regulated by environmental factors.			
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Fungal and bacterial contributions to decomposition in terrestrial and aquatic ecosystems

Margarida Soares



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MADE IN SWEDEN 

Aos meus pais Belinho e Carminho e à minha irmã Isabel

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List of papers

In this thesis the papers are referred to by the following roman numerals:

I. Soares, M., Rousk, J. Microbial growth and carbon use efficiency in soil: links to fungal-bacterial dominance, SOC-quality and stoichiometry. *Soil Biology & Biochemistry* (2019) 131, 195-205.

II. Silva-Sánchez, A.; Soares, M., Rousk, J. Testing the dependence of microbial growth and carbon use efficiency on N availability, pH, and organic matter quality. *Soil Biology & Biochemistry* (2019).

III. Soares, M., Rabow, S., Rousk, J. Microbial carbon-use efficiency under stress: responses to long-term and short-term heavy metal exposure. Manuscript.

IV. Soares, M., Persson, P., Kritzberg, E., Rousk, J. Microbial plant litter decomposition in terrestrial and aquatic boreal systems. Manuscript.

V. Soares, M., Kritzberg, E., Rousk, J. Labile carbon 'primes' fungal use of nitrogen from submerged litter. *FEMS Microbiology Ecology* (2017) 93, 1-9.

Author Contributions

I. MS and JR designed the experiment. MS and JR conducted the field work, and MS collected the data, and did the laboratory experiments. MS and JR analysed all data. MS wrote the manuscript and all authors provided comments on manuscript drafts.

II. MS, AS, and JR designed the experiment. MS, AS and JR conducted the field work, and AS and MS collected the data, and did the laboratory experiments. AS and JR analysed all data. All authors contributed to writing the paper.

III. MS, SR, and JR designed the experiment. MS, SR and JR conducted the field work, and SR and MS collected the data, and did the laboratory experiments. MS analysed all data. MS wrote the manuscript and all authors provided comments on manuscript drafts.

IV. MS, EK, and JR designed the experiment. MS conducted the field work, collected the data, and did the laboratory experiments. MS and PP analysed the FTIR data, while MS, EK and JR analysed all other data. MS wrote the manuscript and all authors provided comments on manuscript drafts.

V. MS and JR designed the experiment. MS did the laboratory experiments, and collected the data. MS, EK and JR analysed all data. MS wrote the manuscript and all authors provided comments on manuscript drafts

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SR – Sandra Rabow

Abstract

Microbial decomposers process the majority of net primary production in the biosphere and thereby regulate carbon (C) and nutrient cycling. Microbial communities are extremely diverse and often not explicitly represented in global C-cycling models, but one strategy to overcome this challenge is to focus on the major decomposer groups: fungi and bacteria. These groups have distinct life strategies and are differently affected by environmental conditions. During microbial decomposition of organic matter (OM), the fraction of C assimilated into microbial growth or used in cell maintenance and respiration is defined as microbial carbon-use efficiency (CUE). Since soils represent a large C pool with a critical role in regulating atmospheric carbon dioxide concentrations, CUE is a key parameter central to understanding the soil C-cycling and its feedback to environmental change.

In this thesis I compared the bacterial and fungal contributions to decomposition by developing conversion factors to measure microbial growth in units of C. I estimated CUE and studied the influence of environmental factors on the fungal-to-bacterial ratio (F:B) and how that affected CUE. These approaches were applied to a survey of field sites and verified in laboratory microcosms. When all sites were compared high CUE was associated with low F:B ratios in high C:N ratio soils which could be an index of low fertility. However, within each field site higher CUE was associated with soils with higher fertility (high N). Using microcosms, higher CUE resulted from low F:B ratios in treatments with low mineral N and high pH, with no effect of OM quality. This indicated that CUE in our field survey was also regulated by a component of soil fertility other than mineral N, and I suggest that plant community traits such as litter and rhizosphere inputs may influence F:B ratio and CUE. I also investigated the effect of long and short term-stress on CUE in a subarctic region. CUE was unaffected by increasing heavy metal concentrations along a gradient of long-term contamination. Fungi were less affected by heavy metal pollution than bacteria but F:B and CUE were unrelated. In experimental microcosms I tested the effect of short-term stress with heavy metal additions both in soils previously exposed to stress and unexposed soils. CUE decreased only in unexposed soils, showing that in soils previously exposed to stress microbial decomposers had grown tolerant to heavy metals and CUE was unaffected.

Differences between fungal and bacterial decomposition of plant litter in aquatic and terrestrial systems were explored in a boreal catchment forest site, where litter bags were installed in soils and adjacent streams along a pH gradient and resolved during 1.5 years. Fungal growth and litter leaching were higher in streams than in soils but overall mass loss was higher in soils. Litter decomposition was explored with IR spectroscopy and litter transformations in terms of chemical functional

groups (carbohydrate and aromatic compound loss) were fundamentally different between systems.

I investigated the priming effect - increased mineralization of native OM in response to an external labile C source- on biofilms associated with plant litter. In these systems the spatial proximity between photosynthetic algae and microbial decomposers allows for products of metabolisms to be exchanged. I found that labile C of photosynthetic algae origin did not affect the decomposition of plant litter in terms of mass loss, but increased the fungal removal of N from plant litter.

In conclusion, microbial growth rates in C units and CUE can now be estimated in natural environments. This thesis provided a deeper understanding of the fungal and bacterial contributions to decomposition in different systems, and how F:B and CUE are regulated by environmental factors.

Aim and Objectives of the thesis

The aim of the thesis was to understand the bacterial and fungal contribution to decomposition. In order to meet this aim, the following objectives were set:

1. Estimate growth rates in C units to understand fungal and bacterial contributions to decomposition in soils:

In order to understand the individual contributions of bacteria and fungi to decomposition, we estimated microbial growth rates in comparable units - units of C. Microbial colonization of litter was studied in laboratory microcosms. Fungal and bacterial growth rates were measured (growth-based methods) at several time points during the exponential growth phase occurring during initial period of litter colonization. Bacterial and fungal biomass formation during this period of net growth was estimated and transformed into biomass-C using conversion factors found in relevant literature. A relationship was established between the cumulative growth of fungi and bacteria and the fungal and bacterial biomass formation, allowing for conversion factors to be calculated. Assuming similarities between aquatic and soil microorganisms in terms of the relationship between growth and biomass formation, bacterial and fungal growth were estimated in C-units. This provided a direct comparison of the contributions by fungi and bacteria to the decomposition of terrestrial C. Objective 1 was met in **paper I**.

2. Identify the environmental controllers of bacterial and fungal growth rates and microbial carbon use efficiency (CUE):

- in soils from different ecosystems and land uses;
- in experiments controlling the availability of nitrogen, the soil pH and the OM quality
- in soils exposed to long- and short-term stress events

Using the conversion factors for growth rates in C units (developed in objective 1) the contributions of bacteria and fungi to C use were assessed. Objective 2 was achieved by calculating the partitioning of C between microbial growth (in C units) and respiration (carbon dioxide production), known as carbon-use-efficiency (CUE). In a field survey, CUE was estimated in a range of soils from different

ecosystems and land uses. To resolve the effect of environmental factors expected to control microbial use of C resources, a factorial experiment with laboratory microcosms was used where the effect of mineral nitrogen availability, pH and OM quality on microbial growth and CUE was tested. Further, we assessed the effect of long-term stress exposure on fungal and bacteria contribution to C use and CUE using field sites along a gradient of long-term heavy metal contamination. Laboratory microcosms were also constructed to test whether CUE and bacterial and fungal contributions to C-use in soils previously exposed to stress (points along metal contamination gradient) were affected by a new stress event (the exposure to Cu). Objective 2 was met in papers I, II and III.

3. Determine the effect of environmental factors on the fungal and bacterial decomposition of plant litter in terrestrial and aquatic systems.

In order to study the environmental factors controlling litter decomposition such as ecosystem fertility factors (nutrient availability and pH), and determine the differences in litter decomposition between terrestrial and aquatic systems, a field experiment was conducted. To address objective 3, field sites in a boreal catchment system differing in soil pH were selected, given that soil pH is known to be a powerful regulator of fungal and bacterial activity in soils. We assessed the colonisation of litter by fungi and bacteria (growth-based techniques) and the endpoint of litter decomposition (mass loss rates and carbon dioxide production). This was achieved by installing mesh bags with one litter type (birch) found to be present in both forest soils and adjacent streams, which provided an opportunity to compare the microbial colonisation and decomposition of the same litter in both systems. Sampling and microbial measurements were done over the course of 16 months in a logarithmic time series, with more frequent sampling in the first 3 months to capture initial colonisation of litter by microbes and mass loss. In addition, this was combined with the characterization of chemical changes in litter over time (Infrared-spectroscopy). Objective 3 was met in paper IV.

4. Determine the effect of a labile C source on the microbial decomposition of plant litter in aquatic systems

For the purpose of assessing the interaction between labile C and plant litter in aquatic settings, motivated by the extensive body of research in this field in soil sciences (investigating the Priming Effect), laboratory microcosms with submerged plant litter were used. In these systems, light availability was used to promote the growth of photosynthetic algae on submerged plant litter. Growth-based methods and the endpoint of decomposition (carbon dioxide production and litter mass loss) were used to estimate the effect of photosynthetic algae on the microbial

colonisation and decomposition of plant litter. These microbial dynamics and decomposition rates were compared to measurements in systems without light, and other systems where glucose-C was continuously delivered. By comparing the effects of photosynthetic algae vs glucose-C we assessed whether the effect of algae on microbial dynamics and decomposition was driven by the provisioning of an additional C source. Objective 4 was met in paper V.

Introduction

Microbial growth and CUE

Heterotrophic microorganisms are the agents that control and regulate decomposition of OM, carbon (C) and nutrient cycling and energy flow in the biosphere (Waksman & Gerretsen 1931; Schmidt *et al.* 2011). However, the connection between the microbial decomposer organisms and their contribution to carbon dioxide (CO₂) emissions is determined by their growth efficiency, i.e. the partitioning of C-resources used by the microorganisms into growth and respiration (Manzoni *et al.* 2012). Microbial CO₂ emissions are six-fold higher than anthropogenic emissions (Trivedi, Anderson & Singh 2013) and the regulation of C stocks is governed by microbial decomposers (Bradford *et al.* 2016; Liang, Schimel & Jastrow 2017). Furthermore, microbial CUE is used in C-cycling and decomposition models and is a very important parameter needed to understand how climate change will affect microbial processes (Frey *et al.* 2013; Geyer *et al.* 2019) and how the microbial contribution to C cycling is regulated.

Microbial decomposers have a starving-survival life style due to limited resources and complex habitat structure in natural environments (Morita 1988; Hobbie & Hobbie 2013). Spatial heterogeneity is characteristic for the soil environment (Stocker 2012) but is less important in aquatic systems. Soil structure interferes with the availability of water required for transport and solubility of resources (Schimel 2018), and low resource availability is a constraint for microbial life since it limits the amount of available energy and the availability of other compounds required for biomass formation (Hobbie & Hobbie 2013). This, in turn, affects the amount of resources allocated to growth or used for energy and cell maintenance because when resources are scarce, catabolism and anabolism are uncoupled increasing the energy allocated for maintenance functions (Roller & Schmidt 2015). Because of that, the life history of microbes has probably been selected to cope with resource limitation where efficient growth and mechanisms for substrate uptake and use have been optimised and are under selection in most environments (Roller & Schmidt 2015). Even though the genetic determinants underlying efficient growth are yet to be discovered, modelling work suggests that when resources are available fast-growing cells operate close to their optimal energy efficiency (Maitra & Dill 2015). It has also been suggested that oligotrophic marine bacteria can grow faster than

copiotrophic bacteria when resources are low (Kirchman 2016), meaning that high growth efficiency is possible by oligotrophic microorganisms adapted to low nutrient conditions. Studies with pure cultures have shown that efficient growth can also be favoured when resources are low and spatial heterogeneity is high (Pfeiffer, Schuster & Bonhoeffer 2001). Spatial heterogeneity in concert with low resources can increase the allocation of resources to biomass production (Stocker 2012), because it might influence competition between populations of microorganisms with different traits, and favour those adapted to low resource conditions (Roller & Schmidt 2015).

Aquatic systems are not as constrained in terms of habitat structure as soils are. An important difference between the two systems is that while soil microbes are several orders of magnitude more abundant per unit volume than aquatic microbes, resource concentrations are at the micromolar level in soils while in aquatic environments they are at the nanomolar level (Hobbie & Hobbie 2013). It has been suggested that this could be an indication of resources being less available to microbes in soils than in aquatic environments, but also that the methods currently in use might be overestimating resource concentrations in soils (Hobbie & Hobbie 2013). However, this could as well be an indication that abundance or biomass are not good predictors of microbial activity and resource use because microbial decomposers can be dormant or in a state of low or high activity (Rousk & Bååth 2011; Blagodatskaya & Kuzyakov 2013). Biomass and abundance estimates are probably poor predictors of rates of biomass production (Blagodatskaya & Kuzyakov 2013), and in order to capture resource use estimates of growth rates are required (Rousk 2016).

Unlike other fields within ecology, the advances in microbial ecology as a discipline have been guided by method development due to the difficulty in observing the organisms being studied in their natural habitat, and quantifying their contribution to the processes they are involved in. In aquatic systems, heterotrophic bacteria perform major functions in the transformation of OM, however, for several decades most bacteria were considered metabolically inactive (Zobel 1946). The first assessments of bacterial abundance were done with plate counts, but later microscopic counts showed two-fold higher bacterial abundances because it allowed for the quantification of unculturable organisms (Hobbie, Daley & Jasper 1977). Only when bacterial growth rates were quantified with *in situ* growth-based methods such as ³H-leucine incorporation (Fuhrman & Azam 1980) was the idea that most bacteria were metabolically inactive abandoned. Studies have shown that oceanic primary production and bacterial production are correlated and the mineralization of OM from primary production provides energy to heterotrophic bacteria (Cole, Findlay & Pace 1988; Ducklow & Carlson 1992). The production of new bacterial biomass is controlled by predation and fuels higher trophic levels (“The Microbial Loop” – Azam *et al.* 1983) and recent advances have pointed out that bacterial mortality by viruses is as large as mortality by other types of predation (Fenchel

2008). It is now widely accepted that heterotrophic bacteria play a key role in marine and freshwater environments by using resources to produce new bacterial biomass and by processing large quantities of C and nutrients despite their low population sizes.

The development of new methods for measuring bacterial activity contributed to a better understanding of the role of bacteria in aquatic environments, and therefore estimates of bacterial growth rates have seen great development in aquatic systems (del Giorgio & Cole 1998; Franco-Vidal & Moran 2011; Kirchman 2016). Bacterial growth estimates with ^3H -leucine incorporation into bacterial protein (Kirchman, Knees & Hodson 1985) and ^3H -thymidine incorporation into bacterial DNA (Fuhrman & Azam 1980; Fuhrman & Azam 1982) involve a short incubation of a radioactively labelled precursor followed by removal of non-incorporated isotope, and final quantification in a scintillation counter. In soils, initially the tracer was added to a slurry followed by acid/base extractions (Bååth 1990; Michel & Bloem 1993). The introduction of low-speed centrifugation and homogenization (Bååth 1992; Bååth 1994a; Bååth 1994b), and later the use of a series of washing steps and centrifugation (Bååth, Pettersson & Söderberg 2001) allowed for better measurements of growth rates because the precursor did not bind to soil particles as in the slurry method, and lower concentrations of tracer could be used (Rousk & Bååth 2011). Bacterial growth rates have also been measured with other labelled substrates. The addition of H_2^{18}O to soil followed by extraction and quantification of DNA is currently in use (Spohn *et al.* 2016a; Spohn *et al.* 2016b). This method assumes constant DNA content in newly-formed and mature cells, and that water is the only oxygen source for growth, and therefore it has been suggested that it might over or underestimate growth (Geyer *et al.* 2019).

With regards to fungi, initial estimates of biomass were based on measuring the length of fungal hyphae (Barlocher & Kendrick 1974). This was very time-consuming, and probably resulted in biomass underestimation due to numerous assumptions during the conversion between hyphal length to biovolume and further to biomass (Gessner & Newell 2002). Later, fungal biomass was measured with biomarkers such as ATP, chitin and ergosterol (Krauss *et al.* 2011). However, ATP does not persist long after cell death but is produced by both bacterial and fungal cells, chitin persists after cell death and is present in fungal and insect cells (Krauss *et al.* 2011). Ergosterol is a lipid found in the cell membranes of most fungal groups with the exception of chytrids and oomycetes (Gessner & Newell 2002). Since ergosterol is rapidly degraded after cell death, it is a good indicator of living biomass, and more accurate estimates have been possible with the use of ergosterol concentrations as a proxy for fungal biomass and growth rates (Krauss *et al.* 2011). Using ^{14}C -acetate incorporation into the fungal-specific ergosterol, first developed for litter decomposing fungi in streams (Newell & Fallon 1991; Newell 1996) and later on applied to soil fungi (Bååth 2001; Rousk & Bååth 2007), estimates of fungal

growth rates became possible and a high number of samples could be processed. ^{13}C -labeled substrates and their fate into microbial biomass have also been used to estimate biomass of different microbial groups with ^{13}C tracking into phospholipid fatty acids in the cell membrane (PLFAs) (Yao *et al.* 2015) or the partitioning of ^{13}C -position specific CO_2 into biosynthesis and respiration (Dijkstra *et al.* 2011a; Dijkstra *et al.* 2015).

Methods to study CUE

In aquatic systems, estimates of CUE have been done extensively for aquatic environments because methods to measure growth rates have been long available, and CUE values have been found to range from 0.05-0.60 (del Giorgio & Cole 1998; Manzoni *et al.* 2012). Nevertheless, these estimates derive from measurements of aquatic heterotrophic bacteria, or bacterial growth efficiency, because the contribution of fungi in aquatic systems is often disregarded (Wurzbacher, Barlocher & Grossart 2010). Most research on fungal decomposition has been developed in studies focusing on plant litter in forested streams (Cebrian 1999), while in lentic systems such as lakes and ponds the topic has been almost completely neglected (Wurzbacher, Barlocher & Grossart 2010). In marine systems the distribution of fungi is mostly associated to patches of terrestrially-derived wood and debris (Raghukumar 2017) but the topic has not drawn much attention, and the fungal contribution to marine C cycling has not been quantified in CUE assessments.

Estimates of CUE for soil microorganisms often fall within the range of 0.30-0.55 (Sinsabaugh *et al.* 2013), and the theoretical thermodynamic maximum for CUE is set to 0.8 (Gommers *et al.* 1988) because a fraction of C is needed to maintain cell energy levels. Several methods are currently used to estimate CUE depending on the system used, from pure cultures to environmental studies, but estimates lack consistency and result in a wide range of values (Geyer *et al.* 2019). Studies at the organism-level do not represent in situ conditions and might lead to an overestimation of CUE when culture studies with optimal conditions are used (Manzoni *et al.* 2018). Empirical methods track the utilisation of ^{13}C -labeled substrates such as glucose, and their fate into microbial respiration and biomass incorporation (Brant, Sulzman & Myrold 2006) or the partitioning of ^{13}C -position specific CO_2 into biosynthesis pathways (Dijkstra *et al.* 2011a; Dijkstra *et al.* 2015). Since microbial decomposers are C-limited, low-molecular weight compounds such as glucose are quickly used when available (Hobbie & Hobbie 2013). Therefore the use of C-labeled substrates provides information about the use efficiency of that specific substrate at the concentration and level of availability induced by the addition of an aqueous substrate solution (Frey *et al.* 2013; Sinsabaugh *et al.* 2013), and does not capture the complex mix of substrates available in a natural

environment (Hobbie & Hobbie 2013; Geyer *et al.* 2019). As a consequence, CUE values estimated in these studies can reach 0.77, nearly as high as the theoretical maximum (Dijkstra *et al.* 2011b; Frey *et al.* 2013), and probably overestimate CUE in natural environments and is also strongly dependent on the time-frame of the incubation (Geyer *et al.* 2019). Calorespirometry has also been used to estimate CUE and assumes that soil heat rate is proportional to net microbial growth and that CUE can be estimated from the ratio of heat rate to respiration (Hansen *et al.* 2004). This method requires substrate amendments therefore might also translate into substrate-use efficiency and will not reflect *in situ* conditions. Substrate-independent assessments consider the presence of organisms in different states of activity decomposing the available resources from the environment. As an example, incorporation of ¹⁸O-labeled water into microbial DNA has been measured in environmental samples, and along with respiration measurements, this method has yielded estimates of CUE converging around 0.2-0.3 (Spohn *et al.* 2016a; Spohn *et al.* 2016b). This results in lower CUE than substrate-based methods, but it probably reflects the overall microbial activity and capture the environmental conditions microbes live in. CUE has also been estimated through modelling of elemental stoichiometry of OM and microbial biomass. Studies have analysed how CUE changes with temperature (Manzoni *et al.* 2012) and resource availability in soils (Manzoni *et al.* 2012; Sinsabaugh *et al.* 2016), looking at the rate of physical loss of organic compounds during litter decomposition (Manzoni *et al.* 2010), and the kinetics of litter degradation (Manzoni 2017). Overall these studies have highlighted the stoichiometric and temporal controls of decomposition and how that affects CUE, yielding lower values than substrate-based methods.

Recognizing the need to establish a new method to estimate CUE in soils, in **paper I** we developed conversion factors (CF) to measure microbial growth rates in C-units. Combined with respiration measurements (carbon dioxide production) it was possible to calculate CUE according to equation 1:

$$CUE = (Bacterial\ growth + Fungal\ growth) / (Bacterial\ growth + Fungal\ growth) + Respiration \quad (Eq. 1)$$

This study was developed in microcosms with submerged litter and followed a few assumptions. We studied the microbial colonisation of plant litter over time because this substrate is found in both aquatic and terrestrial systems. We considered that microbial resource use, microbial growth and CUE and their dependence on environmental factors were similar in aquatic and terrestrial environments, as other studies have assumed (Manzoni *et al.* 2012; Hobbie & Hobbie 2013; Sinsabaugh *et al.* 2013). We also assumed that the relationship between microbial growth and biomass formation during litter colonisation would be similar in aquatic and soil environments. Similar turnover times for microbes in aquatic and soil environments have been calculated with ³H-thymidine incorporation into bacterial DNA and ¹⁴C-

acetate-incorporation into-ergosterol (Rousk & Bååth 2011; Su, Kuehn & Phipps 2015).

Using these methods, bacterial and fungal growth rates were estimated in submerged litter systems in **paper I**. To assess bacterial biomass we extracted and quantified DNA (Tranvik 1997). We used data on bacterial DNA content and cell weight (Simon & Azam 1989) and estimated bacterial biomass-C, assuming that a linear relationship between bacterial DNA and bacterial biomass C exists, and that the increase in biomass-C over time can be calculated based on the increase in DNA over time (Anderson & Martens 2013; Spohn *et al.* 2016a). We used the slope of this linear relationship (Fig. 1a) to calculate conversion factors for bacterial growth rates in C units. Fungal biomass was estimated from measurements of ergosterol concentrations in the litter microcosms. We assumed 5 μg ergosterol corresponded to 1 mg fungal biomass (Jørgensen 2000; Ruzicka *et al.* 2000) and 45% biomass-C content and calculated fungal biomass-C production. We plotted the relationship between cumulative fungal growth and fungal biomass-C (Fig. 1b) and used the slope of this relationship to estimate fungal growth in C units.

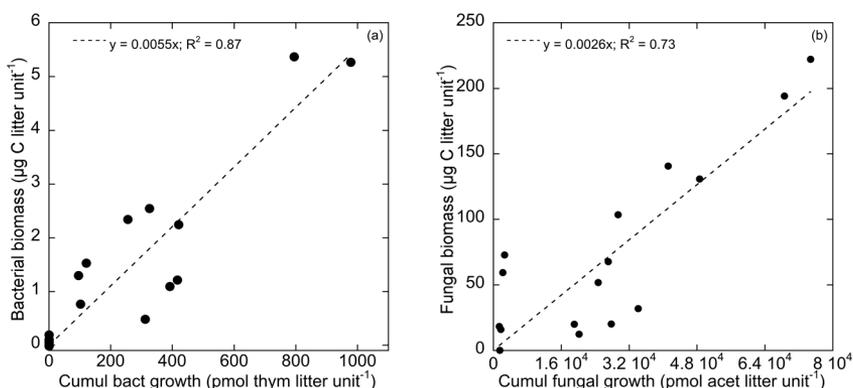


Figure 1 – (a) Relationship between bacterial biomass-C and cumulative bacterial growth (^3H -thymidine incorporation); (b) Relationship between fungal biomass-C and cumulative fungal growth (^{14}C -acetate incorporation into ergosterol). A type-II linear regression using the Least Squares method was applied to (a) and (b) in order to test for a relationship between biomass and growth.

For bacteria, the conversion factor that allowed the transformation of growth rates from pmoles of incorporated thymidine to growth rates in μg of C was 0.0055 (slope of the fitted line in Fig. 1a). This resulted from selecting a bacterial cell size from a list of possible values found in literature, and we acknowledge that the choice of other cell sizes corresponding to smaller or larger cells would have resulted in differences in growth rates of bacteria measured in C units, which is discussed in **paper I**. However, this conversion factor resulted from selecting a bacterial cell size which corresponded to the size of most bacteria in soil.

The conversion factor between pmoles of incorporated acetate into fungal ergosterol to growth rates expressed in μg of C was 0.0026 (slope of the fitted line in Fig. 1b). This value was similar to other conversion factors found in literature (see details on other conversion factors for fungi in **paper I**).

Environmental controls of CUE

In **paper I** CUE was estimated in a dataset of arctic and temperate forest soils and temperate arable soils, each with two levels of fertility. CUE values ranged from 0.03-0.3 (Fig. 2a). CUE is thought to be controlled by environmental factors, resource availability, stoichiometry, microbial physiological activity and the composition of the microbial community (Manzoni *et al.* 2012; Sinsabaugh *et al.* 2013). Therefore, reported estimates for CUE are often lower than the theoretical maximum of 0.8 (Gommers *et al.* 1988). With regards to abiotic factors, microbial CUE is expected to change with temperature (Allison, Wallenstein & Bradford 2010) and generally CUE declines with increasing temperatures in aquatic and terrestrial systems because temperature has larger effects on respiration than on growth rates (Sinsabaugh *et al.* 2013). Water availability is also a strong control of microbial growth and respiration (Schimel 2018) because apart from drought stress on cells it also reduces resource diffusion and transport in soils (Manzoni *et al.* 2012) which in turn affects CUE, while this is rarely an issue in aquatic environments.

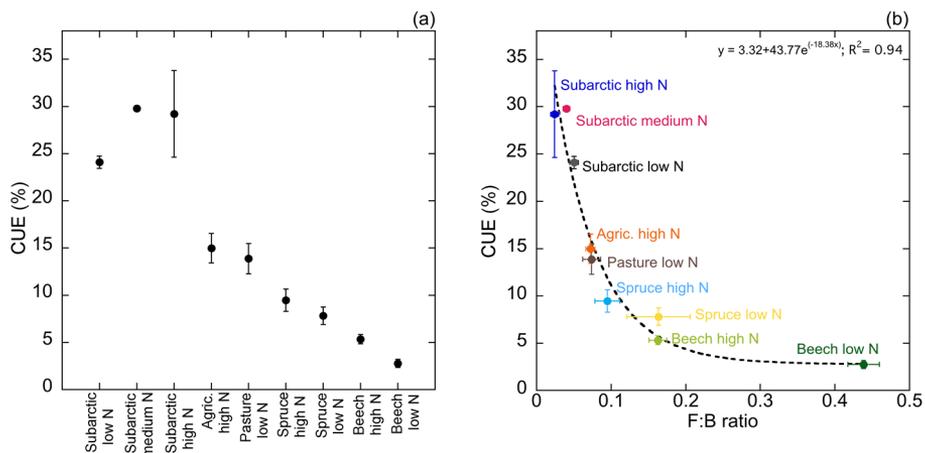


Figure 2 – (a) Microbial CUE estimates (%) and (b) relationship between CUE and F:B ratio in subarctic and temperate forest soils, and arable soils with different levels of fertility (N levels). Data points show mean values \pm 1 SE; for some data points the error bars are smaller than the symbol itself.

Microbial CUE can be affected by OM quality and by the metabolic pathways used for substrate utilization (Roller and Schmidt, 2015). Simple substrates or low molecular weight compounds such as glucose or amino acids can be easily transported inside the cell and require less activation energy and result in high CUE (Ågren & Bosatta 1987). In contrast, larger or more complex molecules require enzyme production and multiple oxidation steps, which often are associated with low CUE (Fierer et al. 2005; Fierer et al. 2006). Microorganisms respond to substrate availability by adapting their enzyme production (Sinsabaugh et al. 2013), and even when the resource is provided in large enough quantities, CUE still depends on the energy content of each C source (Roller & Schmidt 2015).

What seems to also determine CUE is the balance of resource-C to nutrient ratios since microbial decomposers follow relatively strict stoichiometric requirements (Manzoni et al. 2012), and therefore adapt their foraging strategies and C allocation to the availability of resources in the environment (Roller & Schmidt 2015). In both terrestrial and aquatic systems low nutrient availability per C availability is thought to lead to uncoupling of anabolism and catabolism because microbes cannot assimilate all substrate C and therefore a high fraction of it is lost (Sinsabaugh et al. 2013). This leads to overflow mechanisms, low biomass production, and high respiration rates yielding low CUE (Larsson et al. 1995; Russell & Cook 1995). During OM decomposition, as for example plant litter, substrates have usually low nutrient content in comparison to the microbial needs, and CUE increases with increased nutrient availability during the course of the decomposition process (Cotrufo et al. 2013; Frey et al. 2013; Sinsabaugh et al. 2013). At later stages of decomposition labile compounds are exhausted, substrate quality is progressively decreased and recalcitrant compounds tend to accumulate (Berg & Laskowski 2005). This development is thought to lower CUE because a greater investment in enzyme production is required, and the allocation of resources to enhance the uptake of essential nutrients imposes additional effects on growth and respiration (Manzoni 2017).

In addition, there are expectations that fungal and bacterial decomposers in soil have fundamentally different levels of CUE (Six et al. 2006). Microbial necromass highly contributes to the resistance of OM to degradation (Simpson et al. 2007) meaning that the long-term sequestration of soil C seems to be dependent on microbial C-use (Liang, Schimel & Jastrow 2017; Sokol, Sanderman & Bradford 2019). It has been argued that greater presence of fungal necromass can lead to an accumulation of recalcitrant organic matter in soils (Six et al. 2006; Clemmensen et al. 2013). This has been explained by fungal products having higher chemical resistance to degradation (Martin & Haider 1979) and being protected by associations with clay-minerals and soil aggregates (Simpson et al. 2004). Apart from that, there are expectations that fungal groups have higher CUE than bacteria (Holland & Coleman 1987), even though little empirical evidence is available to support this supposition

(Six et al. 2006). Nevertheless, a fungal dominated system is expected to have higher CUE and promote C stabilization and subsequent SOC accumulation (Beare et al. 1997; Bailey, Smith & Bolton 2002).

Fungi and bacteria are the main decomposers of organic matter (OM) but are thought to have different metabolisms and life-strategies. Apart from the already mentioned r-K dynamics (Fig. 11), it has been proposed that fungi have lower nitrogen (N) requirements than bacteria (Sterner & Elser 2002; Strickland & Rousk 2010). This has been concluded from fungal biomass having higher C:N ratios than bacterial biomass (Strickland & Rousk 2010). This difference can limit the access to high C:N-OM by bacteria while favouring fungal groups, even though other factors such as soil pH, moisture, and vegetation might indirectly contribute to these patterns (Fierer et al. 2009). The fungal and bacterial responses to N availability in natural systems have shown contrasting results. N-fertilizer additions have resulted in higher fungal dominance in unfertilized compared to N-fertilized pasture (Bardgett & McAlister 1999) and grassland soils (de Vries et al. 2006), and in forest soils under N deposition (Demoling, Nilsson & Bååth 2008). However, it has also been shown that fungal dominance did not increase even when N-fertilizer application had ceased (Bardgett & McAlister 1999), or even decreased in response to lower fertilisation (Mulder & Elser 2009).

Additionally, soil pH is a powerful regulator of the division between fungal and bacterial energy channels within the detrital food web (Rousk, Brookes & Bååth 2009; Fernandez-Calvino & Bååth 2010). Fungi are considered more tolerant to acidic conditions than bacteria possibly because the pH optimum of fungi is wider than the one for bacteria (Bååth 1996). So far, few studies have evaluated the effect of soil pH on microbial CUEs, but a relationship between higher fungal dominance in low pH soils resulting in higher CUE has been suggested (Sinsabaugh et al. 2016).

Environmental factors that modulate the balance between fungi and bacteria in decomposition such as soil pH and nutrient availability (Rousk, Brookes & Bååth 2009), should also be dominant regulators of CUE. Resolving bacteria and fungi in terms of how they process OM can reveal important patterns, but so far differences between the two groups remain unclear (Thiet, Frey & Six 2006; Bradford et al. 2016) and have been determined with methods that do not allow for absolute comparisons. To date, F:B is been mostly estimated with 3H-leucine/thymidine incorporation and acetate incorporation into ergosterol (van Groenigen et al. 2010; Rousk, Brookes & Baath 2011), PLFAs (Bardgett, Hobbs & Frostegard 1996; Herman et al. 2012; Malik et al. 2016) and SIR with inhibitors (Bååth & Anderson 2003).

In **paper I** I developed conversion factors to estimate both bacterial and fungal growth in units of C and resolved their contribution to C use. While links between a stronger fungal dominance of decomposition and higher level of soil C storage, suggestive of increased CUEs, have been reported in artificial soils early in soil formation (Kallenbach, Frey & Grandy 2016) and during litter decomposition in agricultural soils (Malik *et al.* 2016), this relationship did not seem to extend when ecosystems were compared in **paper I**. Within sites, bacterial dominance of decomposition was higher in soils with higher C/N ratio, and higher pH. Contrary to our expectations, estimates of CUEs were higher in soils with lower F:B ratio (Fig. 2b), resulting in a negative link between dominance of fungi and microbial CUE. Within ecosystems, relatively higher nutrient availabilities resulted in lower F/B and higher CUE. I explored the regulating environmental factors of microbial CUE in **paper II** by evaluating the effects of mineral nitrogen (N) additions, increased pH, and increased OM quality (plant litter addition) on CUE in beech and spruce forest soils with two levels of soil fertility (Fig. 3) over the course of 60 days.

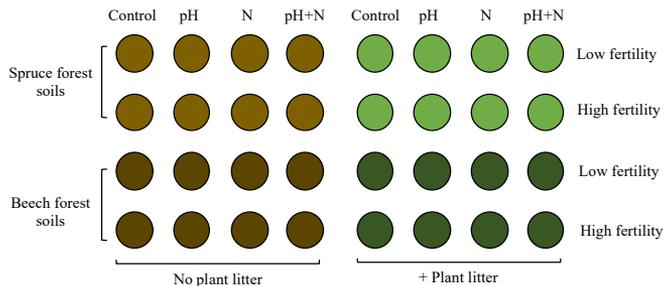


Figure 3 – Experimental design used for the microcosms study in **paper II** where the effects of pH, N, and OM quality on CUE was tested in forest and beech soils with high and low fertility levels.

Overall, estimated levels of microbial CUE ranged from <0.05 to 0.5 (Figure 4). These values fell within the range reported by other studies using substrate independent methods (Spohn *et al.* 2016a; Walker *et al.* 2018; Geyer *et al.* 2019; Zheng *et al.* 2019). Differences in CUE were linked to the dominance of fungi or bacteria, with higher CUE values corresponding to higher bacterial dominance over fungi (**papers I and II**). In **paper II**, higher levels of CUE were associated with higher dominance of bacteria in soils with higher pH and lower N availability. When bacterial growth was inhibited by mineral N additions or low pH soils, a competitive release resulted in a stimulated fungal growth and detrital C-use, which yielded reduced CUEs. Interestingly, the links between soil N availability and microbial CUE observed in **paper I** were not supported by the microcosms work in **paper II**, which indicated that there was an indirect link between nutrient availability and CUE probably explained by plant community productivity. In addition, the strong link to the relative dominance of C-use by fungi and bacteria in **paper I** (Fig. 2b)

was verified in **paper II** both when the fungal-bacterial growth ratios changed with N or soil pH. This emphasized that the dominance of fungi or bacteria can determine the ability of soil to store or lose C (Liang, Schimel & Jastrow 2017). Our study also highlighted how temporally dynamic microbial CUEs are, emphasizing the need to better constrain the influence on CUE by environmental controllers that are prone to vary over time in natural systems.

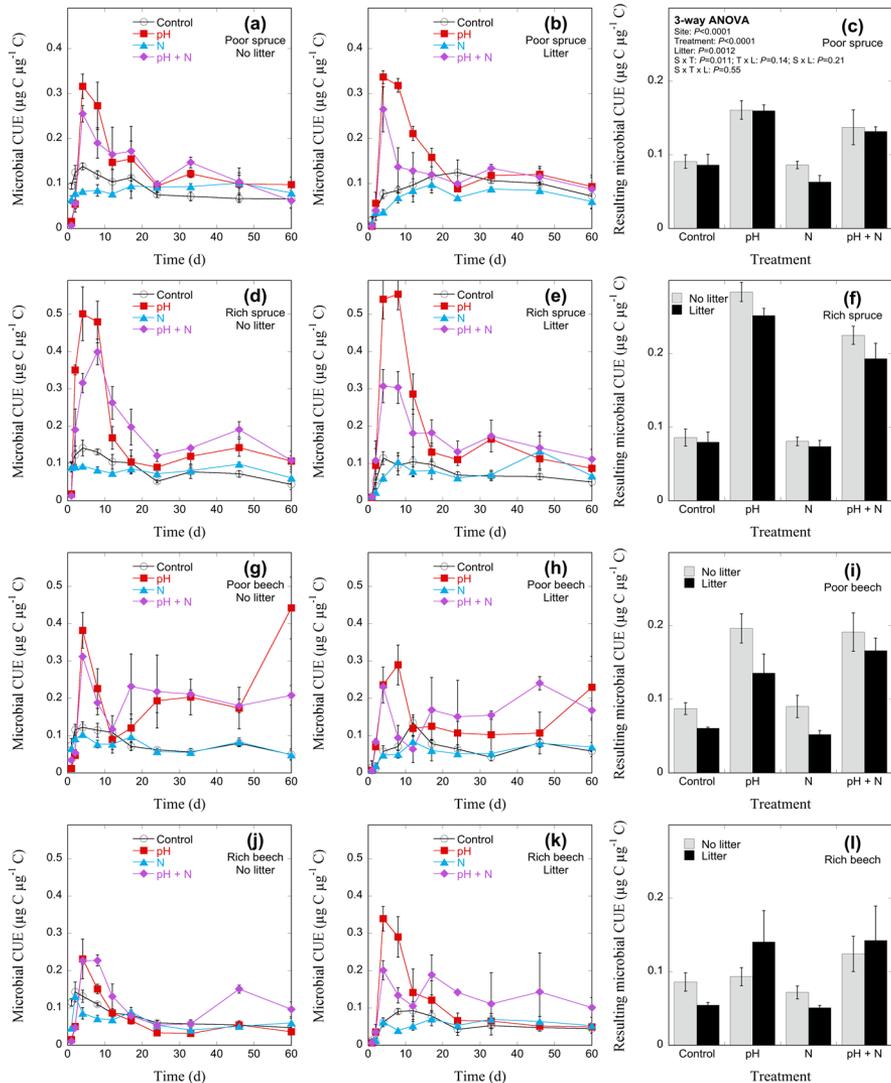


Figure 4 - Microbial carbon use efficiency without litter additions (left-hand panels) and with litter additions (centre panels), and resulting microbial carbon use efficiency (panels g-l) in spruce forest stands (panels a-f) and beech forest stands (panels g-l). Data points show mean values \pm 1 SE; for some data points the error bars are smaller than the symbol itself.

Microbial CUE and stress

Many environmental factors contribute to the regulation of microbial decomposition and CUE, also through the effect on fungal-to bacterial ratio, but microbial processes can also be affected by stressors. Stress is a change in the environment that creates physiological challenges to microbial function and survival (Schimel, Balser & Wallenstein 2007), and therefore affect CUE due to an increased allocation of resources to maintenance instead of growth (Manzoni *et al.* 2018). Previous studies on the effects of stress on soil microbial processes have focused on theories of ecological stability. Under this framework, short-term disturbance decreases the systems efficiency in conserving energy while long term disturbed systems adapt to be more efficient (Odum 1969; Odum 1985). In order to assess soil functions many studies have measured respiration and growth because they reflect stress effects on physiological functions and how that affects major ecological processes including decomposition (Tobor-Kaplon, Bloem & De Rooter 2006). In **paper III** we investigated the effects of stress in soils using a method that captured the amount of C resources allocated for growth and for respiration. As such, we considered that CUE would capture the system's energy use efficiency and the degree of disturbance (Odum 1985), and therefore would be a good proxy for evaluating how the soil microbial community responds to long term and short-term stress.

In soils and water, common sources of stress are drought, freezing-thawing cycles, salinity, and environmental pollution (Nies 1999; Schimel, Balser & Wallenstein 2007; Wilson *et al.* 2012; Rath & Rousk 2015; Karaouzas *et al.* 2018). In **paper III** we evaluated the effects of stress on CUE by investigating a gradient of long-term heavy metal contamination. The presence of heavy metals in soil, resulting from e.g. industrial and mining activities (Trevors & Cotter 1990), can exert acute or chronic stress to microorganisms (Tobor-Kaplon *et al.* 2005). Nevertheless heavy metals are essential trace elements in biochemical reactions but they cannot be synthesized or degraded by the cell (Nies 1999) and therefore persist in the environment. As a result, metal toxicity can arise at high concentrations (Bååth 1989) therefore the influx, efflux, and intracellular concentrations of heavy metals are strictly regulated by the cell (Ladomersky & Petris 2015).

The impact of heavy metal stress on microorganisms can depend on the duration and intensity of exposure. During a short-term exposure to stress, microbial cells experience toxicity effects and suffer additional physiological stress induced by detoxification mechanisms (Nies 1999; Ianieva 2009) which shifts energy allocation from growth and reproduction to survival and maintenance (Sibly & Calow 1989). Thus, heavy metal stress can lead to decreased cell functioning and cell death (Nies 1999; Ianieva 2009; Dupont, Grass & Rensing 2011). During long-term stress events, even though stress negatively impacts community diversity due to species loss (Giller, Witter & McGrath 1998; Torsvik *et al.* 1998), the removal of sensitive

groups releases resources to more resistant members within the microbial community (Tobor-Kaplon *et al.* 2005). This transfer of resources might increase the turnover of more tolerant groups, allowing for more energy to be allocated towards growth and reproduction (Tobor-Kaplon, Bloem & De Ruiter 2006). During a short stress event the effects of stress in microorganisms can lead to changes in energy allocation and/or cell death. In contrast, these changes probably occur to a lesser extent during long-term stress due to the selection of more tolerant groups within the community.

In **paper III** we investigated the effect of long-term exposure to heavy-metal contamination on microbial growth, CUE and F:B ratio and sampled soils along a gradient of heavy metal contamination in Skellefteå, Sweden (Fig. 5). We found wide spans of concentrations for all measured metals along the gradient. Copper (Cu) had the widest range of all (20-4000 mg kg⁻¹) and Cu concentrations had the highest R² value when plotted against the distance to the source of heavy metal pollution (R²=0.90). Therefore, we decided to use Cu as a proxy for metal contamination.



Figure 5 - Map showing the sampling points and the Rönnskär smelter, the source of heavy metal pollution (Nordgren *et al.* 1986). Stars refer to where the samples were taken and the numbers correspond to the sample #s in Table 1 in **paper III** (Rabow 2018).

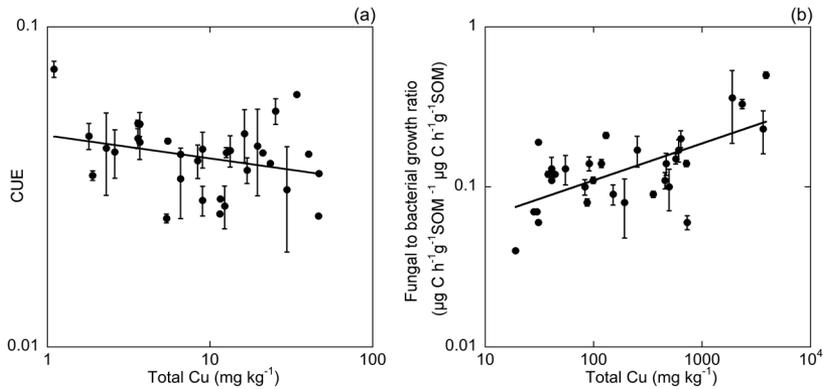


Figure 6 - a) Microbial carbon-use efficiency (CUE) and b) fungal-to-bacterial ratio (F:B) in relation to the total concentration of Cu in soils from the field samples. Data points show mean values \pm 1 SE; for some data points the error bars are smaller than the symbol itself.

CUE was not affected by increasing metal concentrations along the gradient (Fig. 6a). We found that fungal groups were more tolerant to metal stress than bacteria since F:B ratio was higher with increasing metal concentrations in soils exposed to long-term metal contamination (Fig. 6b). Different microbial groups have different strategies to cope with stress and dividing microbial groups into fungi and bacteria is a characterization with many assumptions but that can be a good starting point to understand resource allocation during stress events (Schimel, Balsler & Wallenstein 2007). Fungi have a lower surface to volume ratio than bacteria which results in lower diffusion of contaminants to the cell, and fungal hyphae can also direct growth towards areas without toxicants (Cooke & Whipps 1993). Therefore, it has been suggested that fungi are usually less affected by metal stress than bacteria (Pennanen et al. 1998; Rajapaksha, Tobor-Kaplon & Bååth 2004; Turpeinen, Kairesalo & Haggblom 2004), which we show in **paper III**.

As shown in **papers I** and **II**, we explored the link between CUE and F:B, yet, differences in F:B ratio or metal concentrations along the gradient of contamination could not explain differences in CUE (Fig. 6).

In **paper III** we also investigated the short-term effect of stress in soils previously exposed to heavy metals at low and high background metal concentrations. Using laboratory microcosms, we tested whether short-term exposure to Cu would result in lower CUE due to increased energy allocation towards maintenance and survival (Schimel et al., 2007). We were also interested whether microbes exposed to short-term stress would have higher CUE in soils previously exposed to high background Cu concentrations in comparison to those exposed to low background Cu concentrations, as a result of species sorting during the initial stress event and selection for tolerant groups (Tobor-Kaplon et al. 2005).

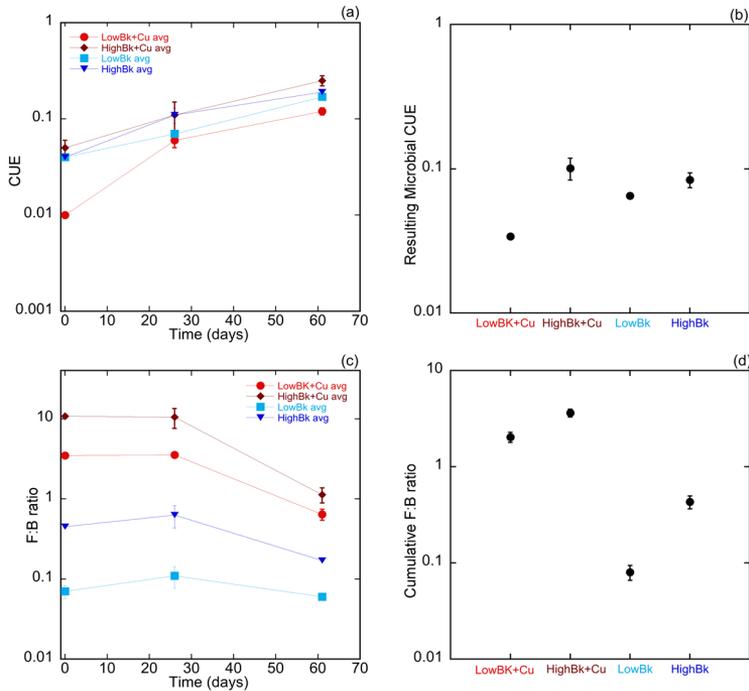


Figure 7 – (a) Microbial carbon-use efficiency (CUE), (b) resulting microbial (c) fungal to bacterial ratio and (d) resulting F:B ratios calculated with cumulative rates by the end of the experimental period. LowBk+Cu - low Cu background soil concentration spiked with Cu; HighBk+Cu - high Cu background soil spiked with Cu; LowBk - low Cu background control soil; HighBk - High Cu background control soil. Data points show mean values \pm 1 SE; for some data points the error bars are smaller than the symbol itself.

Short term-exposure to stress lead to an initial fungal dominance over bacteria, but we could not conclude that this had an effect on CUE because CUE increased towards the end of the experiment and was probably linked to bacterial recovery (Fig. 7a, b). Thus, F:B ratio and CUE were unrelated also in the context of short-term stress (Fig. 7c, d). Cu additions decreased CUE only in soils not previously exposed to metals therefore we could conclude that soils previously exposed to stress were more stable in terms of energy conservation. We concluded that CUE was unaffected by long term exposure to stress or by re-exposure to metal stress due to long-term changes in community structure that favored groups with higher physiological adaptations to heavy metals.

Microbial plant litter decomposition in terrestrial and aquatic boreal systems

Terrestrial plants produce 120 Pg of organic C every year (Beer *et al.* 2010), and microbial decomposers process nearly 90% of new plant production (Cebrian 1999). Litter decomposition is a process of great importance to nutrient and C cycling in terrestrial and aquatic systems (Wallace *et al.* 1999). As such, leaf litter is the main energy source for microbial decomposers in both forest soils and forested streams where riparian canopies supply leaf litter while limiting light availability and primary production (Wallace *et al.* 1997; Webster 2007).

Fungi are the main decomposers of plant litter because they can use low-quality substrates, have the ability to penetrate plant tissue with their hyphae and produce extracellular enzymes that decompose lignin and cellulose (Suberkropp, Arsuffi & Anderson 1983; Baldy, Gessner & Chauvet 1995; de Boer *et al.* 2005). In contrast, and with the exception of documented ligninolytic activity by actinomycetes, only few reports show that bacterial groups can also breakdown lignin (Janusz *et al.* 2017; Wilhelm *et al.* 2019). In general, during the decomposition process, bacteria mainly breakdown polymeric compounds after fungal groups have started the decomposition process (Baldy, Gessner & Chauvet 1995; Romani *et al.* 2006). Therefore bacteria are active during the initial leaching phase where low molecular weight dissolved compounds are available, or in later stages of the decomposition process when products of fungal decomposition can provide bacteria with energy and nutrients (Berg & Laskowski 2005).

In both systems, environmental conditions such as temperature, moisture and pH, nutrient availability, community composition of microbial decomposers and detritivores, and leaf litter quality are important factors regulating litter processing (see Garcia-Palacios *et al.* 2016 and references therein). Microbial decomposers control the mineralization of nutrients from plant litter by balancing the acquisition of C and N during growth conditions (Sinsabaugh & Moorhead 1994), with faster decomposition rates when substrate C to nutrient ratios match the organisms requirements (Melillo, Aber & Muratore 1982). Initially microbes immobilize N from the environment, and only later from plant residues, so typically relative N concentrations in litter increase during decomposition (Suberkropp & Chauvet 1995; Berg & Laskowski 2005). In both aquatic and terrestrial systems, fungi have more flexible nutrient requirements than bacteria due to the wider span of fungal C:N ratios (Strickland & Rousk 2010; Danger, Gessner & Barlocher 2016). Soil fungi have the ability to actively translocate resources between hyphae in nutrient depleted areas and areas with higher nutrient availability (Boberg *et al.* 2010), and nutrient transfer between different decomposing litter species can also occur (Schimel & Hättenschwiler 2007). However, it is unlikely that these mechanisms

are as important in aquatic systems because decomposition products are rapidly washed away and therefore not as available to microbial decomposers as in terrestrial systems (Gessner *et al.* 2010). With regards to pH, it is widely accepted that soil pH is also an important determinant of the dominance of fungi or bacteria during decomposition (Rousk, Brookes & Bååth 2009) and therefore indirectly affect litter decomposition. In aquatic systems the effect of pH has not been explored except for the effects on detritivores (Dangles & Guerold 2001).

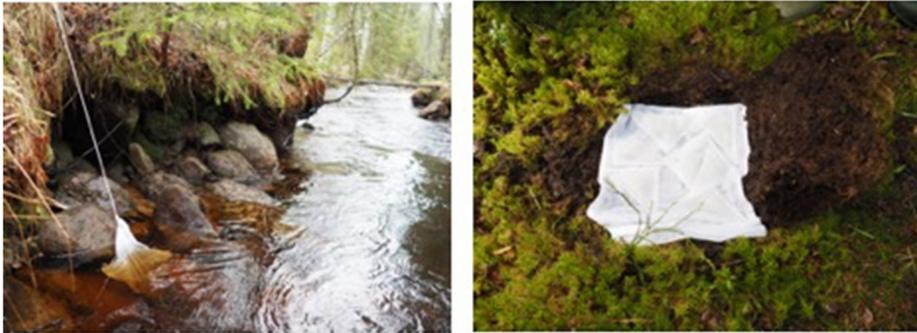


Figure 8 – Litter bags installed in streams and soils (photo by Johannes Rousk).

Paper IV describes a 1.5-year experiment where plant litter decomposition in terrestrial and aquatic systems was investigated along a pH gradient, focusing on microbial contributions to mass loss and to litter chemical changes over time. Litter bags with dried birch (*Betula pendula*) litter were installed in streams and corresponding soils in a boreal system (Fig. 8), close to the Svartberget Research station in Umeå, Sweden.

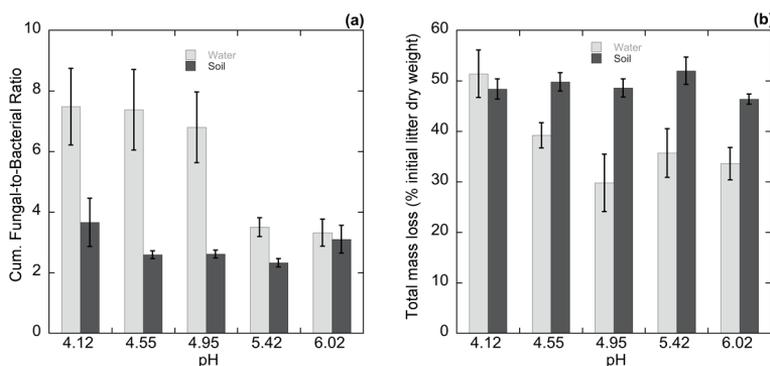


Figure 9 - (a) Fungal-to-bacterial ratio (b) total mass loss rates (% dry weight). Data points show mean values \pm standard error (SE).

We monitored the successional dynamics of fungal and bacterial growth, microbial respiration in leaf litter and litter mass loss over time. Further, the leaf litter composition was qualitatively analyzed with Fourier-transformed infrared absorption (FTIR) spectroscopy. FTIR is a technique that can provide information on the chemical composition of organic matter (Bouskill *et al.* 2016) by generating a spectrum derived from infrared energy that excites molecular bonds and allows for the identification of functional groups (Lammers, Arbuckle-Keil & Dighton 2009). With the FTIR assessment we aimed at identifying major chemical changes in plant litter during the decomposition process and relate that to responses of microbial decomposers to environmental conditions such as soil pH and fertility. We found that the fungal contribution to decomposition seemed to be comparatively larger than the bacterial contribution; a pattern that was particularly pronounced in streams and that was also stimulated by low pHs (Fig. 9a). Although fungi had a dominant role in plant decomposition in streams, the overall mass loss during our study period was higher in soil environments (Fig. 9b).

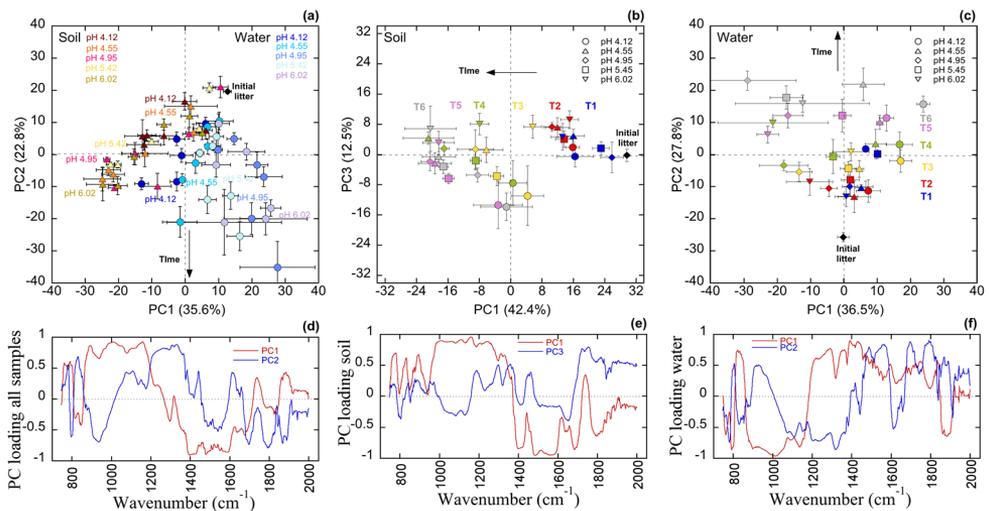


Figure 10 – PCA scores and loadings of the FTIR spectra of leaf litter obtained from all (a, d), soil (b, e) and water samples (c, f) during the whole experiment. Only 6 time points were included. Data points in (a), (b) and (c) show mean values ± 1 standard error (SE).

With regards to chemical changes over time, when all the samples were analyzed PC1 separated the decomposing plant litter in streams and soils samples, and PC2 described the trajectory of decomposition along the time course of the experiment (Fig. 10a). For litter bags buried in soils (Fig. 10b) PC1 showed an increase in a region between 1400-1600 cm^{-1} which corresponded to carboxyl groups, and a decrease in a vibrational region at 1000 cm^{-1} related to carbohydrate groups (Fig. 10e), which was an indication of chemical oxidation of leaf litter. We also observed

a decrease of a peak at 1500-1550 cm^{-1} (Fig. 10e) which could be related to proteins (primary amines) and be an indication of microbial biomass accumulation.

For the litter bags decomposing in streams, PC1 showed no changes in the vibrational region around 1000 cm^{-1} related to carbohydrate stretching which we observed for soil samples (Fig. 10f), but we reported a decrease in a vibrational region around 1600 cm^{-1} which might correspond to aromatic compounds, and this could be an indication that lignin-like compounds were lost from the system. Recently it has been suggested that lignin can be substantially lost from decomposing litter in streams already after 6 weeks (Yue et al. 2016). Also, lignin degradation can be controlled by the availability of easily decomposable carbon sources (Klotzbucher *et al.* 2011) and therefore the rapid decline of these labile C compounds may also accompany lignin decomposition (Yue *et al.* 2018).

In conclusion the bacterial contribution to decomposition was similar to streams and soils and fungal growth was higher in streams than in soils and particularly at low pH sites. We found that litter degradation proceeded in fundamentally dissimilar ways in soils and streams, with higher leaching in streams than soils and with regards to chemical changes in terms of carbohydrates and aromatic compounds.

Priming Effect

Decomposition and the processing of terrestrial organic matter (OM) are central components of carbon (C) cycling. Terrestrial systems are the second largest C reservoir on the planet, and understanding the balance between C mineralization and sequestration is a key aspect of C modelling and future climate projections. It has been argued that rising atmospheric carbon dioxide (CO₂) concentrations could stimulate plant primary productivity and therefore potentially buffer CO₂ elevation by increasing soil C storage (Parton, Ojima & Schimel 1996; Cardon *et al.* 2001). However, there is strong evidence that an increase in primary productivity does not necessarily increase soil stabilisation and C storage. Terrestrial plants produce 120 Pg of biomass every year (Beer *et al.* 2010), and up to 90% of plant production is processed by microbial decomposers (Cebrian 1999), but fresh OM input in soils can actually decrease soil C content. Increased primary production triggers rhizodeposition by plants (Dijkstra & Cheng 2007), which in turn can stimulate microbial processing of soil organic carbon (SOC). Hence, despite the stimulation of primary production by rising CO₂ or the large quantities of plant material incorporated into soil OM, long-term soil C storage appears to be unaffected (Gill *et al.* 2002; Fontaine *et al.* 2004; Dijkstra & Cheng 2007; Kuzyakov & Gavrichkova 2010). One of the proposed explanations for the lack of C sequestration in soil systems is the priming effect (PE), or the changes in OM mineralization triggered by inputs of comparatively more labile OM (Kuzyakov, Friedel & Stahr 2000; Guenet *et al.* 2010; Kuzyakov 2010).

The PE was first described approximately 100 years ago when the addition of fresh green manure to agricultural soils increased the mineralization of SOC (Lohnis 1926). Named some years later (Bingeman, Varner & Martin 1953), the PE has since then received increasing attention and is now an active area of research (Bengtsson, Attermeyer & Catalan 2018). Priming has been detected with various substrates, and has accounted for increases in mineralization of SOM of different qualities. A fresh exogenous C input can increase soil C losses up to 600% in the detritosphere (Kuzyakov *et al.* 2009) and by up to 380% in the rhizosphere (Cheng, Johnson & Fu 2003). Also, the supply of labile OM can stimulate the decomposition of old C pools (Fontaine *et al.* 2007) and of C stored in deep soil layers (Hamer & Marschner 2005).

The PE challenges the current view of how the mechanisms and drivers behind decomposition of OM and C cycling are regulated. Several explanations have been put forward to describe the PE, and two of the main proposed mechanisms are associated with the availability of organic C. The first advocates that priming is a nutritional and energetic competition between microorganisms with different life strategies such as fungi and bacteria. Bacteria are *r*-strategists with regards to C and N use, and grow rapidly when substrate is available and die or become inactive after

substrate depletion (Fontaine, Mariotti & Abbadie 2003; Morris & Blackwood 2005; Soares, Kritzberg & Rousk 2017). Fungi are *K*-strategists and grow slowly while degrading SOM, usually dominating the last stages of decomposition. Within this framework, a fresh input of labile C rapidly stimulates bacterial growth, followed by a gradual increase of fungal groups that produce extracellular enzymes and then increase the SOM breakdown (Fontaine *et al.* 2011). This mechanism can also be further controlled by the availability of growth limiting nutrients, since N availability and labile C can increase the magnitude of SOM mineralization after nutrient limitation is relieved (Chen *et al.* 2014).

A second mechanism considers that priming serves the microbial need for nutrients. In N-limited soils, microbes can use labile OM as an energy source to produce extracellular enzymes that decompose SOM and thus release nutrients (Moorhead & Sinsabaugh 2006). Interestingly, this goes against the “stoichiometric theory” which states that microbial activity and decomposition rates are the highest when stoichiometric demands for microbes are met (Hessen *et al.* 2004). The “stoichiometric theory” predicts that when N is available, SOM decomposition will increase because the microbial needs in terms of C:N ratio are met. In low N environments, *K*-strategists, often associated with fungi, are able to produce extracellular enzymes and have a wider C:N ratio and can tolerate low N environments in comparison to *r*-strategists (Fontaine *et al.* 2011). This provides an explanation for increased SOM mineralization when microorganisms are N-limited, with SOM-mineralization being negatively correlated to N content and positively correlated to C availability (Garcia-Pausas & Paterson 2011).

A third mechanism advocates that non-cellular SOM mineralization can be responsible for the PE. CO₂ emissions have been measured in sterilized soils (Maire *et al.* 2013; Keraval *et al.* 2018) indicating the presence of functional cellular machinery that can process OM when labile C is available (Keraval *et al.* 2016). This is thought to be a result of either oxidation of aromatic compounds, extracellular decarboxylation of metabolites supported by enzyme release during cell death, or mineralization of SOM through extracellular oxidative metabolism (Keraval *et al.* 2018). This non-cellular CO₂ production is thought to be ubiquitous and account for up to 24% of total CO₂ production (Keraval *et al.* 2018).

In priming studies, CO₂ production and/or N mineralization rates are usually measured rather than SOM turnover directly (Blagodatskaya & Kuzyakov 2008). In many studies the use of isotopic-labelled substrates provides a distinction between CO₂ sources and allows for the identification of its origin: microbial biomass turnover, labile substrate itself or SOM decomposition (Blagodatskaya *et al.* 2007; Chen *et al.* 2014). When labile C input increases SOM-mineralization we are in the presence of “Real Priming”, whereas “Apparent Priming” refers to changes in CO₂ originated from turnover of microbial biomass proportional to substrate quantity,

but with no effect on SOM mineralization (Dalenberg & Jager 1981). “Real” and “Apparent” priming can occur after the addition of a labile substrate to soil, as seen in Figure 11. Both real and apparent priming can be considered positive or negative. “Real negative priming” occurs when fresh C additions result in decreased SOM mineralization due to a preferential substrate utilization mechanism from low available SOM to labile C and “apparent negative priming” is related to decrease CO₂ production rates as a result of diminished microbial activity (Kuzyakov 2002), yielding, in my opinion, a complex jargon to navigate through.

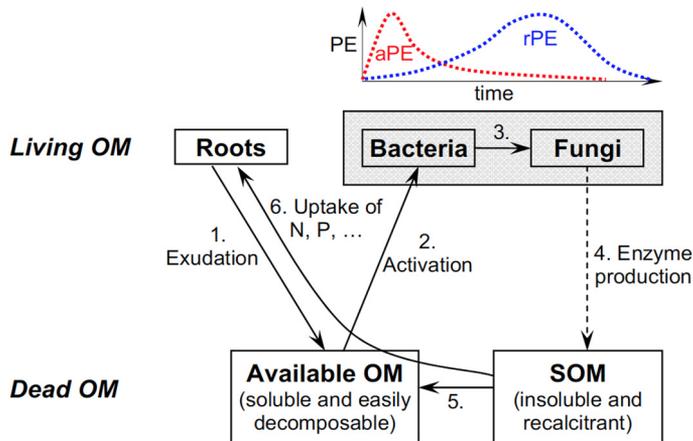


Figure 11 – Mechanisms of real and apparent priming effects (Kuzyakov 2010).

Priming effect in terrestrial systems

The PE in terrestrial environments is often characterised by the quality and type of delivery of labile OM. The differences in OM quality and its wide range of resistance to degradation (Parton *et al.* 1987; Fontaine & Barot 2005) challenge the understanding of how OM processing is regulated by microbial decomposers. The decomposition of SOM is affected by the amount and quality of SOM and its availability to microbes as well as the abiotic environment (Dijkstra & Cheng 2007; Chen *et al.* 2014). The input of labile OM to soils can occur through pulses or continuous additions and can ‘prime’ the decomposition of OM comparatively more resistant to degradation (Kuzyakov 2010). The rapid decomposition of microbial, animal and plant cells provide continuous additions of C-rich compounds for short periods of time (Fontaine, Mariotti & Abbadie 2003; Nottingham *et al.* 2009) whereas roots, leaves and shoot residues provide a continuous C-input over a longer time-frame (Kuzyakov 2010). In the detritosphere, while additions of complex substrates (i.e. with higher resistance to enzymatic breakdown) such as green

manure, wheat straw and ryegrass have caused real positive priming effects, additions of compounds such as glucose or fructose result in contrasting effects on SOC mineralization (Fontaine, Mariotti & Abbadie 2003; Fontaine *et al.* 2004). This can be explained by a preferential substrate utilisation mechanism, where microorganisms selectively switch from SOM to labile C uptake (Bradford, Fierer & Reynolds 2008; Nottingham *et al.* 2009).

In the rhizosphere, labile and soluble compounds such as sugars and amino acids are commonly released by roots and can be easily taken up by microorganisms (Jones & Edwards 1998; Kogel-Knabner 2002). PE is influenced by root density, root growth dynamics and plant photosynthesis; i.e. in grassland soils C inputs cause higher microbial activity and larger PE in densely rooted areas in comparison with areas with less roots (Kuzyakov 2010). Fresh C inputs can also change the structure of microbial communities by activating dormant and/or less active microbes, or selecting for specific microbial group (Griffiths *et al.* 1999; Nottingham *et al.* 2009). High root density and exudate quantity can lead to activation of microbes during the course of exudate use, but energy can be provided for SOM decomposition leading to priming of native OM. The presence of labile-C in the rhizosphere can enhance N supply in N-limited systems (Dijkstra *et al.* 2013) through the N mining mechanism. Also, even though this mechanism as not been explored, the remains of functional cellular machinery in the areas surrounding roots can potentially result in native OM mineralization, as shown in bulk soils (Keraval *et al.* 2018).

Priming effect in aquatic systems

PE has received considerably less attention in aquatic than in terrestrial systems. But terrestrial-aquatic similarities in terms of microbial actors, abiotic factors, nature of OM (Guenet *et al.* 2010) have recently inspired more studies in this field. Moreover, since PE challenges the understanding of the decomposition process and terrestrial organic material is also processed in inland waters with origin in soil transport and plant litter, investigating its contribution to C dynamics is crucial.

An important fraction of terrestrial organic material is processed in inland waters with origin in soil transport and erosion and plant litter (Tank *et al.* 2018; Tranvik, Cole & Prairie 2018). Networks of streams, lakes, and rivers store, process and release at least one half of the C they receive from terrestrial ecosystems (Battin *et al.* 2009). Terrestrial aquatic systems are net sources of CO₂ to the atmosphere by transporting and processing terrestrial OM in addition to a smaller fraction of internally fixed C. Because terrestrial inputs to inland waters can dominate C cycling and subsidize food webs (Wallace *et al.* 1997; Webster 2007), understanding the processing and fate of terrestrial C is of high priority. Until recently, terrestrial C budgets excluded freshwater systems, thus ignoring its role on the global C cycle (Tranvik, Cole & Prairie 2018). Lakes, rivers and reservoirs were

regarded as pipe transports of C to the ocean (Cole *et al.* 2007). Only in 2013 did the Intergovernmental Panel for Climate Change rethink the pipeline perspective, and included C budgets from inland waters (Tranvik, Cole & Prairie 2018) into global C cycle assessments. Given the substantial amount of terrestrial C processing in inland waters and that the priming effect has been extensively studied in soil systems, it is surprising that only recently has priming in aquatic environments been investigated.

Early reports on the priming effect have started to emerge mostly after a review by Guenet (2010) which suggested that PE should be revisited in aquatic ecology research. Since then a high number of studies in rivers, lakes, estuaries and marine environments focusing on interactions between different substrates. Priming has been measured on leaf litter mass loss (Halvorson *et al.* 2016) and riverine DOM (Ward *et al.* 2016), plant litter (Kuehn *et al.* 2014), leaf (Bianchi *et al.* 2015) and soil leachates (Guenet *et al.* 2014; Morling *et al.* 2017) in pond and lake habitats. A recent meta-analysis investigated 26 studies across freshwater and marine environments and reported no priming effects in aquatic systems, even though a positive but not statistically significant trend was detected (Bengtsson, Attermeyer & Catalan 2018).

Previously, it has been suggested that mechanisms similar to the ones behind the priming effect in soils could apply to aquatic systems (Guenet *et al.* 2010). It is likely that r and K dynamics can explain priming effect in aquatic systems, with the uptake of labile compounds performed by bacteria which have been regarded as r -strategists. However, fungal diversity in freshwaters is lower than in terrestrial systems (Bärlocher & Boddy 2016). And even though aquatic fungi are capable of hydrolyzing a wide range of plant polymers, the effective fungal degradation of lignin has not been well documented (Gessner *et al.* 2010). This could have potential implications on the magnitude of priming effects in aquatic systems and on the r and K dynamics. Other mechanisms of priming such as non-cellular mineralization of OM have not been explored in aquatic studies, and can probably be ruled out due to low residual microbial biomass in aquatic systems (Hobbie & Hobbie 2013). In the case of N-mining, it has been proposed that labile OM in aquatic systems (i.e. algal exudates) is more N-rich than terrestrial OM (Meyers 1994), and this could be a reason for the absence of N-mining mechanisms.

Due to its physical structure, the potential for interaction between different organic matter pools in soils is greater than for most aquatic habitats, with the exception of biofilms and sediments. For example, in the rhizosphere, spatial proximity and substrate availability to microbial decomposers drive priming dynamics. However, an analogous system to the rhizosphere is biofilms developing in leaf litter, where microbial decomposers are in close spatial proximity with periphytic algae and can utilize carbon (C) exudates released during photosynthesis (Wetzel 1990,

Goldsborough et al. 2005) resulting in an increase in OM mineralisation. **Paper V** investigated whether labile C provided by photosynthetic exudates could modify the microbial colonization and use of submerged plant litter, and stimulate litter decomposition through the priming effect. In a parallel experiment we mimicked the delivery of algal labile C by continuously adding glucose in order to experimentally assign the effect of algal products to labile C delivery, and tested for a C-driven mechanism behind the priming effect. We observed that labile C, added as natural algal exudates or as glucose, resulted in an increased fungal contribution to litter decomposition relative to bacteria, and generated a higher N use from litter during the active period of fungal growth (Fig. 12). Thus, although labile C did not stimulate total mass loss, it did trigger, or prime, an increased N acquisition from litter, which coincided with fungal growth. We interpreted this as a change towards a fungal dominated litter degradation induced by labile C which raises important questions with regards to C cycling since fungal products are known to be more resistant to degradation (Six *et al.* 2006).

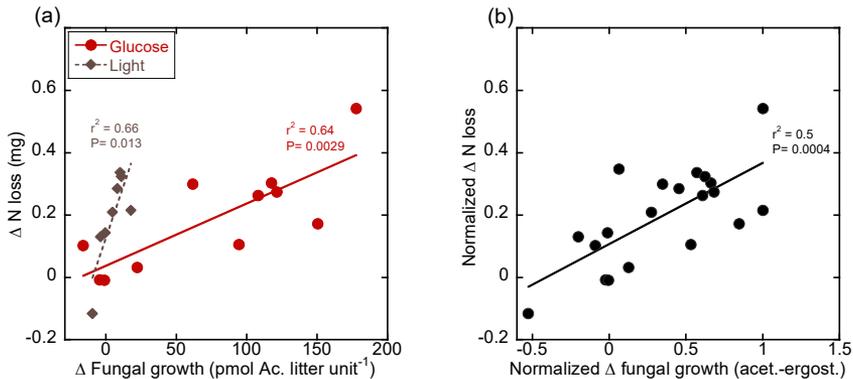


Figure 12 - (a) Effect of treatment labile C administered through glucose or photosynthetic algal exudates on the relationship between fungal growth and N loss in light (brown diamonds) and glucose (red circles). To show treatments effects, values for control treatments have been subtracted. (b) All data points presented in (a) were normalized to the highest value; the regression lines were obtained with least squares method.

Conclusions and future perspectives

In this thesis the contributions of fungi and bacteria to OM decomposition were investigated. We resolved between these two major decomposer groups due to the important functional differences anticipated between fungi and bacteria, and because environmental conditions, such as pH, nutrient availability and OM quality, can affect fungi and bacteria in contrasting ways.

In **paper I** conversion factors for measuring microbial growth in C units were estimated (Objective 1). With this revised set of methods, the resource-C use by fungi and bacteria could finally be compared in a range of soils and environmental conditions. The regulatory power of pH in shaping the dominance of fungi or bacteria in soils was confirmed: bacteria had a higher contribution to C-resource use in high pH conditions and even though both bacteria and fungi were stimulated by increased OM quality, there was a shift towards fungal dominance in N fertilized soils and towards bacterial dominance in high pH conditions. These patterns were evaluated both in a survey of field sites and in response to factorial experiments in laboratory conditions, and **papers I** and **II** provided strong evidence that F:B growth ratio determines CUE (Objective 2). In a survey of forest and arable sites, higher CUEs corresponded to high fertility soils with higher bacterial rather than fungal contribution to C use. Although F:B growth ratio decreased with high C:N when all sites were considered, higher within-site fertility corresponded to lower F:B growth ratio and higher CUE. This relationship was experimentally confirmed in laboratory microcosms. Interestingly, in laboratory microcosms, mineral N fertilization resulted in reduced bacterial activity and lower CUE, particularly in acidic soils. This indicated that soil fertility influenced CUE through another fertility component rather than mineral N availability. One possibility is that plant community traits such as litter quality and rhizosphere inputs influence F:B ratio and CUE. This particular effect deserves further exploration in future studies; how do differences in litter quality input influence F:B ratio, and in concert with pH and nutrient availability affect CUE? How do other environmental variables such as temperature, precipitation, moisture shape these relationships?

Expectations that soils with higher fungal than bacterial contribution to decomposition would have higher CUE (Holland & Coleman 1987; Six *et al.* 2006) were not confirmed in the field survey (**paper I**) or the laboratory studies (**paper II**). In fact, CUE was the highest in soils with higher bacterial dominance over fungi,

opposite to expectations. It has been suggested that higher CUE will lead to higher SOC formation due to the contribution of microbial biomass to C stabilization (Liang, Schimel & Jastrow 2017; Sokol, Sanderman & Bradford 2019). However, it has yet to be addressed whether the high CUE in bacterial-dominated soils will lead to increased C stocks because the fate of bacterial biomass in soil was not studied here. The “Carbon Stock Efficiency” has recently been explored and related to CUE (Manzoni *et al.* 2018) and certainly deserves further consideration in the future.

In field surveys and laboratory experiments, CUE was higher in bacterial-dominated soils and regulated by environmental conditions through their effects on the relative dominance of fungi and bacteria. The effect of stress on microbial CUE were also explored in this thesis (Objective 2 – **paper III**). A survey of long-term exposure to heavy metal stress confirmed the expectation that fungal groups were more resistant to metal stress than bacteria, evident from increasing F:B growth ratios in soils with higher metal concentrations, but higher fungal contribution to SOM decomposition did not result in higher CUE. Instead, microbial CUE was unaffected by increasing metal concentrations along a gradient of long-term contamination. In experimental microcosms the effect of heavy metal additions was tested in both soils previously exposed to stress and unexposed soils. CUE decreased only in soils previously unexposed to metals. Therefore, additional metal stress showed that in soils previously exposed to stress microbial decomposers had grown tolerant to heavy metals and CUE was not affected.

Since few studies exist that directly compare the microbial decomposition of plant litter in aquatic and terrestrial systems, this was one of the thesis’ objectives (Objective 3, **paper IV**). We found that the fungal contribution to decomposition seemed to be comparatively larger than the bacterial contribution; a pattern that was particularly pronounced in streams and that was also stimulated by low pHs. Although fungi had a dominant role in plant decomposition in streams, the overall mass loss during our study period was higher in soil environments. Changes in chemical functional groups of the decomposing litter was resolved with IR spectroscopy. We found that litter degradation proceeded in fundamentally dissimilar ways in soils and streams in terms of (carbohydrate and aromatic compound loss. The mechanisms behind these results remained unresolved but the distinct enzymatic abilities of aquatic and terrestrial microorganisms and the role of other environmental conditions are likely candidate reasons that could be further explored by future studies. A better understanding of the biological and environmental controls of litter decomposition could provide an insight on the regulatory mechanisms that lead to the litter fractions that contribute to OM formation.

Paper V studied the microbial dynamics on biofilms growing on submerged plant litter and assessed whether the effect of photosynthetic algae on decomposition was driven by the provisioning of an additional C source (Objective 4). Labile C of photosynthetic algae origin did not affect the decomposition of plant litter in terms of mass loss but increased the fungal N removal from plant litter suggesting a N-mining mechanism.

In conclusion, this thesis increased the understanding of the differences and similarities between terrestrial and aquatic microbial ecology. The contributions of fungi and bacteria to decomposition in natural environments can now be estimated in comparable units, a development that also enables the partitioning of C between biomass and respiration, or CUE.

In summary:

- CUE is controlled by environmental factors through their effects on F:B ratio
- CUE is higher in soils where the bacterial contribution to C use is higher
- Environmental stress caused by heavy metal pollution does not affect microbial CUE in soils previously exposed to heavy metal stress.
- Litter decomposition in terrestrial and aquatic systems is fundamentally different in terms of the higher contribution of fungal groups and the resulting chemical changes in litter over time.
- Labile C promotes fungal N removal of litter decomposing in aquatic systems

Popular science summary

Microorganisms play a key role in breaking down dead organic matter such as wood, branches and leaves, and during this process nutrients are released and made available for uptake by living plants. These microscopic decomposers consist of bacteria and fungi. While bacteria are small single-celled organisms, fungi form long tubular structures called hyphae. Both bacteria and fungi are present in a wide range of environments, such as forest and arable soils, deserts, streams and deep-sea sediments, and in a table spoon of soil billions of bacteria and kilometres of fungal hyphae can be found. In this thesis, I compared the contribution of bacteria and fungi to organic matter decomposition because these two groups have distinct life-styles and respond differently to environmental conditions such as pH, nutrient fertilization, and the quality of available organic matter.

During organic matter decomposition by microbes, a fraction of acquired carbon is used for cell growth, while another fraction is used for cell maintenance - this is often called microbial carbon use efficiency. I am interested in this topic because the amount of carbon devoted to biomass or growth can remain in soil for very long time as dead cells, while the fraction of carbon used for maintenance produces carbon dioxide, which is one of the main gases responsible for changes in the global climate. Carbon dioxide emissions from microbes are six times larger than those from human activities, so it is crucial to study carbon use efficiency and understand what determines how microbes divide carbon between growth and carbon dioxide production. Researchers have suggested that fungi have higher carbon use efficiency than bacteria due to the characteristics of the fungal cells, and therefore when decomposition is dominated by fungi less carbon dioxide is produced and more carbon remains in soil. In this thesis I revised a method that allowed me to measure bacterial and fungal growth in comparable units – units of carbon, and along with carbon dioxide measurements I estimated carbon use efficiency. I studied 9 sites from arctic and temperate regions in Sweden, including forest and agricultural sites which were different in fertility, and fertility is usually associated with high pH and high amounts of fertilizers such as nitrogen. I evaluated the environmental factors that stimulate bacterial and fungal contributions to decomposition and how that affected carbon use efficiency. I found that bacteria contributed more to decomposition than fungi and that resulted in high carbon use efficiency, contrary to what was expected. This was true for sites with low fertility. But each of the 9

sites (forest, agriculture, etc) was divided in fertile and unfertile plots. When I analysed each of these plots I discovered that fertile plots resulted in high carbon use efficiency and high contribution of bacteria. In the laboratory I ran experiments and discovered that carbon use efficiency was still higher when bacteria dominated decomposition in soil at high pH conditions. But when I added nitrogen fertilizer this resulted in decomposition being dominated by fungi which seemed contradictory. However, I think that most likely the presence of different plant communities in each of the sites (arctic forest, agriculture soils, etc) also contributed to the effect of fertility on carbon use efficiency.

I also tested the effect of stress on fungal and bacterial contributions to decomposition and resulting carbon use efficiency in a gradient of long-term heavy metal contamination and discovered that soil fungi are more tolerant to heavy metals than are bacteria. By adding more heavy metals to soils previously unexposed and also to those previously exposed to heavy metal contamination, I found out that that once microorganisms had grown accustomed or tolerant to high heavy metal concentrations, carbon use efficiency was unchanged by more heavy metal contamination, presumably because sensitive groups had been removed and replaced by others with higher tolerance to heavy metals.

The presence of easily available organic compounds such as sugars released by plant roots or algae during photosynthesis can also affect the contribution of fungi and bacteria to decomposition, and can also change the rate of ('prime') decomposition of organic matter, often called the Priming Effect. The Priming effect has been extensively studied in soils but it has not been very much explored in aquatic systems. In my thesis, I studied biofilms often found growing on decomposing plant litter in streams where they are exposed to sunlight, and where algae, bacteria and fungi live in close proximity. Even though easily available organic compounds released by photosynthetic algal origin did not increase the microbial decomposition of plant litter, fungi used this extra energy source to remove nutrients from plant litter.

Plant litter decomposition differs in aquatic and terrestrial environments but the specific comparison between system has rarely been made. In my thesis I investigated the roles of bacteria and fungi in plant litter decomposition along a fertility gradient in boreal forest floors and adjacent streams. I concluded that bacteria were similarly active in both streams and soils but that fungi dominated the decomposition processes in streams, a difference that was especially pronounced in low pH sites. The difference in fungal and bacterial dominance of litter decomposition led to distinct chemical changes of plant litter in streams and soils during the course of decomposition.

In summary, my thesis provided a deeper understanding of the fungal and bacterial contributions to decomposition in different systems and environmental conditions, and how that regulates CUE.

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List of papers

I. Soares, M., Rousk, J. Microbial growth and carbon use efficiency in soil: links to fungal-bacterial dominance, SOC-quality and stoichiometry. *Soil Biology & Biochemistry* (2019) 131, 195-205.

II. Silva-Sanchéz, A.; Soares, M., Rousk, J. Testing the dependence of microbial growth and carbon use efficiency on N availability, pH, and organic matter quality. *Soil Biology & Biochemistry* (2019).

III. Soares, M., Rabow, S., Rousk, J. Microbial carbon-use efficiency under stress: responses to long-term and short-term heavy metal exposure. Manuscript.

IV. Soares, M., Persson, P., Kritzberg, E., Rousk, J. Microbial plant litter decomposition in terrestrial and aquatic boreal systems. Manuscript.

V. Soares, M., Kritzberg, E., Rousk, J. Labile carbon 'primes' fungal use of nitrogen from submerged litter. *FEMS Microbiology Ecology* (2017) 93, 1-9.