Decomposition of Soil Organic Matter under a Changing Climate

Tobias Bölscher

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A Matter of Efficiency?

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Cover: Schematic illustration of substrate-use efficiency. Background photo shows a Stagnic Cambisol under forest close to Osnabrück, Germany (photo and figure: T. Bölscher)

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Decomposition of Soil Organic Matter under a Changing Climate. A Matter of Efficiency?

Abstract

Soil organic matter is the largest carbon (C) pool in the terrestrial C cycle, and soil CO_2 emissions surpass anthropogenic emissions from fossil fuel combustion by a factor of nine. Therefore, mechanisms controlling C stabilisation in soils and its feedback to climate change are widely debated. During decomposition, microbial substrate-use efficiency is an important property because it determines the allocation of substrate C to biosynthesis and respiratory losses. High efficiency values indicate that C primarily remains in soils while low efficiency implies that C is primarily lost into the atmosphere. Despite empirical evidence that efficiency is temperature sensitive, traditional Earth system models treat this property as a constant.

The aim of this thesis was to improve our mechanistic understanding of drivers regulating substrate-use efficiency with special consideration to climate change. It investigated the impacts of (i) temperature, (ii) microbial community composition and (iii) substrate quality on substrate-use efficiency. Within the thesis, a microbial energetics approach was applied and further developed using isothermal calorimetry. Further, the thesis compared common approaches for measuring microbial substrate-use efficiency, and the implications of the resultant empirical data for projected C stocks were tested using a modelling approach.

Substrate-use efficiency was generally temperature sensitive and decreased with increasing temperature. The observed temperature responses were non-linear and varied across land use management systems. The changes in substrate-use efficiency with temperature were driven rather by changes in microbial physiology than by shifts in active microbial communities. Nevertheless, fungi and Gram-negative bacteria tended towards relatively higher efficiencies. Efficiencies varied among utilised substrates, but substrate quality per se was a poor proxy for efficiency. Projected losses from soil C stocks varied across land use management systems and were up to 39 % and 15 % for grassland and forest systems, respectively. Results from the modelling approach confirmed that substrate-use efficiency is one of the factors to which soil C stocks react most sensitively. Findings from this thesis emphasise the importance of furthering our understanding of substrate-use efficiency for reliable climate projections.

Keywords: soil organic matter, substrate-use efficiency, temperature, land use, microbial community, substrate quality, isothermal calorimetry, carbon modelling

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Dedication

To my parents and brother

Our heads are round so our thoughts can change direction.

Francis Picabia

Also remember, you are a scientist – it is not your job to be right. It is your job to be thoughtful, careful, and analytical; it is your job to challenge your ideas and try to falsify your hypotheses; it is your job to be open and honest about uncertainties in your data and conclusions.

Joshua Schimel – Writing Science

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Bölscher T, Wadsö L, Börjesson G, Herrmann AM (2016). Differences in substrate use efficiency: impacts of microbial community composition, land use management, and substrate complexity. *Biology and Fertility of Soils* 52, 547-559.

II Bölscher T, Ågren GI, Herrmann AM. Land use alters the temperature response of substrate-use efficiency. (manuscript)

III Bölscher T, Paterson E, Freitag T, Thornton B, Herrmann AM. Temperature sensitivity of substrate-use efficiency is driven by microbial physiology rather than community composition. (manuscript)

IV Herrmann AM, Bölscher T (2015). Simultaneous screening of microbial energetics and CO_2 respiration in soil samples from different ecosystems. *Soil Biology and Biochemistry* 83, 88-92.

The contribution of Tobias Bölscher to the papers included in this thesis was as follows:

I Planned the study together with the main supervisor. Performed all the laboratory work. Analysed the data and wrote the article together with the main supervisor with inputs from co-authors. Responsible for correspondence with the journal.

II Planned the study together with the main supervisor. Performed all the laboratory work. Involved in the carbon modelling. Analysed the data with inputs from all co-authors. Wrote the manuscript together with all co-authors.

III Responsible for experimental design with inputs from all co-authors. Performed all the laboratory work. Analysed the data and wrote the manuscript together with the main supervisor and inputs from co-authors.

IV Involved in the practical work and writing of the article.

Abbreviations

C	Carbon
C:N	Carbon-to-nitrogen ratio
CUE	Carbon-use efficiency
DNA	Deoxyribonucleic acid
N	Nitrogen
NPP	Net primary production
P	Phosphorus
PLFA	Phospholipid fatty acid
SOC	Soil organic carbon
SOM	Soil organic matter

1 Introduction

Globally, soils contain more than three times the amount of carbon (C) present in the atmosphere and four times the amount of above-ground biomass C (Jobbágy & Jackson, 2000; Tarnocai *et al.*, 2009; Ciais *et al.*, 2013). They are therefore major components in the terrestrial C cycle (*Figure 1*). Furthermore, soil CO_2 emissions surpass anthropogenic emissions from fossil fuel combustion by a factor of nine (Schlesinger & Andrews, 2000; Ciais *et al.*, 2013). These numbers illustrate the fact that small changes in soil C fluxes could seriously modify global climate conditions as CO_2 is an important greenhouse gas contributing to global warming. Yet, projections of future soil C stocks as well as CO_2 emissions under a changing climate remain inconclusive despite extensive research (Davidson & Janssens, 2006; Kirschbaum, 2006; Heimann & Reichstein, 2008).

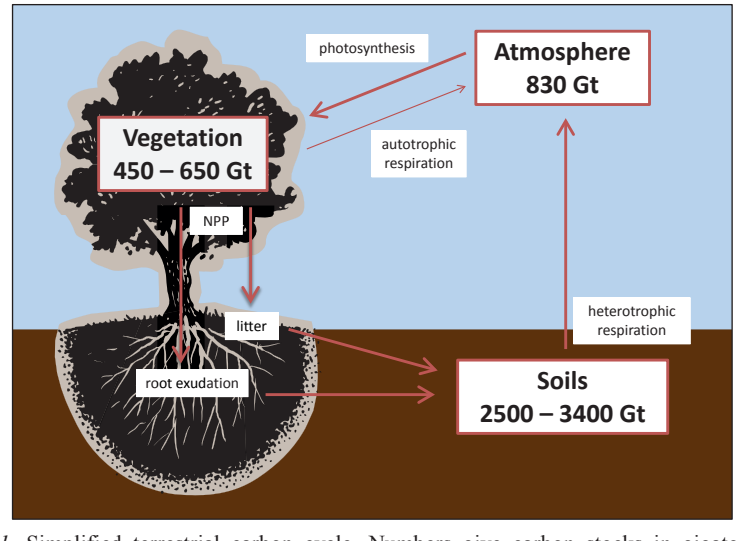

Figure 1. Simplified terrestrial carbon cycle. Numbers give carbon stocks in gigatones (Gt) (Tarnocai *et al.*, 2009; Ciais *et al.*, 2013). NPP = net primary production.

During decomposition of soil organic matter (SOM), microorganisms liberate parts of the organic C and emit it as CO_2 into the atmosphere (Schlesinger & Andrews, 2000). Thus, they are key players in governing the terrestrial C cycle (Schimel & Schaeffer, 2012). Their metabolic performance regulates the allocation of soil organic carbon (SOC) to biosynthesis and respiratory losses. This partitioning is referred to as microbial substrate-use efficiency describing the ratio of C allocated towards biosynthesis to the total C utilised by microorganism. High efficiency values indicate that C primarily remains in soils while low efficiency implies that C is primarily lost into the atmosphere. Traditional Earth system models (Parton *et al.*, 1987; Ågren & Bosatta, 1998; Coleman & Jenkinson, 2014) often consider the microbial biomass as a *black box* with substrate-use efficiency being a constant irrespective of environmental conditions (Allison *et al.*, 2010; Schimel, 2013; Wieder *et al.*, 2013). Yet, empirical evidence has revealed that substrate-use efficiency is temperature sensitive (Devêvre & Horwáth, 2000; Steinweg *et al.*, 2008; Frey *et al.*, 2013; Tucker *et al.*, 2013; Schindlbacher *et al.*, 2015), and recent modelling approaches emphasise that temperature sensitive substrate-use efficiency has a large effect on projected SOC stocks (Allison *et al.*, 2010; Wieder *et al.*, 2013). These modelling approaches have replaced the implicit microbial *black box* by explicit consideration of microbial biomass and physiology (Allison *et al.*, 2010; Schimel, 2013; Wieder *et al.*, 2013). Their results stress the importance of furthering our mechanistic understanding of key factors influencing substrate-use efficiency. Improved knowledge is required to enhance our understanding of the terrestrial C cycle and to improve future climate projections.

This PhD thesis investigates drivers of substrate-use efficiency with special consideration to temperature, applying a microbial energetics approach. This approach adapts thermodynamic efficiency equations proposed by Battley (1960, 1987) to environmental soil systems (Harris *et al.*, 2012; Herrmann *et al.*, 2014).

2 Aim

The overall aim of my thesis was to improve our mechanistic understanding of drivers regulating substrate-use efficiency of microbial communities in terrestrial ecosystems. The work focused on microbial communities in various land use management systems taking into consideration climate change aspects. Specifically, I investigated effects of (i) temperature, (ii) microbial community composition as well as (iii) substrate quality on substrate-use efficiency. The scientific hypotheses were: substrate-use efficiency of soil microbial communities (i) decreases with increasing temperature, (ii) changes with shifts in composition of active microbial communities and (iii) is altered by the quality of the substrate undergoing decomposition.

The specific objectives were:

- To determine the temperature sensitivity of substrate-use efficiency (Papers II & III).
- To study links between microbial community composition and substrate-use efficiency (Papers I & III).
- To assess effects of substrate quality on substrate-use efficiency (Papers I – III).
- To evaluate projections of future SOC stocks based on empirically obtained substrate-use efficiencies (Paper II).
- To develop a high-throughput screening method for estimating substrate-use efficiency (Paper IV).
- To critically evaluate the concept of substrate-use efficiency by scrutinising the underlying theory and approaches.

3 Background

Theoretical models and empirical evidence suggest that substrate-use efficiency is influenced by various environmental drivers (Manzoni *et al.*, 2012; Sinsabaugh *et al.*, 2013; Geyer *et al.*, 2016). Potential major drivers are: (i) temperature, (ii) microbial community composition and (iii) substrate quality (*Figure 2*). In the following, I will discuss these three factors in more detail.

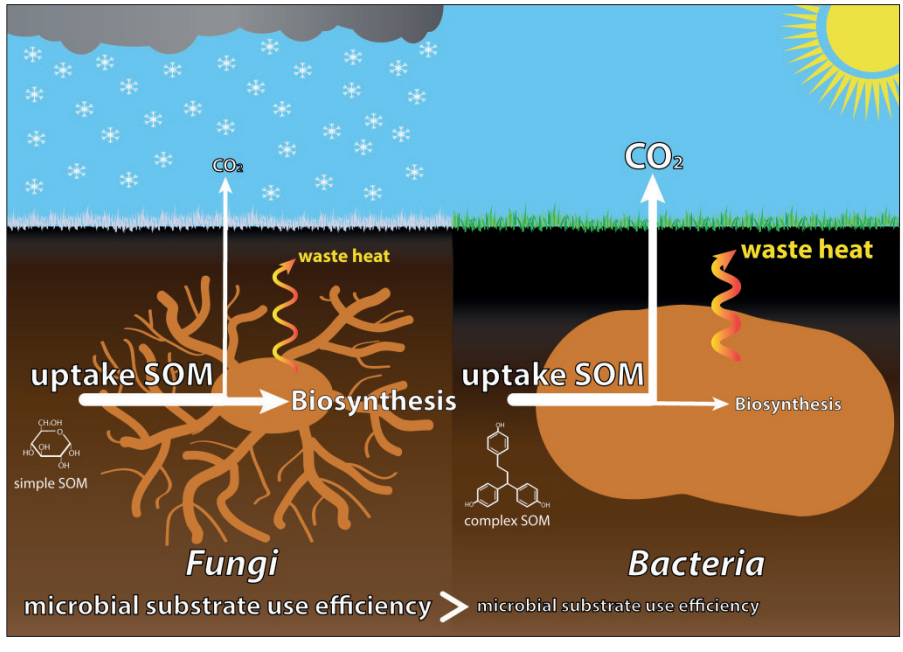

Figure 2. Schematic scheme showing major drivers of substrate-use efficiency. Low temperature, utilisation of simple soil organic matter (SOM) and relatively more fungi in microbial communities are thought to result in high efficiencies (left). High temperature, utilisation of complex SOM and relatively more bacteria are thought to result in low efficiencies (right).

3.1 Drivers of Substrate-Use Efficiency

3.1.1 Temperature

In general, a decrease in microbial substrate-use efficiency with increasing temperature has been observed in empirical studies (*Table 1*; Devêvre & Horwáth, 2000; Steinweg *et al.*, 2008; Tucker *et al.*, 2013; Schindlbacher *et al.*, 2015). Yet, the number of studies is limited and some studies discovered more complex temperature responses such as a decline in substrate-use efficiency over a limited temperature range (Wetterstedt & Ågren, 2011) or substrate dependent temperature sensitivity (Frey *et al.*, 2013). Also, other studies found no response in substrate-use efficiency to temperature changes (Dijkstra *et al.*, 2011; Hagerty *et al.*, 2014). Differences in temperature sensitivity may be due to the fact that the there is a plethora of approaches available in the literature (see Chapter 3.2), and that often only one substrate was examined. It is noticeable that substrate-use efficiency was only temperature insensitive in experiments using glucose or oxalic acid (*Table 1*). Oxalic acid, on the one hand, is generally utilised with low efficiency (< 5 %; Brant *et al.*, 2006; Frey *et al.*, 2013). It is highly oxidised (Nunan *et al.*, 2015), making it unattractive for anabolic use. Glucose, on the other hand, is an easy-to-use substrate which is taken up rapidly by soil microorganisms, and its metabolisation is therefore likely to be functionally redundant (Hill *et al.*, 2008). Hence, it may be used with constant efficiency under many circumstances.

Research outcomes from empirical studies are supported by theoretical reflections stating that substrate-use efficiency should decrease with increasing temperature (Manzoni *et al.*, 2012; Sinsabaugh *et al.*, 2013). The theory is based on considerations regarding (i) different effects of temperature on microbial respiration and substrate uptake and (ii) increasing maintenance requirements with temperature rise.

The first argument states that microbial respiration and substrate uptake into biomass vary in their temperature sensitivity. Respiration increases strongly with warming whereas substrate uptake increases less and reaches a plateau above an optimum temperature (Manzoni *et al.*, 2012).

The second argument recognises that maintenance requirements increase with increasing temperature. Maintenance requirements increase because, for example, proteins or membrane structures turn over faster at higher temperatures. Therefore, a higher fraction of C and energy needs to be allocated to maintenance, leaving fewer resources for growth (Steinweg *et al.*, 2008; Dijkstra *et al.*, 2011; Manzoni *et al.*, 2012).

Table 1. *Overview of empirical studies investigating temperature responses of substrate-use efficiency using soil incubation experiments. All studies measured microbial respiration and calculated carbon-use efficiency (CUE). The CUE results are expressed as a range across applied incubation temperatures and display mean values.*

Substrate	Temperature sensitive	Temperature (°C)	CUE range[1]	CUE approach	Study
Glucose	No	4 – 20	0.73	Biomass-based[2]	Dijkstra *et al.* (2011b)
Glucose	Yes	1.5 – 22.5	0.90 – 0.54	Biomass-based	Tucker *et al.* (2013)
Glucose	No	5 – 20	0.72	Biomass-based[2]	Hagerty *et al.* (2014)
Cellobiose	Yes	15 – 25	0.81 – 0.66	Substrate-based	Steinweg *et al.* (2008)
Glucose	No	5 – 25	0.72	Biomass-based	Frey *et al.* (2013)
Glutamic acid	Yes		0.66 – 0.46		
Oxalic acid	No		0.04		
Phenol	Yes		0.42 – 0.19		
Mixture[3]	Yes	3 – 23	0.8 – 0.6	Biomass-based	Schindlbacher *et al.* (2015)
Rice straw	Yes	5 – 25	0.61 – 0.34	Substrate-based	Devêvre & Horwáth (2000)

1 Some of the studies had several sample treatments. Here, only results for one treatment are shown. General findings regarding temperature sensitivity of CUE were always similar across treatments.
2 Substrate uptake assumed to be 100 %.
3 Substrate mixture contained sugars, amino sugars, organic acids, and amino acids.

3.1.2 Microbial Community Composition

Soil microorganisms are key players in the terrestrial C cycle and can be regarded as the biological engine, driving large parts of the C cycle. After SOM is taken up, its fate is under control of the microorganisms. Therefore, microbial community composition may affect the allocation of C (i.e. substrate-use efficiency) if microorganisms differ in functional traits (Schimel & Schaeffer, 2012). More specifically, fungi are considered to utilise substrate with higher efficiency than bacteria, because of high C accumulation in soil systems with relatively high abundance of fungi (Holland & Coleman, 1987; Ohtonen *et al.*, 1999; Six *et al.*, 2006). Further, a positive correlation was observed between efficiency and the abundance of Gram-negative bacteria (Harris et al., 2012). Yet, direct measurements of substrate-use efficiency have rarely been compared across soil microbial communities (Brant *et al.*, 2006; Thiet *et al.*, 2006; Harris *et al.*, 2012).

Theoretical considerations regarding microbial community composition being a driver of substrate-use efficiency are based on two aspects:

(i) differences in life history strategies (Pianka, 1970; Six *et al.*, 2006; Schimel & Schaeffer, 2012) and (ii) varying nutrient demands (Keiblinger *et al.*, 2010; Manzoni *et al.*, 2012; Sinsabaugh *et al.*, 2013).

Microbial species have developed distinct life history strategies during evolution. A broad concept describing such life history strategies is the r- and K-selection continuum (MacArthur & Wilson, 1967). Although criticised (Reznick *et al.*, 2002), it is used to describe strategies regarding the competition for resources which influence the efficiency of substrate use. Essentially, r-strategists maximise their growth rate when resources are abundant, but do so at the cost of inefficient substrate utilisation. In contrast, K-strategists keep low growth rates, but maximise their efficiency (Pianka, 1970; Fierer *et al.*, 2007). Different life history strategies may thus explain temperature responses of substrate-use efficiency, because community composition shifts towards r-strategists as temperature increases (Bradford, 2013). Depending on the phyla, fungi and bacteria can, however, be classified as either r-strategists or K-strategists (Fierer *et al.*, 2007; Keiblinger *et al.*, 2010), indicating that the general assumption of fungi having high substrate-use efficiencies may be too simplistic.

Varying substrate-use efficiencies may also be caused by different nutrient demands among microbial groups. Soil fungi have generally a higher carbon-to-nitrogen (C:N) ratio than soil bacteria (C:N approx. 10 for fungi and approx. 4 for bacteria; Sylvia *et al.*, 2005; Keiblinger *et al.*, 2010). Soil organic matter has commonly an even higher C:N ratio, but its ratio is closer to the ratio of fungi than to the ratio of bacteria (Keiblinger *et al.*, 2010). Therefore, fungi need to direct less C towards overflow respiration (i.e. waste C) than bacteria to match their C:N stoichiometry under N limitations. Hence, fungi should have higher substrate-use efficiency than bacteria when N is limited (Keiblinger *et al.*, 2010; Manzoni *et al.*, 2012; Sinsabaugh *et al.*, 2013).

3.1.3 Substrate Quality

The thermodynamic argument suggests that metabolic reactions breaking down structurally complex, low quality substrate have higher activation energies than reactions metabolising structurally simpler, high quality substrate (Bosatta & Ågren, 1999). Thus, utilisation of complex, low quality substrates requires higher initial energy costs for enzyme production and (i) these enzymatic requirements reduce substrate-use efficiency (Manzoni *et al.*, 2012; Cotrufo *et al.*, 2013; Sinsabaugh *et al.*, 2013). Further, (ii) the element ratio of substrates (Hessen *et al.*, 2004; Keiblinger *et al.*, 2010; Manzoni *et al.*, 2012; Sinsabaugh *et al.*, 2013) as well as (iii) the energy stored per C may influence microbial substrate-use efficiency (Gommers *et al.*, 1988; Manzoni *et al.*, 2012).

Evidence for this theory is, however, limited but some laboratory experiments support this notion (Devêvre & Horwáth, 2000; Frey *et al.*, 2013).

Extracellular enzymes are required to decompose most organic substrates and the production of such enzymes demands initial C and energy investments. Because of the investments, less substrate is available for biomass production and substrate-use efficiency should thus decrease in proportion to the number of required enzymatic steps for substrate decomposition (Manzoni *et al.*, 2012; Cotrufo *et al.*, 2013). The decrease in efficiency may be reinforced by the generally narrow C:N ratio of enzymes, because overflow respiration becomes more likely (Cotrufo *et al.*, 2013). Further, it is unlikely that microorganisms maintain high efficiency by decreasing enzyme production, as enzymes are required to maintain the supply of utilisable substrates (Schimel & Weintraub, 2003).

The element ratio of the metabolised substrate can control substrate-use efficiency in so far as the decomposer community requires C and nutrients in a relatively fixed proportion of approx. 60:7:1 (i.e. C:N:phosphorus; Cleveland & Liptzin, 2007). The C-to-nutrient ratio of metabolic products is commonly lower than the ratio of SOM (see Chapter 3.1.2), and to meet their nutrient demands, microorganisms can mineralise SOC in excess. Organic C is then directed towards overflow respiration and wasted, thus decreasing substrate-use efficiency (Keiblinger *et al.*, 2010; Manzoni *et al.*, 2012; Sinsabaugh *et al.*, 2013). This effect interacts, however, with inorganic nutrient supply because SOM is not the only nutrient source (Keiblinger *et al.*, 2010; Manzoni *et al.*, 2012).

As for nutrients, microorganisms require C and energy in a certain ratio. If the decomposers and the decomposed substrate have a similar C-to-energy ratio (i.e. the amount of chemical energy per mole of C), substrate-use efficiency can reach its theoretical maximum. This is approximately the case for carbohydrates. When, however, the C-to-energy ratio is different between decomposers and substrate, the microbial metabolism is either C or energy limited and substrate-use efficiency may stay below its theoretical maximum (Manzoni *et al.*, 2012).

3.2 Substrate-Use Efficiency: an Ambiguous Concept...

At first glance, substrate-use efficiency is a coherent concept: it expresses the ratio of substrate allocated towards biosynthesis in relation to the total substrate utilised during metabolism. Yet, if examined more closely, it becomes a diffuse concept (Geyer *et al.*, 2016): approaches vary in (i) measurement units, (ii) the consideration of which metabolic processes contribute to an efficient use of

substrate, and (iii) the ecological scale. Moreover, almost a dozen terms are circulating in the scientific literature (e.g. Geyer *et al.*, 2016). To avoid ambiguity the terms used in this thesis will be defined and differences between approaches pointed out.

3.2.1 …Concerning Measurement Units

Microorganisms convert C and energy during their metabolism and the rates of these two are closely linked (Battley, 1987). Consequently, substrate-use efficiency can be investigated by measuring either C or energy flows. Common approaches quantify C losses by means of CO_2 respiration (Frey *et al.*, 2001; Geyer *et al.*, 2016). Recently, alternative approaches have been developed which quantify the heat production from metabolism (Barros *et al.*, 2010; Harris *et al.*, 2012). Heat production can be used as a proxy for respiration, because anabolic processes produce negligible amounts of heat (Sparling, 1981a; b; Li *et al.*, 2009). Carbon and energy approaches are therefore alternatives, yet complementary, because heat production accounts for incomplete decomposition (Herrmann *et al.*, 2014). In this thesis, *substrate-use efficiency* will refer to the general concept, while *carbon-use efficiency* and *thermodynamic efficiency* will refer to approaches measuring the efficiency of microbial substrate use in units of C or energy, respectively.

3.2.2 …Concerning Metabolic Processes

In terrestrial ecosystems, substrate allocated to biosynthesis and remaining in soil is quantified by three broad approaches: (i) substrate-based, (ii) biomass-based, and (iii) growth-based efficiency, respectively (Manzoni *et al.*, 2012). These three approaches consider different metabolic processes which are now presented in more detail.

Substrate-based efficiency measures changes in substrate concentrations (e.g. Steinweg *et al.*, 2008) and considers those parts of the substrate directed towards biosynthesis that are incorporated into the microorganism or exuded from it. This covers growth and most maintenance process such as protein turnover, osmoregulation, or enzyme production. It does not, however, cover shifts in metabolic pathways or energy spillage as these maintenance processes cause a loss of C or energy from the soil system. When substrate concentrations are analysed after addition of labelled substrate (e.g. ^{13}C), substrate-based approaches approximate biomass-based approaches. This is due to the fact that labelled substrate atoms are incorporated into exudates which are recovered from the soil extracts and thus accounted for as unused substrate.

Biomass-based approaches measure substrate incorporation into biomass (e.g. Tucker *et al.*, 2013). In contrast to substrate-based approaches, they do not consider exudates as directed towards biosynthetic stabilisation. Efficiently used substrate is thought of as used for microbial growth and some of the maintenance processes such as protein turnover and membrane repair. These maintenance processes keep substrate C inside the biomass.

Very recently, a growth-based approach has been introduced (Spohn *et al.*, 2016a; b). This approach is substrate independent and measures incorporation of ^{18}O into DNA. Growth-based approaches acknowledge only the fraction of substrate directed towards biosynthetic stabilisation which is used for growth. They do not consider any substrate used for maintenance processes as efficiently used.

In general, biomass-based approaches seem to be most common. Nevertheless, all substrate-use efficiency approaches are frequently referred to as growth efficiency (Frey *et al.*, 2001; Hagerty *et al.*, 2014). In this thesis, substrate-based (Papers I, II & III) and biomass-based (Paper III) approaches were adopted.

3.2.3 …Concerning Ecological Scale

Investigations of substrate-use efficiency capture different ecological scales, resulting in incoherent and inconclusive results (Geyer *et al.*, 2016). For clarification, Geyer *et al.* (2016) suggested a nested conceptual framework with increasing temporal and spatial scales. The authors distinguish between population-, community-, and ecosystem-scale substrate-use efficiency. Population-scale refers to species-specific efficiency measured mostly in pure culture studies. The community-scale approach analyses the efficiency of microbial communities in, for example, soil samples during short-term incubation studies. The measured substrate-use efficiency is an average efficiency of all active microorganisms in the community comprising diverse species. Ecosystem-scale expands the time frame and covers aspects such as microbial turnover and interactions of the substrate undergoing decomposition with the soil matrix. By moving from one level of the framework to another, more and more drivers of substrate-use efficiency are included, from pure metabolic restrictions to environmental factors and community dynamics (Geyer *et al.*, 2016). This thesis will discuss substrate-use efficiency at the community-scale.

4 Material and Methods

4.1 Soils

Soil samples were collected from long-term field experiments located in Röbäcksdalen and Flakaliden in Northern Sweden (Papers I, II, & IV), Fors, Nåntuna and Jädraås in Central Sweden (Paper IV), and Dundee in Central Scotland (Paper III) (*Figure 3*). The field experiments differ in land use management systems ranging from arable land, ley farming and grassland to forest systems. The field sites in Northern and Central Sweden are exposed to a boreal and humid continental climate, respectively. The Scottish field site is exposed to an Atlantic climate and represents an arable system with different organic amendments. Plots with compost addition and control plots were sampled. Soil types were Cambisol at arable land, ley farming, and grassland sites and Podzol at forest sites (IUSS Working Group WRB, 2006).

Samples were sieved, homogenised, and stored frozen until further use. Prior to incubation experiments, all samples were kept for 2-4 days at room temperature to allow any disturbance from sampling and freezing to subside (Herrmann & Witter, 2002). Afterwards, samples were acclimated for ten more days at experimental temperatures ranging from 5 to 25 °C. Over the entire incubation period, the water content of soils was kept between 45 to 65 % of water holding capacity to ensure optimal conditions for microbial activity.

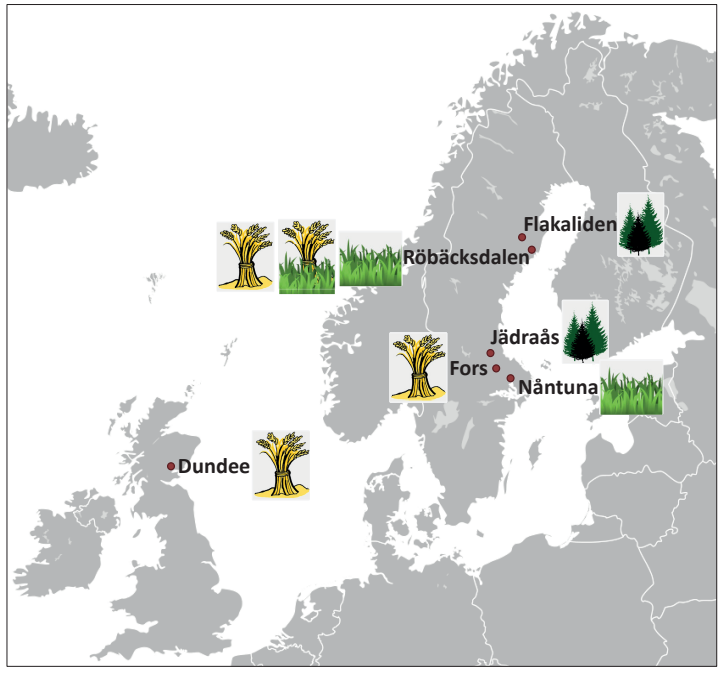

Figure 3. Soil samples were collected from two areas in Sweden and one in Scotland. Field sites represent four land use management system: wheat ears indicate arable land, grass indicates grassland, wheat ears combined with grass indicate ley farming, and trees indicate forest ecosystems. (adapted from Maix, 2007)

4.2 Microbial Energetics

Microbial energetics was investigated by measuring heat production after substrate amendments. The data was used to calculate thermodynamic efficiency (see *Equation 1* below) (Papers I – III) and calorespirometric ratios, i.e. the ratio of heat-to-CO_2 production (see *Equation 3* below) (Papers I & IV). Heat production rates were measured using TAM Air isothermal calorimeters (TA Instruments, New Castle, DE, USA). Aliquots of 5 g soil (dry weights) were placed in 20 ml glass reaction vials and amended with either 50, 500 or 1,800 µg C g^{-1} soil using substrate solutions of either D-glucose (Papers I – IV), D-trehalose (Paper III), L-alanine (Papers I & III), phenol (Paper III), or glycogen (Papers I & II). In all experiments, control samples received ultrapure water. Reaction vials were either closed with a lid containing a rubber septum (i.e. closed vials; *Figure 4a*) or an admix ampoule set up (*Figure 4b*). The admix ampoule set up system consists of two 1 ml syringes, which allows addition of substrate solution after introduction of the reaction vials into the calorimeter and stabilisation of the heat production rate signals. Closed vials

were amended with substrate solutions prior to introducing them into the isothermal calorimeters. Heat production rates were measured continuously up to 32 hours after substrate addition at temperatures ranging between 5 up to 25 °C.

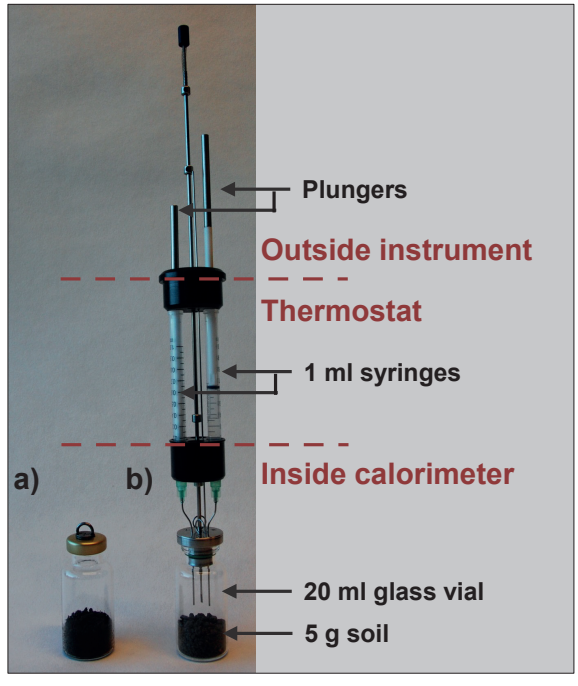

Figure 4. TAM Air reaction vial set up systems used for calorimetric investigations: (a) Closed vial sealed with a lid containing a rubber septum. The hook is used for lowering the vial into the calorimeter. (b) Admix ampoule set up includes two 1 ml syringes; here, substrate solutions can be added to samples during calorimetric measurements, because plungers stay outside the instrument. (Photo: T. Bölscher)

4.3 Soil Respiration

Soil respiration was determined for calculations of the calorespirometric ratio (see *Equation 3* below) (Papers I & IV), the metabolic quotient (Paper I), and carbon-use efficiency (see *Equation 2* below) (Paper III). In Papers I & III, soil respiration was analysed on separate sample sets. Here, aliquots of either 20 or 30 g soil (dry weight, Papers I & III, respectively) were treated similarly to samples used for calorimetric measurements, and all samples were incubated in gastight glass jars. Accumulation of CO_2 was periodically analysed using an infrared gas analyser (EGM-4, PP systems, Amesbury, MA, USA). In Paper III, uniformly ^{13}C labelled substrates were used to determine the amount of

CO_2 respired from substrate addition (Waldrop & Firestone, 2004). Therefore, isotopic C ratios in accumulated CO_2 were analysed on gas samples using a GasBench II interfaced to a Delta Plus^{XP} isotope-ratio mass spectrometer (Thermo Finnigan, Bremen, Germany). In Paper IV, soil respiration was determined in closed calorimetric reaction vials using CO_2-traps (*Figure 1 in Paper IV*) which contained an indicator gel and these were analysed on the basis of a colorimetric approach proposed by Rowell (1995) and Campbell *et al.* (2003).

4.4 Microbial Substrate Utilisation (Papers I – III)

Microbial substrate utilisation was either determined via residual substrate in soil solutions (Papers I – III) or incorporation of substrate into microbial biomass (Paper III).

4.4.1 Residual Substrate in Soil Solution

Residual substrate in soil solution was analysed to calculate thermodynamic efficiency (see *Equation 1* below). In Papers I & II, residual substrate in soil solution was analysed on separate sample sub-sets after 0, 5, 21 and 32 h. Briefly, glucose and glycogen concentrations in 5 g (dry weight) amended soil were determined spectrophotometrically (GENESYS 20, Thermo Scientific, Waltham, MA, USA) after an enzymatic reaction using an glucose assay kit (GAGO-20, Sigma-Aldrich, St. Louis, MO, USA). The procedure for glycogen comprised an initial acid hydrolysation step using 7 M HCl solution (Geary *et al.*, 1981). Quantification of alanine was done by adopting a procedure for animal plasma (Reverter *et al.*, 1997) and using ultra-performance liquid chromatography (Dionex UltiMate 3000 RS, Thermo Scientific, Waltham, MA, USA). Linear models were fitted to data of residual substrate in soil solution. In Paper III, residual substrate in soil solution was obtained from dissolved organic ^{13}C in unfumigated samples after measuring microbial respiration.

4.4.2 Substrate Incorporation into Microbial Biomass

For calculating carbon-use efficiency (see *Equation 2* below), the incorporation of substrate C into the microbial biomass was determined. Soils were initially amended with uniformly labelled ^{13}C substrates and microbial respiration was periodically measured. At the end of the incubation period, microbial biomass C was analysed using the fumigation-extraction method (Vance *et al.*, 1987). The incorporation of substrate C into the microbial biomass was calculated from the ^{13}C signature in soil extracts of fumigated and unfumigated samples

(Brant *et al.*, 2006). Therefore, the dissolved organic C in these extracts was evaporated through addition of sodium persulfate and the ^{13}C signature of the evolved CO_2 analysed using a GasBench II interfaced to a Delta Plus^{XP} isotope-ratio mass spectrometer (Thermo Finnigan, Bremen, Germany).

4.5 Microbial Substrate-Use Efficiency (Papers I – III)

In this thesis, substrate-use efficiency is expressed as thermodynamic efficiency (Papers I – III) or as carbon-use efficiency (Paper III).

Thermodynamic efficiency (η_{eff}) was calculated from heat production and residual substrate in soil solution. Thus, it is a substrate-based approach. In order to calculate η_{eff} , the equation by (Battley, 1960, 1987) was further developed to consider residual substrate in soil solution:

$$\eta_{eff} = 1 - \left(\frac{Q_{substrate}}{\Delta H_{Initial} - \Delta H_{Residual}}\right) \quad (1)$$

where $Q_{substrate}$ (J g^{-1} soil) is the heat production from microbial metabolism after substrate addition, $\Delta H_{Initial}$ (J g^{-1} soil) is the heat of combustion of the initially added substrate, and $\Delta H_{Residual}$ (J g^{-1} soil) is the heat of combustion of the residual substrate (see Chapter 4.4.1). The numerator in *Equation (1)* is the energy content of substrate undergoing decomposition during the incubation period.

Carbon-use efficiency (*CUE*) was applied as a biomass-based approach and calculated from microbial respiration and substrate incorporation into microbial biomass. The equation by Frey *et al.* (2001) was used:

$$\mathrm{CUE} = \frac{\mathrm{d}MBC_{substrate}}{(\mathrm{d}MBC_{substrate} + \sum CO_2 - C_{substrate})} \quad (2)$$

where d$MBC_{substrate}$ (µg C g^{-1} soil) is the change in microbial biomass C from incorporation of substrate C and $\sum CO_2$-$C_{substrate}$ is the cumulative loss of substrate C from respiration.

In Papers I & III, a *time-based approach* was used to calculate substrate-use efficiency, i.e. substrate-use efficiency was determined over the same time period, while in Paper II substrate-use efficiency was calculated using a *consumption-based approach,* i.e. using time periods with same substrate consumption. In the latter, investigation periods varied in their temporal length.

4.6 Calorespirometric Ratio (Papers I & IV)

The calorespirometric ratio γ was tested as a relative measure for substrate-use efficiency. It is the ratio of heat-to-CO_2 production (Hansen *et al.*, 2004):

$$\gamma = \frac{Q}{co_2} \quad (3)$$

where Q (J g^{-1} soil) and CO_2 (mol CO_2 g^{-1} soil or µg CO_2-C g^{-1} soil) are the heat and CO_2 released from samples after substrate addition. Changes in the calorespirometric ratio can indicate variation in microbial substrate-use efficiency, shifts in metabolic pathways and/or changes in organic substrate undergoing decomposition (Hansen *et al.*, 2004; Barros *et al.*, 2011, 2016; Herrmann *et al.*, 2014).

4.7 Microbial Community Composition (Papers I – III)

The composition of microbial communities was determined by phospholipid fatty acid (PLFA) analysis using the method of Frostegård *et al.* (1993). In Paper III, PLFA analysis was combined with stable isotopes and uniformly ^{13}C labelled substrates were used. Substrate incorporation and isotope enrichment into PLFAs was determined on a gas chromatograph coupled to an isotope ratio mass spectrometer (TraceUltra GC interfaced to Delta^{Plus} XP IRMS via GC Combustion III, Thermo Finnigan, Bremen, Germany). Isotope ratios were corrected for C added during the PLFA extraction procedure using a mass-balance equation (Crossman *et al.*, 2004).

5 Results and Discussion

Carbon-use and thermodynamic efficiency revealed overall similar patterns across soil treatments (Paper III), and results are therefore referred to as substrate-use efficiency in the following.

5.1 Temperature Sensitivity (Papers II & III)

Overall, microbial substrate-use efficiency was temperature sensitive and declined with warming (Papers II & III). Decreases in efficiency varied largely across land use and substrate amendments. This general observation is summarised in *Figure 5* and the range was between 0.03 up to 0.53. Similar changes in efficiencies were observed in previous studies (*Table 1* above).

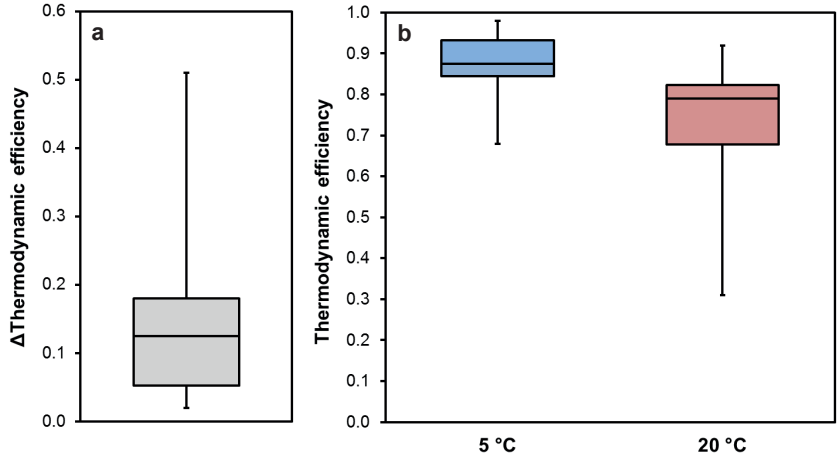

Figure 5. Temperature effects on substrate-use efficiency measured in Papers II & III: boxplots show (a) the decrease in thermodynamic efficiency between 5 and 20 °C and (b) the actual values of thermodynamic efficiencies measured at 5 and 20 °C. Graphs display mean values of similar sample treatments.

The decreases in substrate-use efficiency with warming may be triggered by (i) different temperature sensitivities of microbial respiration and substrate uptake (Manzoni *et al.*, 2012) and/or (ii) increased maintenance requirements at higher temperatures (Steinweg *et al.*, 2008; Dijkstra *et al.*, 2011; Manzoni *et al.*, 2012). The two are indications that there are changes in microbial physiology and the studies in this thesis revealed indications of both: (i) In Paper II & III, microbial respiration and substrate uptake revealed different temperature sensitivities (c.f. *Figure 1 and Table 2 & 3 in Paper II*; *Table 2 in Paper III*). Such a pattern was also found by Schindlbacher *et al.* (2015). (ii) Enzyme production is often considered as a maintenance requirement (van Bodegom, 2007; Dijkstra *et al.*, 2011) which consumes and decreases the amount of resources available for biomass production (Manzoni *et al.*, 2012). Because enzyme turnover is faster at higher temperatures (Wallenstein *et al.*, 2011; Bradford, 2013), costs for enzyme production should increase with temperature (Dijkstra *et al.*, 2011; Manzoni *et al.*, 2012). Consequently, substrate-use efficiency should show a distinct temperature response for substrates which require multiple enzymatic steps for decomposition. Such an effect was revealed in Paper II when comparing efficiencies of glucose and glycogen over a temperature range of 5-20 °C. Glycogen requires more enzymatic steps than glucose and within each land use management system, substrate-use efficiencies decreased more markedly for glycogen than for glucose with increasing temperatures (*Figure 6*).

In Paper II, substrate-use efficiencies were measured across a set of five temperatures. Results revealed a non-linear temperature response of efficiency to warming for ley farming, grassland and forest systems whereas in arable soils, efficiencies were constant over the entire temperature range (*Figure 6*). Values were constant in a temperature range from 5 to 12.5 °C and declined beyond 12.5 °C when efficiencies were temperature sensitive. The decrease beyond 12.5 °C was different across land use management systems and was most pronounced in forest soils with 0.14 and 0.28 for glucose and glycogen amended samples, respectively (*Figure 6*). The decreases in ley farming and grassland systems were similar to each other and reached up to 0.09 and 0.15 for glucose and glycogen amended samples, respectively (*Figure 6*). In theory, the different temperature sensitivities could have been triggered by differences in the composition of the microbial communities (see Chapter 3.1.2), but results from Paper III emphasised that changes in physiology rather than shifts in microbial community composition can be held responsible for temperature responses of substrate-use efficiency (see Chapter 5.2). Further, community profiles using PLFA analysis varied little among all soils and differences were

insignificant between arable, ley farming, and grassland soils (*Table 2 in Paper II*).

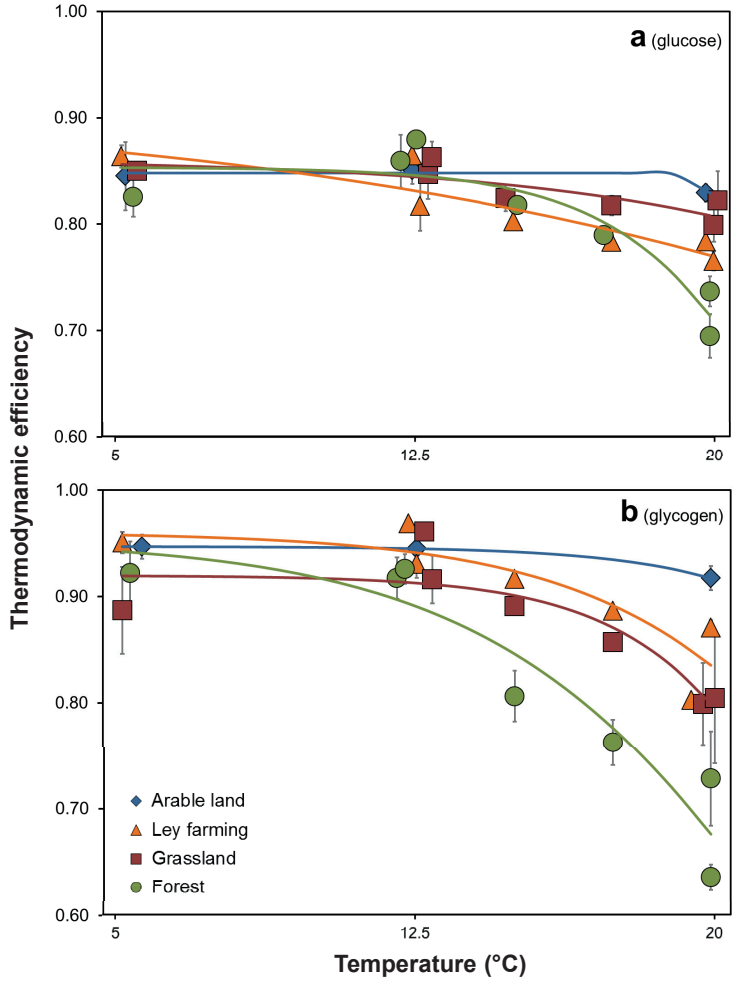

Figure 6. Temperature response of thermodynamic efficiency in soils from different land use management systems amended with (a) glucose or (b) glycogen. Lines show fitted specific temperature response functions for each land use management system. Results are displayed as means (n = 3) and whiskers show standard errors. Symbols are slightly shifted with respect to temperature to improve visibility. Note: x-axis crosses y-axis at 0.60.

In the literature, results from temperature incubations are frequently reported as decrease in substrate-use efficiency per unit °C suggesting a linear decrease in efficiency with warming (Allison *et al.*, 2010; Tucker *et al.*, 2013; Hagerty *et al.*, 2014; Schindlbacher *et al.*, 2015). Yet, curvilinear temperature responses are commonly observed in many studies where samples were incubated at three or more temperatures (Devêvre & Horwáth, 2000; Wetterstedt & Ågren, 2011; Frey *et al.*, 2013; Tucker *et al.*, 2013; Schindlbacher *et al.*, 2015). Linear responses of substrate-use efficiency with warming can also be found, but are less common (Frey *et al.*, 2013). Generally, two curvilinear responses can be distinguished: (i) The first pattern is more frequent and shows a strong decrease in efficiency with warming at lower temperatures (mainly below 15 °C) and levels off at higher temperatures (Devêvre & Horwáth, 2000; Wetterstedt & Ågren, 2011; Frey *et al.*, 2013; Tucker *et al.*, 2013). (ii) In the second pattern, substrate-use efficiency decreases moderately or remains constant with warming at lower temperatures and decreases strongly with further warming above a temperature threshold (roughly above 10 °C) (Schindlbacher *et al.*, 2015; Paper II). This second temperature response pattern may be explained by different temperature sensitivities of microbial respiration and substrate uptake (see Chapter 3.1.1). Nevertheless, underlying mechanisms causing the various temperature responses remain unclear. However, understanding the temperature responses of substrate-use efficiency is crucial for accurate projections of SOC stocks under a changing climate. Recently, SOC models were improved by explicitly considering microbial physiology assuming linear temperature responses of substrate-use efficiency (Allison *et al.*, 2010; Wieder *et al.*, 2013). These models are sensitive to changes in efficiency and may be further improved when non-linear temperature responses are incorporated. Therefore, further studies should aim to understand the drivers behind different temperature responses of substrate-use efficiency.

The modelling study in Paper II revealed that projected losses of SOC varied significantly across land use management systems when land use specific temperature responses of substrate-use efficiency are considered (*Figure 7*). As substrate-use efficiency was temperature insensitive in arable soils, no significant changes in steady state SOC stocks are projected. But, projected losses range from 2.5 to 15.0 % in forest soils and 6.3 to 38.6 % in grassland soils, depending on the location and the assumed temperature increase of either 2 °C or 4 °C (*Figure 7*). Projected losses are higher at locations with higher current mean annual temperature, because substrate-use efficiency declined more strongly at higher temperatures (*Figure 6*). Changes in steady state SOC stocks are huge, although the decrease in efficiency during

model runs were below 0.02 (i.e. 2 % change in absolute efficiency). The modelling approach confirms that substrate-use efficiency is one of the factors to which SOC stocks react most sensitively (Hyvönen *et al.*, 1998). Therefore, understanding the drivers behind varying temperature sensitivities across land use management systems needs to be a next step. This is vital as changes in land use management are proclaimed as a possible mitigation measure against climate change by sequestering C (Post & Kwon, 2000; Lai, 2004; Knorr *et al.*, 2005). Such C sequestration may be overestimated, if land use specific temperature responses of substrate-use efficiency are neglected.

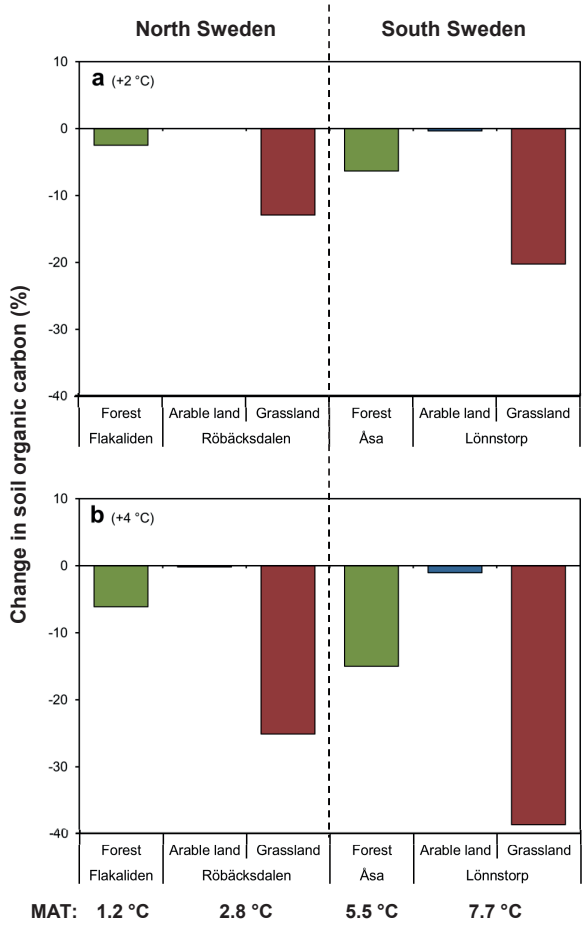

Figure 7. Modelled development of soil organic carbon stocks in soils at case study sites in Flakaliden, Röbacksdalen (northern Sweden) as well as Åsa and Lönnstorp (southern Sweden). Results show projected relative changes in soil organic carbon stocks for an increase in mean annual temperature (MAT) of (a) 2 °C or (b) 4 °C.

5.2 Microbial Community Composition (Papers I & III)

Varying substrate-use efficiencies could be a result of (i) shifts in microbial community composition, (ii) physiological adjustments within the same community, or (ii) a combination of the two (Manzoni *et al.*, 2012; Schimel & Schaeffer, 2012). My results emphasised that major differences in substrate-use efficiency between temperatures are most likely caused by changes in microbial physiology, and shifts in community composition may be of minor importance for varying efficiencies.

In Paper III, substrate-use efficiencies decreased significantly with increasing temperature. Decreases ranged from 0.06 up to 0.53 (*Figure 8*). The relative incorporation of substrate C into biomarker groups changed, however, little within the same groups and across temperature treatments (*Figure 2 in Paper III*). Thus, it is unlikely that specific microbial groups altered their contribution to substrate decomposition much and the minor changes found cannot explain large decreases in efficiency, because substrate-use efficiency is only slightly sensitive to shifts in active microbial communities. Therefore, the observed decreases in substrate-use efficiency were more likely to have been caused by physiological alterations within mostly similar microbial communities. This finding was further supported by the different temperature sensitivities of microbial respiration and substrate uptake described above (*Table 2 in Paper III*, see Chapter 5.1).

Besides the major contribution of microbial physiology to changes in substrate-use efficiency, shifts in the composition of active microbial communities could have contributed to observed efficiency changes to a minor extend. In Paper III, fungi were very active in utilising phenol. Their activity was disproportionally high considering their low total abundance in examined soils (*Figure 2 in Paper III*). The high activity of fungi may point towards a relatively high substrate-use efficiency of fungi, because microbial communities decomposed phenol with the highest efficiency of all substrates (*Figure 8*). This indication is supported by research findings in Paper I. Here, fungi showed a higher abundance in forest soils compared to soils derived from agricultural land use (*Figure 4a and Table 3 in Paper I*) and microbial communities in forest soils had significantly higher efficiencies when compared to arable, ley farming, and grassland soils (*Figure 3a in Paper I*). These findings support the common assumption of fungi having a higher substrate-use efficiency than bacteria (see Chapter 3.1.2; Holland & Coleman, 1987; Ohtonen *et al.*, 1999; Six *et al.*, 2006). Further, Gram-negative bacteria were more abundant (Paper I) or active (Paper II) in soil samples which showed high substrate-use efficiency (*Figure 3a & 4a and Table 3 in Paper I*, c.f. *Figure 1 & 2 in Paper III*). Thus, these results indicate that Gram-negative

bacteria may have relatively high substrate-use efficiency and they confirm results from previous studies (Harris *et al.*, 2012; Creamer *et al.*, 2015). Moreover, a clue that Gram-positive bacteria tend to have relatively low efficiencies compared to other microbial groups was found in Paper III. Gram-positive bacteria were more actively involved in decomposing substrate at 20 °C compared to 5 °C (*Figure 2 in Paper III*) and at 20 °C substrate-use efficiency was generally lower than at 5 °C (*Figure 8*). The indication that Gram-negative bacteria have relatively high and Gram-positive relatively low efficiencies is, however, not in line with the assumption regarding life history strategies. Gram-negative bacteria are assumed to represent fast growing, inefficient r-strategists and Gram-positive bacteria are considered to represent slow growing, efficient K-strategists (de Vries & Shade, 2013). The theory of the r-K continuum is, however, a quite simplified concept and has been criticised as being over-simplistic (Reznick *et al.*, 2002). It may be too simplistic to improve our understanding of substrate-use efficiency.

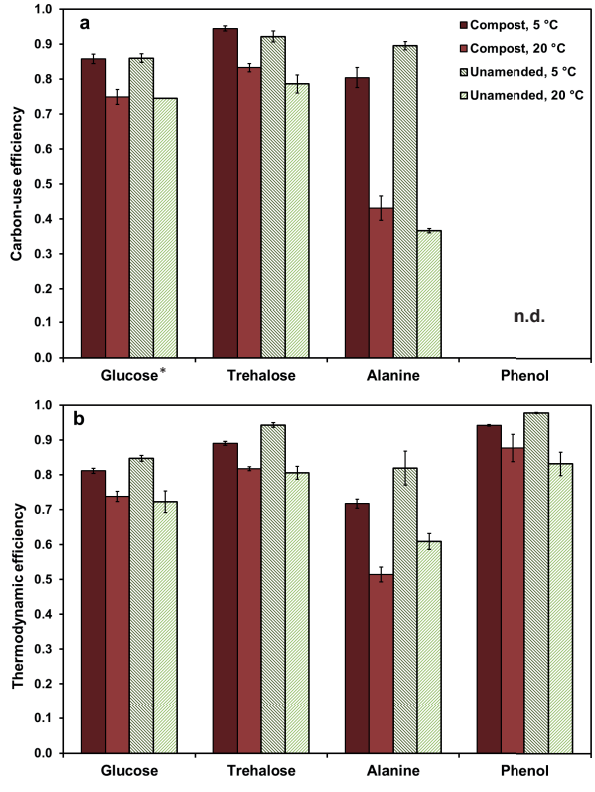

Figure 8. Results of substrate-use efficiency expressed as (a) carbon-use efficiency and (b) thermodynamic efficiency; results are displayed as means (n = 3) and whiskers show standard errors; * = no whiskers indicate data from a single value; n.d. = no data.

5.3 Substrate Quality (Papers I – III)

Microbial substrate-use efficiencies varied among different C sources (Paper I-III). Nevertheless, efficiencies in relation to different C sources were not simply a matter of simple organic matter[1]. In theory, substrate-use efficiency should decrease with increasing substrate complexity, because more enzymatic steps are required for decomposition. In the present thesis, I have used glucose, trehalose and glycogen representing carbohydrates with similar repeating polymer units. In contrast to my initial hypothesis, the monosaccharide glucose was utilised less efficiently than the disaccharide trehalose (*Figure 8*) and the polysaccharide glycogen (*Figure 3a in Paper I* and *Figure 6*). This was despite the fact that trehalose and glycogen require additional enzymatic steps. When costs for enzyme production are higher, substrate-use efficiency should decrease (Manzoni *et al.*, 2012). Yet, a wide range of microorganisms have the capacity to synthesise and degrade trehalose and glycogen. For instance, both substrates are widely used as storage compounds (Argüelles, 2000; Henrissat *et al.*, 2002) and the microbial communities in the investigated soils may have enzymes readily available that are required for degradation of trehalose and glycogen. These substrates may therefore not be 'difficult to decompose' for microorganisms. In this case, investment costs for enzyme production would not have occurred during the short-term incubation period. Decomposition of the more complex substrate glycogen revealed, however, a stronger temperature response of substrate-use efficiency than decomposition of the simple glucose (*Figure 6*). Enzymes should have turned over faster at the higher temperatures (Wallenstein *et al.*, 2011; Bradford, 2013) and enzyme production may have resulted in a decrease of substrate-use efficiency. The stronger decrease in efficiency for glycogen compared to glucose could be explained by the larger number of required enzymatic steps and thus larger number of required enzymes.

Next to glycogen, alanine amended samples showed a large decrease in efficiency with increasing temperature (*Figure 8*). This decrease was more pronounced than the decrease observed in glucose, trehalose, and phenol amended samples. Alanine was the only amino acid, i.e. a source of N, added to soil samples. Alanine addition could therefore have triggered overflow respiration if microorganisms needed to gain N (Keiblinger *et al.*, 2010; Manzoni *et al.*, 2012). As microbial activity usually increases with temperature, overflow respiration may have been accelerated and thus triggered the strong decline of substrate-use efficiency in alanine amended samples.

1. Sentence inspired by Nunan *et al.* (2015).

Although the outcome of the experiments are not in line with the thermodynamic argument (i.e. higher activation energy causes lower efficiency), they emphasise the importance of using several C substrates simultaneously when evaluating efficiency. Otherwise, varying efficiencies among substrates (Frey *et al.*, 2013) may remain concealed and results from single substrate experiments should not be generalised. The application of diverse substrates enlarges, however, the number of required samples. This may not always be feasible but the high-throughput method using the calorespirometric ratio (Paper IV) allows rapid screening of microbial substrate-use efficiency and substrate mixtures could be used (Schindlbacher *et al.*, 2015). The set of applied substrates should not only concentrate on one substrate groups (e.g. sugars), but rather use different carbohydrates, amino and organic acids as well as aromatic compounds. Further, studies analysing the effect of substrate quality on substrate-use efficiency should be combined with analysis of enzymes present (Burns *et al.*, 2012). Such combined analyses would test if investment costs for enzyme production occurred during the experiment.

For the combined analysis of thermodynamic and carbon-use efficiency (Paper III), samples were amended with glucose, trehalose, or alanine. These substrates have equal C-to-energy ratios (Nunan et al., 2015) and the ratios are close to the ratio of microbial biomass (Manzoni et al., 2012). Therefore, substrates supplied C and energy in the ratio needed by the microorganism and neither C nor energy needed to be wasted. This explains the similar results for thermodynamic and carbon-use efficiency (*Figure 8*).

6 What is efficiency?

Defining efficiency comes down to being a philosophical question as it depends on what we measure and why we measure certain properties. From a physical-chemical or economical viewpoint, efficiency is the ratio between output-input, where output is the part of the input which is used for an aimed outcome. In soil systems, microbial substrate-use efficiency is an important property as it determines the allocation of SOC to biosynthesis and respiratory losses. It is of scientific interest, because even small changes may have a large impact on future soil C emissions and stocks of SOC (Hyvönen *et al.*, 1998; Allison *et al.*, 2010).

In the majority of cases, substrate-use efficiency is evaluated over constant time periods, these are time-based approaches (Devêvre & Horwáth, 2000; Frey *et al.*, 2013; Tucker *et al.*, 2013; Dijkstra *et al.*, 2015; Schindlbacher *et al.*, 2015). I have used time-based as well as consumption-based approaches in my PhD thesis and the same data was analysed by applying both approaches: In Paper I, thermodynamic efficiency was measured across four land use management systems at 12.5 °C and over an incubation period of 32 hours. In Paper II, the same data were analysed, but thermodynamic efficiency was calculated when 15 % of the added substrate was consumed (i.e. consumption-based approach). The results differ between the two approaches. The time-based approach (Paper I) revealed significantly higher efficiencies in forest soils compared to soils from other land use systems. In contrast, using the consumption-based approach (Paper II), substrate-use efficiencies were similar across all systems of land use (*Figure 9*). The purpose of both studies was, however, different as Paper I aimed to explore substrate-use efficiencies across land use systems at a single temperature, whereas in Paper II substrate-use efficiency was measured across a set of different temperatures. Geyer *et al.* (2016) pointed out that time is a crucial factor regarding investigations of substrate-use efficiency. Drivers of efficiency may change over time and

confounding effects from microbial turnover (Hagerty *et al.*, 2014) and grazing (Frey *et al.*, 2001) become more pronounced with increased length of the incubation periods. The time scale as considered by Geyer *et al.* (2016) is, however, a proxy for the magnitude to which driving and confounding processes take place. But, the magnitude of these processes varies across temperatures, because temperature accelerates nearly all chemical, physical, and biological processes. Therefore, application of time-based approaches are more appropriate when evaluating efficiency under the same environmental conditions (e.g. temperature, *Figure 10a*), whereas under different environmental conditions (e.g. differences in temperature), a consumption-based approach is more appropriate (*Figure 10b*). Consumption-based approaches ensure that microbial communities experience similar workloads and these approaches make it more likely that non-metabolic drivers of substrate-use efficiency have similar magnitudes in samples under different environmental conditions. Choosing the approach will, however, depend on the question addressed. The question of similar workloads for microbial communities should be further discussed by the scientific community and consequences should be analysed.

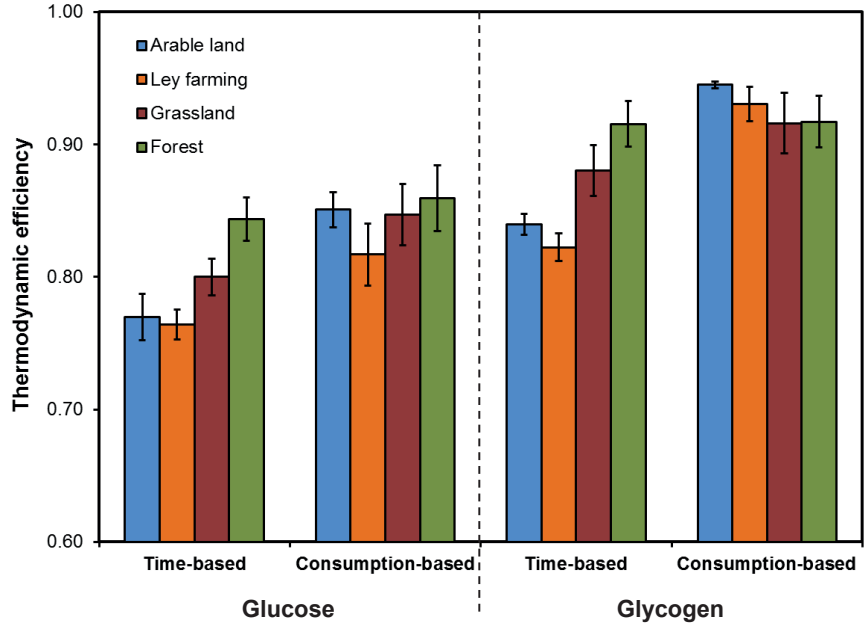

Figure 9. Thermodynamic efficiencies measured across various land use management systems at 12.5 °C and applying a time-based or consumption-based approach. Note: x-axis crosses y-axis at 0.60.

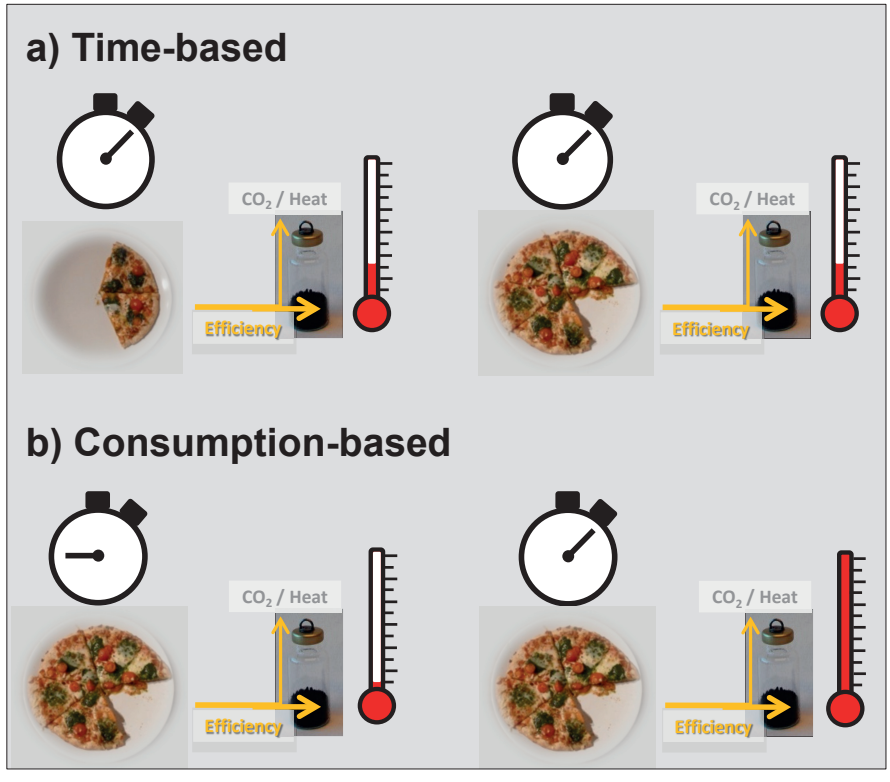

Figure 10. Schematic overview of substrate-use efficiency approaches: (a) time-based approaches measure efficiency over same time periods, but varying substrate consumption; (b) consumption-based approaches measure efficiency over same substrate consumption, but varying time. (Photos: T. Bölscher)

The output fraction of substrate-use efficiency, i.e. C directed towards biosynthesis, comprises all kind of microbial products such as new cells, storage compounds, structural repair, enzymes or other exudates (van Bodegom, 2007). Substrate-based efficiency approaches measure all these microbial products as output (see *Equation 1* above). Biomass-based approaches are, however, primarily used and they do not consider exudates as an output in efficiency calculations (see *Equation 2* above). Independent of the applied approach, scientists often refer to substrate-use efficiency as microbial growth efficiency (e.g. Allison *et al.*, 2010; Manzoni *et al.*, 2012; Geyer *et al.*, 2016), and it is suggested that substrate-use efficiency be measured by applying growth-based approaches, i.e. only new cells are considered as an output (Sinsabaugh *et al.*, 2013). However, recognising substrate-use efficiency as the efficiency of microbial growth is based on the assumption of steady state conditions. This assumption keeps the structure of computer

models simple and makes consideration of soil C fluxes more comprehensible[2]. Growth is here defined as change in microbial biomass between two time points and this change defines C storage in soils. But, common approaches of substrate-use efficiency do not measure changes in biomass. Instead, they measure incorporation of isotopically labelled substrate into biomass (e.g. Frey *et al.*, 2013; Tucker *et al.*, 2013; Schindlbacher *et al.*, 2015) or, less commonly, DNA (Spohn *et al.*, 2016a; b). In both cases, gross rather than net production of microbial biomass is measured, because parts of the biomass are constantly turned over or consumed by grazers (Frey *et al.*, 2001; Hagerty *et al.*, 2014). Approaches using isotopic labelling techniques do not consider this effect as labelled atoms are not only incorporated into additional biomass (i.e. net change), but newly formed biomass also replaces dead cells (Sinsabaugh *et al.*, 2013). Thus, labelling approaches estimate the change in microbial biomass rather than quantifying it directly, i.e. they do not quantify what is needed for steady state assumption. Although results derived from substrate-based efficiency approaches can also be confounded by microbial turnover, they do quantify all parts of the utilised substrate remaining in the soil at present. Thus they may be more appropriate in comparison with biomass-based and growth-based approaches, especially as the steady state assumption can be generally questioned. In soils, environmental conditions change regularly throughout the four seasons, and under consideration of climate change with increasing mean annual temperatures and elevated CO_2 concentrations (Stocker *et al.*, 2013). For general investigations of substrate-use efficiency, substrate-based approaches may be most appropriate. Yet, for assumption of steady state, approaches should be further developed to measure true net biomass production. Measurements of the latter may be appropriate for providing data for certain, i.e. steady state, modelling approaches.

Carbon dioxide emissions are of major concern in respect to future climate scenarios and projected C stocks in soils. Microbial carbon-use efficiency is therefore commonly investigated. Nevertheless, thermodynamic efficiency is an alternative approach, because heat release from microbial metabolism is closely connected to catabolic processes (Sparling, 1981a; b; Li *et al.*, 2009). Compared to measurements of CO_2 production, calorimetric investigations offer the advantage of continuous measurements, they are less labour-intensive, and results are less variable (Paper III). Smaller variation when using thermodynamic efficiency in comparison to carbon-use efficiency may be a result of calorimetric measurements quantifying all heat production from the entire microbial metabolism and not only processes which lead to CO_2 release (Herrmann *et al.*, 2014). Both approaches should, however, come to similar

[2]. Personal communication G.I. Ågren, SLU, Sweden

results when the C-to-energy ratio of substrate is close to the C-to-energy ratio of microbial biomass which is the case for carbohydrates and some amino acids (Manzoni *et al.*, 2012). This was shown empirically in Paper III (*Figure 8*). When the C-to-energy ratios of substrate and microbial biomass differ, results from thermodynamic and carbon-use efficiency approaches may differ as well. Energy limitations should cause carbon-use efficiency to be less than thermodynamic efficiency whereas C limitations should lead to thermodynamic efficiency being less than carbon-use efficiency (Gommers et al., 1988; Manzoni et al., 2012). Simultaneous measurements of both efficiencies could be used to test the effect of various substrate C-to-energy ratios on microbial substrate-use efficiency (Manzoni *et al.*, 2012). Such an investigation should apply various substrates covering a wide range of substrate C-to-energy ratios (LaRowe & Van Cappellen, 2011; Nunan *et al.*, 2015).

The calorespirometric ratio (Paper I & IV) is the relation between heat dissipated per unit CO_2 respired. It is not a direct measure of substrate-use efficiency, because efficiency is generally defined as the ratio between output and input (see discussion above). Nevertheless, the calorespirometric ratio can be used as an index of efficiency, because it tends to decline with increasing efficiency when the same organic material is undergoing decomposition (*Figure 5a in Paper I*; Hansen *et al.*, 2004; Barros *et al.*, 2010; Wadsö & Hansen, 2015). Especially in combination with the proposed high-throughput approach (Paper IV), it can be used as a quick and simple screening method.

For further investigations, I recommend defining substrate-use efficiency based on overall substrate utilisation by microorganisms rather than microbial growth. For measurements at a single temperature, time-based approaches should be applied, whereas consumption-based approaches should be used when temperatures or other environment conditions vary in research studies.

7 Conclusions

Microbial substrate-use efficiency is increasingly recognised as a crucial factor in the terrestrial C cycle and during SOM decomposition (Geyer *et al.*, 2016). The main aim of my thesis was to enhance our mechanistic understanding of substrate-use efficiency and its drivers under special consideration of climate change. From the findings of the conducted studies, the following conclusions can be drawn:

- Substrate-use efficiency was found to generally decrease with increasing temperature. The temperature sensitivity of substrate-use efficiency was non-linear and varied across land use management systems.
- Temperature responses of substrate-use efficiency were driven more by changes in microbial physiology than shifts in the composition of the active microbial community. Nevertheless, indications for varying efficiencies across microbial groups were found with fungi and Gram-negative bacteria tending towards higher efficiencies than other microbial groups.
- Microbial communities utilised various substrates with different efficiencies, but the quality of the substrate was a poor indicator for the magnitude of efficiency.
- Projections of SOC stocks were very sensitive to small changes in substrate-use efficiency.
- The calorespirometric ratio can be used for high-throughput screening of substrate-use efficiencies.
- Consumption-based approaches of substrate-use efficiency are more appropriate if various incubation temperatures are used in the investigation. Time-based approaches are more appropriate for investigations using single incubation temperatures.

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