Differences in substrate use efficiency: Impacts of microbial community composition, land use management and substrate complexity

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Abstract

Microbial substrate use efficiency is an important property in process-based soil organic matter models, but is often assumed to be constant in mechanistic models. However, previous studies question if a constant efficiency is appropriate, in particular when evaluating carbon (C) cycling across temperatures and various substrates. In the present study, we evaluated the relation between substrate use efficiency, microbial community composition and substrate complexity in contrasting long-term management regimes (47-49 years of either arable, ley farming, grassland or forest systems). Microbial community composition was assessed by phospholipid fatty acid analysis and three indices of substrate use efficiencies were considered: (i) thermodynamic efficiency, (ii) calorespirometric ratio and (iii) metabolic quotient. Three substrates D-glucose, L-alanine or glycogen, varying in complexity, were added separately to soils, and heat production as well as C mineralization were determined over a 32-hours incubation period at 12.5 °C. Microbial communities from forest systems were most efficient in utilizing substrates, supporting our hypothesis that maturing ecosystems become more efficient. These changes in efficiency were linked to microbial community composition with fungi and Gram-negative bacteria being important biomarkers. Despite our initial hypothesis, complex substrate such as glycogen was utilized most efficiently. Our findings emphasize that differences in land use management systems as well as the composition of soil organic matter need to be considered when modelling C dynamics in soils. Further research is required to establish and evaluate appropriate proxies for substrate use efficiencies in various ecosystems.

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Introduction

Soil microorganisms are key players in governing the terrestrial carbon (C) cycle (Schimel and Schaeffer 2012). But due to the complexity of soils, they are often not specifically incorporated into simplistic process-based models used to predict soil organic matter decomposition. For example, the Century-model (Parton et al. 1987), RothC model (Coleman and Jenkinson 2014) or Q-model (Ågren and Bosatta 1987) treat the microbial compartment system as a 'black box'. During microbial decomposition, organic C is partitioned between respiratory energy production and substrate assimilated into microbial biomass and stabilized in soil organic matter. This partitioning is often referred to as substrate use efficiency or C use efficiency, and it is an important microbial physiological feature in determining the fate of C during organic matter decomposition in soils. This property is often assumed to be constant in process-based models, but research indicates that substrate use efficiency is (i) temperature dependent (Devêvre and Horwáth 2000; Steinweg et al. 2008; Wetterstedt and Ågren 2011; Frey et al. 2013; Tucker et al. 2013) and (ii) varies among C substrates (Frey et al. 2013). Recent model frameworks emphasize that changes in microbial physiology i.e. varying substrate use efficiency have (i) significant impacts on global soil C stocks (Allison et al. 2010; Frey et al. 2013; Wieder et al. 2013) and (ii) their incorporation may improve future climate change model projections (Allison et al. 2010; Wieder et al. 2013). The underlying process mechanisms of substrate use efficiency are still unclear, but differences in efficiencies have been reported to reflect microbial community composition (Harris et al. 2012; Herrmann et al. 2014; Creamer et al. 2015).

Recent research suggests that differences in substrate use efficiencies may be linked to the relative abundance of fungi and Gram-negative bacteria in an arable ecosystem (Harris et al. 2012; Herrmann et al. 2014) or a forest ecosystem (Creamer et al. 2015). These studies emphasize that microbial community composition may play a significant role in determining substrate use efficiencies within one land use management system. Yet, little is known if and how substrate use efficiencies vary across different land use management systems. Theoretical frameworks suggest that maturing ecosystems along a successional gradient become more complex in terms of their food web and biodiversity, and they increase their efficiency in utilizing resources (Odum 1969; Addiscott 1995). Arable soils are annually disturbed through ploughing and/or other tillage practices whereas grassland ecosystems are less frequently disturbed and forest ecosystems are the end point of a successional gradient. Thus, these land use management systems can be considered as systems representing certain stages within a successional gradient

following disturbance. Microbial community composition changes along this gradient. In comparison to arable and grassland ecosystems, forest ecosystems contain relatively more fungi than bacteria (Bossio et al. 2005; Cookson et al. 2007; Drenovsky et al. 2010), and it is often assumed that soil fungi have higher substrate use efficiencies than soil bacteria (Holland and Coleman 1987; Herrmann et al. 2014). Hence, we would expect that microbial communities residing in forest would have higher substrate use efficiency in comparison with communities residing in arable systems.

Besides differences in substrate use efficiencies, soil fungi (e.g. wood decomposing fungi) have the ability to decompose complex soil organic matter through the production of extracellular enzymes (Bödeker et al. 2009; 2014). The thermodynamic argument suggests that reactions metabolizing structurally complex, aromatic components have higher activation energies than reactions metabolizing structurally simpler, more labile components (Bosatta and Ågren 1999). Thus, utilization of complex substrates requires higher initial energy costs reducing net energy gain (Bradford 2013). Furthermore, metabolic pathways during decomposition are dependent on the nature of the utilized substrate and show varying respiration rates leading to variable substrate use efficiencies (Gommers et al. 1988; Manzoni et al. 2012). Simple carbohydrates such as glucose are primarily used when evaluating microbial substrate use efficiency (e.g. Dijkstra et al. 2011; Tucker et al. 2013; Blagodatskaya et al. 2014). However, soil organic matter consists of heterogeneous organic material with inherent chemical energy stored below-ground which is exchanged within the soil system. Recent research has shown that the use of various substrates resulted in varying efficiencies (Frey et al. 2013) emphasizing the importance to evaluate several substrates when assessing microbial substrate use efficiencies.

Substrate induced respiration in combination with incorporation of C into the microbial biomass is often used to evaluate substrate use efficiency (e.g. Behera and Wagner 1974; Blagodatskaya et al. 2014). Recently, microbial energetics approaches such as thermodynamic efficiency of microbial communities (Harris et al. 2012) and calorespirometric ratio (Barros et al. 2010) have been tested for exploring microbial substrate use efficiencies in soil systems. Isothermal calorimetry is used to determine these indices and its main advantage is that it quantifies all metabolic processes not only those accounted for by CO_2 respiration measurements. Thus, it provides complementary information to the CO_2 respiratory approach (Herrmann et al. 2014). Thermodynamic efficiency is a dimensionless index of substrate use efficiency and high values of this index indicate that microbial metabolism is

efficient (Harris et al. 2012). The calorespirometric ratio is metabolic heat released in relation to CO_2 production (i.e. J mol^{-1} CO_2 or mJ μg CO_2-C). If the same organic material is undergoing decomposition, changes in calorespirometric ratios indicate differences in substrate use efficiencies with decreasing ratios indicating an increase in efficiencies (Hansen et al. 2004). Both indices are independent of the amount of microbial biomass residing in soils. Previously, Anderson and Domsch (1986, 1990, 2010) used the ratio of respiration to microbial biomass (i.e. μg CO_2-C μg^{-1} biomass-C), i.e. the microbial metabolic quotient, as an index for substrate use efficiency of microbial communities. This quotient has been previously criticized (Wardle and Ghani 1995; Nannipieri et al. 2003) and should be therefore used with care. However, the microbial biomass is an important soil property when using the metabolic quotient for assessment of substrate use efficiency. So far, these three indices have not been used in the same study and it is not known if they would lead to similar conclusions.

The aim of the present study was to test the hypotheses that (i) substrate use efficiency increases with maturing ecosystems along a successional gradient, (ii) the increase in efficiency is due to changes in microbial community composition, and (iii) chemically complex substrates result in a decrease in efficiency in comparison with labile organic material. Furthermore, we evaluated various substrate use efficiency indices using linear regression analysis.

Material and Methods

Soils

In August 2012, we sampled soils from the agricultural long-term field experiment in Röbäcksdalen (63°48'N, 20°14'E) and the forest long-term nutrient fertilization experiment at Flakaliden (64°07'N, 19°27'E). Both research sites are closely located to the city of Umeå, Northern Sweden, and exposed to a boreal climate. Mean annual temperature in the area is 2.3 °C; ranging from -8.7 °C in February to 14.4 °C in July (Coucheney et al. 2013). The field experiment in Röbäcksdalen was established in 1965 on an *Eutric Cambisol* (FAO) (Bergkvist and Öborn 2011), and we selected three land use management systems: (i) barley annually (*arable land*), (ii) barley for one year followed by a two-year period with green fallow (*ley farming*) and (iii) barley for one year followed by a five-year period with green fallow (*grassland*) (Bergkvist and Öborn 2011). At the time of soil sampling, the ley farming and grassland management systems were in their second and fifth year of green fallow, respectively. Samples were taken

121 from a depth of 0 – 10 cm in the A horizon. The Flakaliden long-term field experiment was established in 1986 on a
122 forest stand with Norway spruce (*Picea abies*) which was planted in 1963 on a *Haplic Podzol* (FAO) (Linder 1995).
123 We sampled the control treatment from a depth of 2 – 10 cm representing the E horizon. The control treatment
124 received no nutrient addition but soils were irrigated with water in order to avoid biases due to water stress. For each
125 land use management system, we sampled soils from three field replicates, taking 25 to 30 subsamples per replicate
126 which were thoroughly mixed and combined to one sample per replicate. Soils were sieved to 2 mm, plant material
127 removed and soils were then adjusted to 50 % of their water holding capacity. Samples were stored frozen until
128 further use. Additional soil data are given in Table 1.

129 *Incubation experiment*

130 Soils were pre-incubated for 14 days at 12.5 °C to allow the microbial respiration flush from fresh organic matter
131 released due to sampling and freezing procedure to subside (Herrmann and Witter 2002). This temperature
132 corresponded to the mean air temperatures at the long-term field experimental sites during the vegetation period
133 (May-September). After the pre-incubation period, soils were then divided into three sets of subsamples for
134 determination of heat production, C mineralization and evaluation of the soil microbial biomass.

135 The first set was used for calorimetric measurements. For each soil management system, four aliquots of
136 soil (5 g soil dry weight) were placed into 20 ml glass reaction vessels and each vessel was sealed with an admix
137 ampule set up consisting of two 1 mL syringes (Fig. 1). Each admix ampule contained either a solution of D-
138 glucose, L-alanine, glycogen or double deionized water as control. The substrates were selected as they are all water
139 soluble. D-glucose and L-alanine were chosen as representatives for simple substrates, with L-alanine being
140 additionally a nitrogen source, whereas glycogen was chosen as a representative for complex substrates (Henrissat et
141 al. 2002). Prior to the start of the experiment, we tested soluble starch but this substrate precipitated shortly after
142 dissolution and was thus not applicable for the experiment. Therefore, glycogen was used as a complex substrate as
143 it is water soluble, has a similar structure to starch and is used as a storage compound by soil microorganisms
144 (Dijkstra et al. 2015). The samples where then introduced into a TAM Air isothermal calorimeter (TA Instruments,
145 USA) with the thermostat set to 12.5 °C. The calorimeter was then sealed and the samples were allowed to
146 equilibrate for 18 to 19 h. After equilibration (at time 0 h), substrate solutions (75 µl g^{-1} soil) were added drop-wise
147 providing 500 µg C g^{-1} soil. All substrate solution additions increased the water content of the samples to 65 % of

148 their water holding capacity. Heat production rates were measured continuously over 32 h after substrate addition.
149 The syringes were thoroughly cleaned with ethanol and repeatedly rinsed with deionized water after each use. At the
150 end of the incubation period, samples were freeze-dried for subsequent determination of residual substrate in soil
151 solution as described below. Initial substrate in soil solution was determined at the start of the incubation i.e. at time
152 0 h, on separate aliquots of soils that were amended with C substrates and immediately freeze-dried.

153 The second set of four aliquots (20 g soil dry weight) was amended with 500 μg C g^{-1} soil of D-glucose, L-
154 alanine or glycogen solution or double deionized water as control. Samples were placed into 0.5 l airtight glass
155 containers and incubated for 32 hours at 12.5 °C, and evolved CO_2 was analyzed consecutively every five to nine
156 hours with an infrared gas analyzer (EGM-4, Environmental Gas Monitor, PP systems, UK).

157 The third set was used to evaluate the biomass and composition of microbial communities in soils at the
158 end of the 14-days pre-incubation period. Microbial biomass was determined using the chloroform-fumigation-
159 extraction method (Vance et al. 1987) with minor modifications (Dahlin and Witter 1998). Extracted organic C was
160 measured as total organic C (TOC-5000A, Shimadzu, Japan), and the extracted C by fumigation was converted to
161 microbial biomass C using a k_{ec} factor of 0.45 (Wu et al. 1990). Phospholipid fatty acid (PLFA) analysis was used to
162 assess the microbial community composition using the method of Frostegård et al. (1993b). Briefly, phospholipids
163 were extracted from 1 g of freeze-dried soil using a chloroform, methanol and citrate buffer in the ratio 1:2:0.8
164 (v/v/v), fractionated by solid phase extraction and then derivatized by mild alkaline methanolysis (Börjesson et al.
165 1998). The resultant fatty acid methyl esters were analyzed by gas chromatography (6890 Series GC System,
166 Hewlett-Packard Company, USA). Fungal-to-bacterial ratio (F:B ratio) was based on the abundance of the fungal
167 PLFA biomarker 18:2ω6 and 18:1ω9 (Federle 1986) and the sum of 11 bacterial PLFA biomarkers (i15:0, a15:0,
168 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, cy17:0, 17:0, 18:1ω7 and cy19:0; Frostegård et al. 1993a).

169 *Substrate in soil solution*

170 Utilized substrate has to be taken into account when estimating the thermodynamic efficiency of soil microbial
171 communities (see Eq. 1). However, assays for the quantification of D-glucose, L-alanine and glycogen in soil
172 solution are not readily available within soil research. We, therefore, tested if a commercial assay kit and assays
173 established within animal sciences are applicable for soil samples. Unless indicated otherwise, soils from one field

174 replicate (5 g dry soil) of each land use management were amended with 375 µl of either (i) D-glucose,
175 (ii) L-alanine or (iii) glycogen solution (for substrate concentrations see supplementary Table S1). Substrates were
176 dissolved in 0.25 M K_2SO_4 or 0.1 M HCl to ensure the same background matrix for standard curves as well as for
177 the determination of substrate recovery in soil solution (see below). Because it was necessary to store amended soil
178 samples for a significant amount of time prior analysis, we also tested if freeze-drying prior soil extraction will have
179 an effect on the amount of substrate measured in soil solution, i.e. fresh soil versus freeze-drying soil prior substrate
180 extraction were compared. Three technical replicates were used for each treatment.

181 For D-glucose quantification, we used a commercial available enzymatic assay kit (Glucose (GO) Assay
182 Kit, GAGO-20, Sigma-Aldrich, USA). D-glucose in soil samples was extracted by shaking with 0.25 M K_2SO_4
183 (soil:extractant mass-to-volume ratio of 1:4) for 30 minutes followed by centrifugation (3 minutes at 740 xg) and
184 filtration using a 0.2 µm nylon syringe filter. 0.5 ml of the filtrate were then mixed with 1.0 ml of enzymatic assay
185 reagent and the mixture was placed into a dark water bath at 37 °C. To stop the enzymatic reaction, 1 ml 6 M H_2SO_4
186 solution was added after 30 minutes and the absorbance was measured at 540 nm (GENESYS 20, Thermo Scientific,
187 USA). A calibration curve of absorbance (x-axis) versus glucose concentrations in freeze-dried samples (y-axis) was
188 established and absorbance data from glucose concentration in soil solutions in the incubation experiment were
189 fitted to a linear model ($R^2 = 0.98$) as follows: $y = 156.5x - 9.2$.

190 Quantification of L-alanine was done by adapting a method used for animal plasma (Reverter et al. 1997):
191 L-alanine in soil samples was extracted by shaking with 0.1 M HCl (soil:extractant mass-to-volume ratio of 1:4) for
192 30 minutes followed by centrifugation (3 minutes at 740 xg). 800 µl of the supernatant were then mixed with 30 %
193 (w/v) 5-Sulfosalicylic acid and centrifuged for 30 minutes at 14 000 xg. 200 µl of the mixture were transferred into
194 30 kDa centrifugal filter units (Microcon-30 with Ultracel-30 membrane, Merck Millipore, USA) and centrifuged
195 for 10 minutes at 14 000 xg. The filtrate was diluted to an estimated concentration of 250 pmol/µl and analyzed
196 using Ultra-Performance Liquid Chromatography (UPLC) (Dionex UltiMate 3000 RS, Thermo Scientific, USA)
197 after derivatization (AccQ Tag Ultra, Waters, UK). The amino acid norvaline was used in a dilution series to
198 establish a calibration curve ($R^2 = 1.0$).

199 Results from the D-glucose and L-alanine assays indicate that freeze-drying prior substrate extraction did
200 not affect substrate concentrations (data not shown) and therefore only freeze-dried samples were analyzed in the

glycogen assay. Glycogen in soil samples was extracted by shaking with 0.25 M K_2SO_4 (see above), and glycogen levels were quantified as D-glucose equivalent after 7 M HCl hydrolysis (extractant:HCl ratio of 1:1; 1 hour in boiling water bath to accelerate hydrolysis), followed by neutralization with 3.5 M K_2CO_3 (Geary et al. 1981). D-glucose concentrations were quantified on 0.5 ml of soil extract using the enzymatic D-glucose assay kit (see above). Recovery of substrate in soil solutions varied among land use management systems, and therefore individual calibration curves were established for each management system (supplementary Fig. S1).

Microbial substrate use efficiency indices

We calculated microbial substrate use efficiencies with three indices: (i) thermodynamic efficiency, (ii) calorespirometric ratio and (iii) metabolic quotient of soil microbial communities.

Thermodynamic efficiency (η_{eff}) was expressed by adapting the equations of Battley (1960; 1987) and Harris et al. (2012). In the present study, added substrate was not completely decomposed during the incubation period and therefore utilized substrate needs to be taken into account when calculating thermodynamic efficiency:

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

where Q (J g^{-1} soil) is heat produced from microbial metabolism, $\Delta H_{Initial}$ (J g^{-1} soil) and $\Delta H_{Residual}$ (J g^{-1} soil) is the combustion enthalpy of initial substrate in soil solution and residual substrate in soil solution at the end of the incubation period, respectively. The difference between $\Delta H_{Initial}$ and $\Delta H_{Residual}$ is the combustion enthalpy of the utilized substrate, i.e. it is the theoretically available energy for metabolic processes during the incubation period. The standard enthalpy of combustion ΔH_c° is 2,816.8 kJ mol^{-1} for D-glucose, 1,626.1 kJ mol^{-1} for L-alanine and 2,841.3 kJ mol^{-1} for the smallest repeating polymer unit of glycogen (Washburn 2003). These values were used to calculate $\Delta H_{Initial}$ and $\Delta H_{Residual}$. In the present work, 19.5 J g^{-1} soil for D-glucose, 22.6 J g^{-1} soil for L-alanine and 19.8 J g^{-1} soil for glycogen were added to the soil samples.

The calorespirometric ratio γ (J mol^{-1} CO_2 or mJ μg^{-1} CO_2-C) is the ratio of heat production and CO_2 production (Hansen et al. 2004):

$$\gamma = \frac{Q}{CO_2} \quad (2)$$

where Q (J g^{-1} soil) and CO_2 (mol CO_2 g^{-1} soil or μg^{-1} CO_2-C g^{-1} soil) are the heat and CO_2 production after substrate addition, respectively.

The microbial metabolic quotient (qCO_2) is the ratio of CO_2 production per unit microbial biomass C (Anderson and Domsch 1985a; 1985b):

$$qCO_2 = \frac{CO_2}{C_{mic}} \quad (3)$$

where C_{mic} (μg C g^{-1} soil) is the microbial biomass C determined by fumigation extraction (see above).

Statistical analysis

Resultant data (i.e. thermodynamic efficiencies, calorespirometric ratios and metabolic quotients) were analyzed by two-way ANOVA (i.e. land use management system and C substrate were the two explanatory variables and the interaction effect between these variables was also tested) and homogeneous groups of mean established using Tukey's HSD test (Minitab 17 Statistical Software, 2010). Within each land use management, results were analyzed by one-way ANOVA followed by Tukey's HSD test. All data were tested for normality using Anderson-Darling test and equal variances using Levene's test. If necessary, data was log or square root transformed prior analysis to obtain normal distribution and equal variance. For analysis of microbial community composition and efficiency profiles, we used R version 3.0.0 (R Core Team 2013) and the 'vegan: Community Ecology Package' version 2.0-9 (Oksanen et al. 2013). Profiles were examined with principle component (PCA) analysis using normalized covariance of mole percent PLFA, thermodynamic efficiency values or calorespirometric ratios. Significant differences between land use management systems along ordination axes were analyzed by post hoc one-way ANOVA followed by Tukey's HSD on PC scores. The associations between thermodynamic efficiency, calorespirometric ratios and PLFA data were determined by comparing the dissimilarity matrices of each of the data sets using the Mantel test based on the Pearson product-moment correlation coefficient (999 permutations). Linear regression analysis was used to evaluate equivalence between thermodynamic efficiency (X-axis) and calorespirometric ratio or metabolic quotient (Y-axis).

Results

In forest management systems, heat production rates were constant when soils were amended with glucose or glycogen (Fig. 2a and c) whereas in alanine amended samples heat production rates increased from ca. 2 $\mu W\ g^{-1}$ soil to ca. 10 $\mu W\ g^{-1}$ soil (Fig. 2b) during the 32-hours incubation period. The other three land use management systems increased slightly up to ca. 45 $\mu W\ g^{-1}$ soil during the incubation (Fig. 2), but based on heat flow data exponential microbial growth was not observed in any of the amended soil systems. Land use management systems and substrate type had significant effects on cumulative heat produced from microbial metabolism over the 32 hours incubation period (Table 2). Overall, heat production of soils from different land use managements decreased in the order ley farming > arable land/grassland > forest ecosystems ($P < 0.01$; Table 2). Across all land uses, D-glucose amended management systems produced significantly more heat in comparison with systems amended with L-alanine or glycogen ($P < 0.01$, Table 2). These patterns were mirrored in CO_2 production (Table 2), but utilized substrate ($\Delta H_{Initial} - \Delta H_{Residual}$) resulted in a different pattern. Although we observed significant differences in heat production between management systems, substrate utilization was similar within each substrate class ($P = 0.06$, Table 2). Across all management systems, utilization of D-glucose and glycogen were significantly higher than L-alanine utilization ($P < 0.01$, Table 2). The largest total heat production was observed in the ley farming system amended with D-glucose (Table 2). Taking this heat production, Thornton's rule, and the ideal gas equation into account, O_2-concentration decreased from 21 to 17 % at the most indicating that sufficient O_2 was present in the reaction vials throughout the entire incubation period (data not shown).

Taking the heat output and utilized substrate into account, thermodynamic efficiency of soil microbial communities (Eq. 1) ranged between 0.63 and 0.92 with significant differences among land use management systems as well as applied C substrate (Fig. 3a). Generally, microbial communities residing in forest soils used substrates most efficiently and microorganisms in ley farming systems were least efficient in substrate use among the four management systems ($P < 0.05$, Fig. 3a). Thermodynamic efficiencies of microbial communities residing in arable land and grassland systems were in between but not significantly different to the ley farming system (Fig. 3a). Glycogen was used most efficiently by soil microorganisms followed by glucose and the efficiency of microbial L-alanine use was the lowest among the three substrates ($P < 0.01$, Fig. 3a). Mean calorespirometric ratios (Eq. 2) ranged from 22 to 59 mJ μg^{-1} CO_2-C (Fig. 3b). Here, only land use management had a significant effect on calorespirometric ratios. Forest soils revealed on average the lowest ratio of 28 mJ μg^{-1} CO_2-C indicating highest microbial substrate use efficiency among the four land use management systems (Fig. 3b). Ley farming systems

277 resulted in the highest calorespirometric ratios (on average 44 mJ μg^{-1} CO_2-C), i.e. lowest substrate use efficiency (P
278 < 0.05). The ratios of arable land and grassland management systems were in between (both on average 40 mJ μg^{-1}
279 CO_2-C) with no significant differences to the other two land use management systems (Fig. 3b). The microbial
280 metabolic quotient (Eq. 3) was highest for arable land use and then decreased in the following order: ley farming,
281 grassland and forest management systems (Fig. 3c). Furthermore, D-glucose amended soils showed the highest
282 metabolic quotient among the three substrates ($P < 0.01$) indicating that microorganisms used glucose less
283 efficiently in comparison with L-alanine and glycogen.

284 The PCA of PLFA data revealed a clear separation between communities of different land use management
285 systems ($P < 0.01$), and the eigenvalues of the first two components of the PLFA data together accounted for 78 %
286 of the total variance of the PLFA profiles (Fig. 4a). Forest soil microbial communities were separated from arable
287 land, grassland and ley farming communities along PC1 ($P < 0.01$). Along PC2, microbial communities of arable
288 land were separated from communities of grassland and ley farming soils ($P < 0.01$). Biomarkers of actinomycetes
289 (10Me16:0; Zelles 1999), Gram-negative bacteria (18:1ω7c; Frostegård et al. 1993a) and fungi (18:1ω9c; Federle
290 1986; Bååth 2003) were the main drivers responsible for the separation of microbial communities (Fig. 4a). Pairwise
291 comparison of dissimilarity matrices between microbial community and efficiency profiles revealed significant
292 similarities between the two (Mantel $R = 0.59$, $P < 0.01$, cf. Fig. 4a and B; Mantel $R = 0.53$, $P < 0.01$, cf. Fig. 4a and
293 c). Total amount of fungi, Gram-negative biomarkers and the F:B and Gram-negative:Gram-positive ratios were
294 significantly higher in the forest system in comparison with the other three management systems (Table 3).

295 The correlation coefficients of the linear regression between the efficiency indices were poor when using
296 data across all land uses and substrate amendments together ($R^2 = 0.32$ and 0.01 for calorespirometric ratios and
297 microbial metabolic quotient, respectively). However, when we analyzed the data separately for each substrate,
298 thermodynamic efficiency and calorespirometric ratio showed a significant negative correlation for soils amended
299 with L-alanine and glycogen amendments, but there was no relation between the two indices when D-glucose was
300 added to soils (Fig. 5a). Substrate specific correlations between thermodynamic efficiency and metabolic quotient
301 were not significant for D-glucose and L-alanine amended samples and poor for glycogen amended samples ($R^2 =$
302 0.09, 0.10 and 0.54, respectively; see Fig. 5b).

303 **Discussion**

Substrate use efficiencies across land use management systems

Our initial hypothesis that substrate use efficiencies increase along a successional gradient due to changes in microbial community composition was partially confirmed. Irrespectively of efficiency indices, microbial communities in forest soils were most efficient in using substrates and separated clearly from arable, grassland and ley farming management systems (Fig. 3). Forest soils had a higher abundance of fungi and Gram-negative bacteria as well as higher F:B and Gram-negative:Gram-positive ratios in comparison with the other three management systems (Table 3). Our results therefore support the common assumption that fungi are more efficient in utilizing soil organic matter in comparison to bacteria (Holland and Coleman 1987; Herrmann et al. 2014), and that the abundance of Gram-negative bacteria may be of importance for differences in substrate use efficiencies (Harris et al. 2012; Creamer et al. 2015). The Mantel tests for dissimilarities support the hypothesis that there may be a link between the composition of the microbial community and substrate use efficiency. Differences in microbial substrate use efficiencies among arable land, grassland and ley farming management systems were not significant, but grassland systems tend to have higher efficiencies than ley farming and arable systems (Fig. 3). This observation supports our initial hypothesis that substrate use efficiencies increase with maturing ecosystems along a successional gradient. These three management systems were located at the same field site in Röbäcksdalen. Here, microbial community composition was similar except that arable land use management differed from ley farming and grassland systems due to a higher abundance of actinomycetes (Table 3; Fig. 4). However, there was no relationship between actinomycetes and microbial substrate use efficiencies ($R^2 = 0.26$ for thermodynamic efficiency), and we therefore conclude that actinomycetes are unlikely an important biomarker that could be made responsible for differences in substrate use efficiency. We expected that 47 years of various soil management systems at Röbäcksdalen would result in significant differences in soil microbial communities as observed in various land use management systems (e.g. Bossio et al. 2005; Cookson et al. 2007; Drenovsky et al. 2010). Although, along two successional gradients at the Kellogg Biological Station (Michigan State University, USA), Jangid et al. (2011) showed that legacy effects of past management still have an influence on soil microbial community composition, particularly in early succession sites. Our grassland management system consists of a five-year period with green fallow followed by one year barley and therefore, it represents a management system in early succession. Thus, the timeframe and/or type of land use managements at Röbäcksdalen were not sufficient to alter soil microbial

community composition significantly. This in turn may explain why we did not observe significant differences in microbial substrate use efficiencies among the management systems at the Röbäcksdalen site.

Differences in microbial substrate use efficiencies between the sites at Röbäcksdalen and Flakaliden (forest system) could also be due to differences in general soil characteristics such as soil texture and pH (Table 1) (Blagodatskaya and Anderson 1998; Bååth and Anderson 2003; Rousk and Bååth 2011; Manzoni et al. 2012), and we cannot conclude firmly that differences in microbial community composition are the driver for changes in microbial substrate use efficiencies. To test Odum's (1969) and Addiscott's (1995) theory of increasing substrate use efficiency along a successional gradient, future studies could consider (i) the use of selective inhibition of microbial groups (Rousk et al. 2008) or (ii) gamma radiation following re-inoculation with microbial communities of different complexities obtained through a combination of soil fumigation, dilution and filtering techniques (Griffiths et al. 2004). Such studies would avoid confounding effects of major soil properties such as soil texture and/or pH (Table 1; Delmont et al. 2014), and they should provide unequivocal evidence on the relationship between microbial community composition and microbial substrate use efficiency along a successional gradient.

Carbon chemistry and substrate use efficiency

Our initial hypothesis that chemically complex substrates are utilized with a lower efficiency than labile substrates was not confirmed. Out of the three substrates used, we regarded glycogen as the most complex substrate as it is a multi-branched polysaccharide. Decomposition of glycogen requires debranching enzymes and further degradation by glycogen phosphorylases or glycosidases to glucose, glucose-1-phosphate or maltose (Henrissat et al. 2002). Bosatta and Ågren (1999) defined substrate quality as the number of enzymatic steps required for breaking down a substrate, and they state that low quality, complex structures have therefore higher activation energies. Thus, we assumed that investments costs for microbial decomposition of glycogen should be higher in comparison with D-glucose resulting in a lower substrate use efficiency of glycogen. But, conversely to our hypothesis, this substrate had either the highest (Fig. 3a and c) or similar substrate use efficiencies (Fig. 3b) compared with D-glucose. Most microorganisms have the capacity to synthesize and degrade glycogen as they use it as intracellular storage compound for energy (Henrissat et al. 2002). High efficiencies of glycogen may be the result that microbial communities have the enzymatic set for decomposition of this substrate readily available in these soils, and therefore glycogen may have been utilized without initial investment costs. Furthermore, we deem it unlikely that evolution

would select for a widely common storage compound which requires large energy losses during decomposition. Future studies should include analysis of enzymes present in the soil (Burns et al. 2012; Nannipieri et al. 2012).

Implications for soil organic matter modelling

Research has implied that substrate use efficiency is temperature dependent (Devêvre and Horwáth 2000; Steinweg et al. 2008; Wetterstedt and Ågren 2011; Tucker et al. 2013; Frey et al. 2013), and assuming varying substrate use efficiencies in modelling frameworks have significant consequences for projections of global soil C stocks (Allison et al. 2010; Frey et al. 2013; Wieder et al. 2013). Temperature dependency of substrate use efficiencies is therefore of major concern when modelling terrestrial C cycling. In our study, thermodynamic efficiencies varied up to 32 % across various land use management systems and C substrates (mean values varied between 0.63 and 0.92). Within the same substrate amendment, thermodynamic efficiencies varied between 10 and 20 % across land use management systems (i.e. mean values varied between 0.76 to 0.84, 0.63 to 0.80 or 0.82 to 0.92 for D-glucose, L-alanine or glycogen, respectively; Fig. 3a). Similarly, within the same land use management system but across various substrates, thermodynamic efficiencies varied between 10 and 30 % (mean values varied between 0.71 to 0.84, 0.64 to 0.83, 0.63 to 0.88 or 0.80 to 0.92 for arable land, ley farming, grassland or forest, respectively; Fig. 3a). These variations are in a similar range as temperature induced changes in microbial substrate use efficiencies when temperature changes by 10 °C (Devêvre and Horwáth 2000; Steinweg et al. 2008; Tucker et al. 2013; Frey et al. 2013). A recent model framework showed that relative alteration in substrate use efficiency by 10 to 30 % could result in a change of 1 to 4 % of total organic C stored below-ground within 98 years, corresponding to approx. 90 to 270 g C m^{-2} in a forest system (Frey et al. 2013). Thus, our results emphasize that variation in substrate use efficiency across land use management systems and various substrates are equally important as temperature induced changes in efficiencies. The composition of soil organic matter changes significantly along a successional gradient (Quideau et al. 2001; Garnier et al. 2004; Merilä et al. 2010), and future research should focus on evaluating potential proxies for microbial substrate use efficiencies that are applicable in modeling approaches of soil organic matter dynamics.

Soil C models commonly assume values of substrate use efficiency ≤0.55 (Parton et al. 1987; Ågren and Bosatta 1987; Coleman and Jenkinson 2014). In comparison, our thermodynamic efficiency values are above this value, i.e. they are between 0.67 and 0.92 (Fig. 3a). Such high efficiencies were reported previously (Steinweg et al.

2008; Dijkstra et al. 2011; Tucker et al. 2013; Frey et al. 2013; Hagerty et al. 2014) but these values are criticized being experimental biases due to a combination of short incubation time and storage compound synthesis (Nguyen and Guckert 2001; Hill et al. 2008; Sinsabaugh et al. 2013; Reischke et al. 2014; Blagodatskaya et al. 2014; Reischke et al. 2015). Dijkstra et al. (2015) could not confirm that high efficiencies are related to an experimental bias using position-specific labelled substrates, and they emphasized that the hypothesis of high efficiencies warrants further testing. Despite high values in substrate use efficiencies, our results confirm previous studies (Devêvre and Horwáth 2000; Frey et al. 2013) showing that microbial substrate efficiencies varies among C substrates. This should be taken into account when modelling soil organic matter decomposition in ecosystems. Our research emphasizes that further research in evaluating microbial substrate use efficiencies should focus on a set of different C substrates which may help to improve our mechanistic understanding of terrestrial C cycling.

Compatibility of different efficiency indices

The three efficiency indices resulted in the same overall conclusion, namely that the microbial community of the forest soil was most efficient in using the substrates. However, efficiency is generally defined as the ratio between an output and an input, and in most cases it is good if efficiency is high. From the indices used in the present study, only thermodynamic efficiency uses an input-output approach (Eq. 1). It is similar to approaches traditionally used for microbial C use efficiency where biomass production (output) is related to utilized substrate (input) (Frey et al. 2001). Modelling of C dynamics in soils requires such values of substrate use efficiency which are based on the concept of input-output. Still, measuring the amount of utilized substrate (Eq. 1) may not always be feasible and/or rapid screening of microbial substrate use efficiency is required (Herrmann and Bölscher 2015). Under such circumstances, calorespirometric ratios may be a good proxy for relative substrate use efficiency, but only within the same substrate class (Fig. 5). Furthermore, the calorespirometric ratio has the potential for combined investigations of substrate use efficiency, substrate quality and metabolic pathways (Barros et al. 2016). It should be noted that calorespirometric ratios varied substantially, but this variation could be reduced, if heat and CO_2 production are measured in the same sample (Barros et al. 2011; Herrmann and Bölscher 2015). The metabolic quotient is not clearly related to thermodynamic efficiencies (Fig. 5) and applying this quotient may result in different overall conclusions in comparison with the thermodynamic efficiency index (see e.g. Harris et al. 2012). The underlying assumption in the metabolic quotient is that the amount of biomass is of importance when evaluating microbial

412 substrate use efficiency, but it has been questioned if the microbial biomass is a pivotal characteristic for C turnover
413 in soils (Kemmitt et al. 2008). Therefore, we consider that the metabolic quotient should be applied with care when
414 evaluating microbial substrate use efficiencies.

415 **Conclusions**

416 Our study revealed differences in substrate use efficiencies among land use management systems with
417 microorganisms residing in forest systems utilizing resources most efficiently. These findings support our
418 hypothesis that microbial efficiencies increase with ecosystem maturity. The composition of microbial community
419 may determine substrate use efficiency, and fungi as well as Gram-negative bacteria appear to be important
420 biomarkers for differences in efficiencies. Furthermore, substrate use efficiency varied among resources, but
421 complexity was not a good proxy for changes in efficiencies. Hence, the hypothesis that chemically complex
422 substrates are metabolized with lower efficiency in comparison with labile organic material could not be confirmed.
423 Our proposed thermodynamic efficiency provides values necessary for soil organic matter modelling, but the
424 calorespirometric ratio could be used as an alternative when rapid screening of microbial substrate use efficiency is
425 required. This study emphasizes that differences in land use management systems as well as the composition of soil
426 organic matter may need to be considered when modelling C dynamics in terrestrial ecosystems. Our results warrant
427 further investigation into establishing and evaluating appropriate proxies for substrate use efficiencies in various
428 ecosystems.

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623

624

Figure Legends

Fig. 1 Schematic representation of the TAM Air 20 ml Admix ampule set up system

Fig. 2 Heat production rates over 32 h incubation from (A) D-glucose, (B) L-alanine and (C) glycogen amended soil samples. Values displaying means (n = 3) and whiskers show standard errors

Fig. 3 Results of substrate use efficiency expressed as (A) thermodynamic efficiency, (B) calorespirometric ratios, and (C) microbial metabolic quotient (qCO_2). Values display means (n = 3) and whiskers show standard errors; common symbols after land use management regimes (see legend) and substrates (see x-axis) indicate homogenous means analyzing of overall effects (two-way ANOVA and Tukey's HSD test at 5 % significance level). Values of calorespirometric ratios are expressed as mJ μg^{-1} CO_2-C and kJ mol^{-1} CO_2 to facilitate comparison with previous studies

Fig. 4 Microbial community composition and substrate use efficiency profiling. Principle component analysis representing (A) microbial community composition by PLFA, (B) thermodynamic efficiency profiles and (C) calorespirometric ratio profiles in different land use management systems. Values in parentheses on axis labels denote % variation accounted for by the respective components

Fig. 5 Substrate specific correlations between thermodynamic efficiencies (x-axis) and (A) calorespirometric ratios (y-axis) and (B) microbial metabolic quotient (qCO_2) (y-axis)

Fig. 1

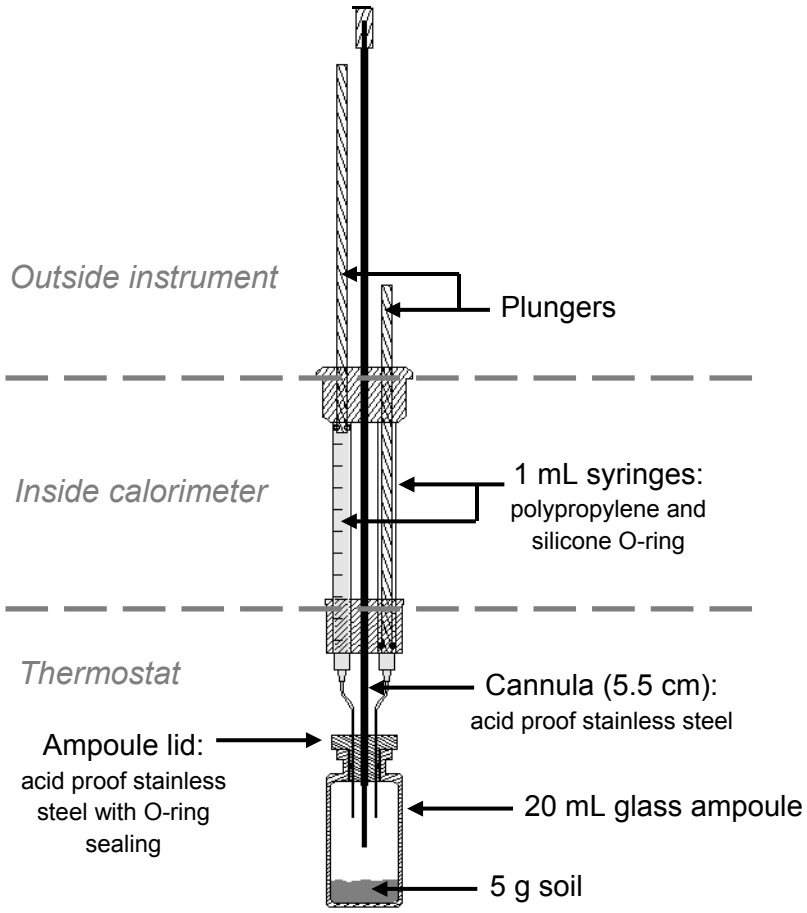

Outside instrument
Plungers
Inside calorimeter
1 mL syringes:
polypropylene and
silicone O-ring
Thermostat
Cannula (5.5 cm):
acid proof stainless steel
Ampoule lid:
acid proof stainless
steel with O-ring
sealing
20 mL glass ampoule
5 g soil

Fig. 2

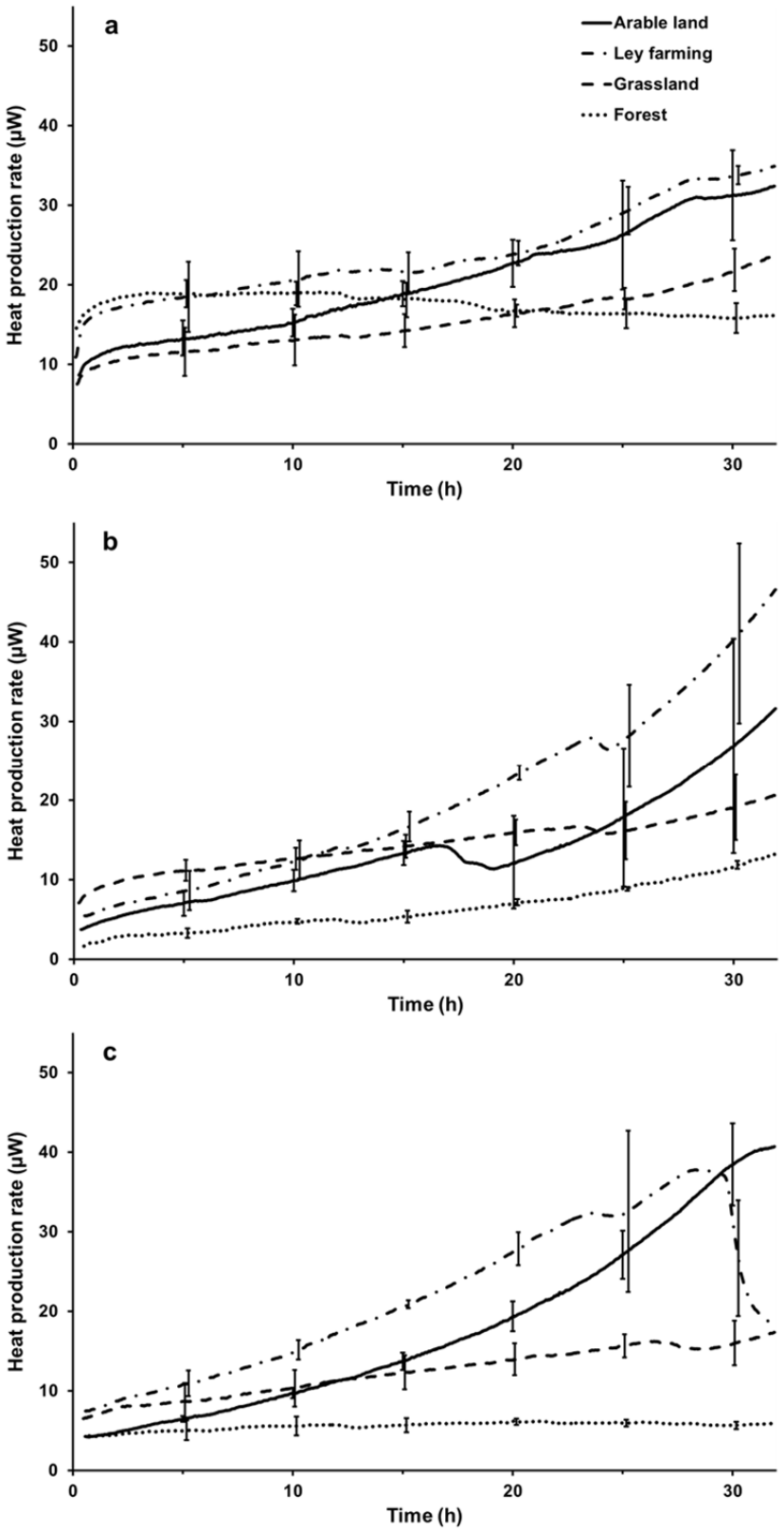

a
Arable land
Ley farming
Grassland
Forest
Heat production rate (µW)
Time (h)
b
Heat production rate (µW)
Time (h)
c
Heat production rate (µW)
Time (h)

Fig. 3

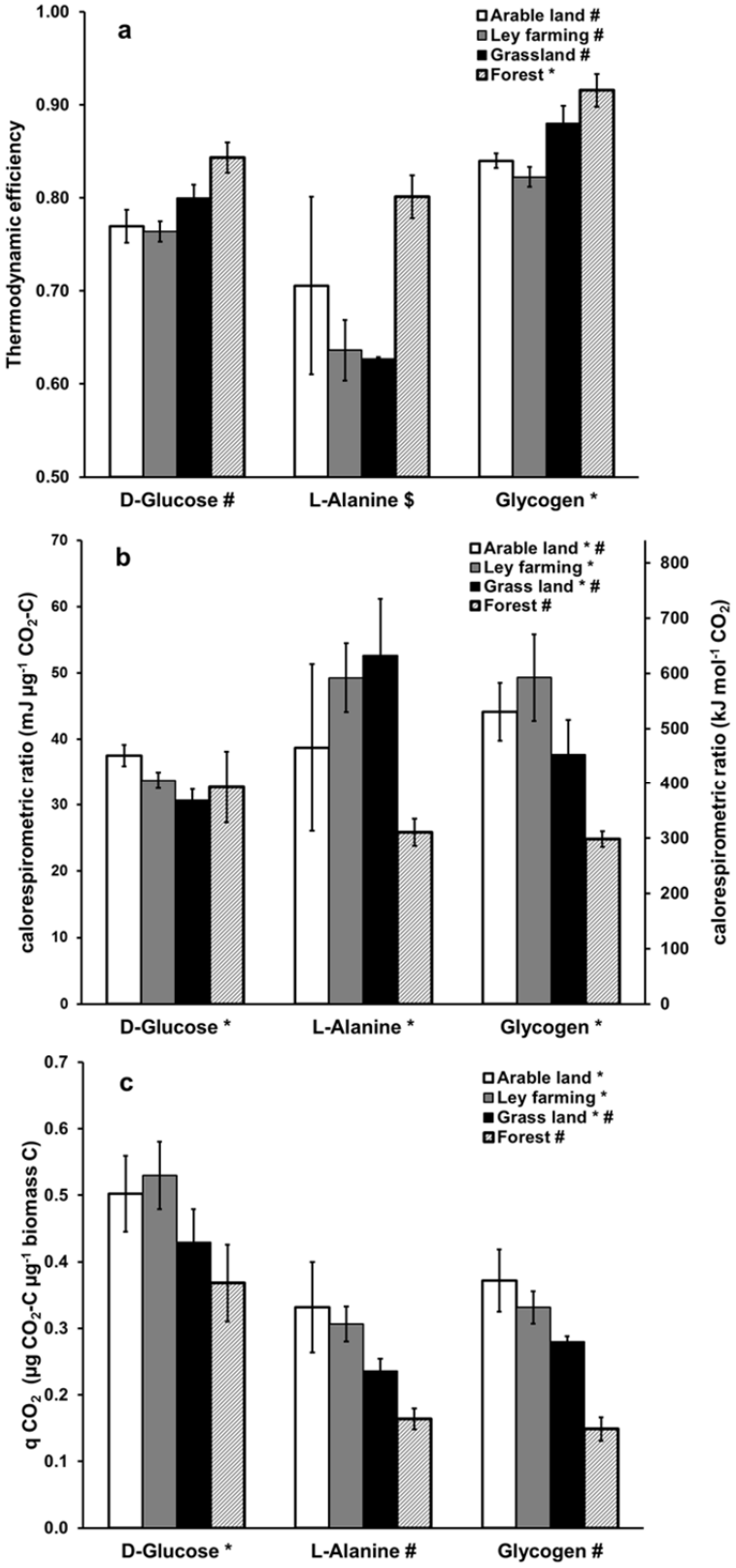

a
Arable land #
Ley farming #
Grassland #
Forest *
Thermodynamic efficiency
1.00
0.90
0.80
0.70
0.60
0.50
D-Glucose #
L-Alanine $
Glycogen *
b
Arable land * #
Ley farming *
Grass land * #
Forest #
calorespirometric ratio (mJ µg-1 CO2-C)
calorespirometric ratio (kJ mol-1 CO2)
70
60
50
40
30
20
10
0
800
700
600
500
400
300
200
100
0
D-Glucose *
L-Alanine *
Glycogen *
c
Arable land *
Ley farming *
Grass land * #
Forest #
q CO2 (µg CO2-C µg-1 biomass C)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
D-Glucose *
L-Alanine #
Glycogen #

Fig. 4

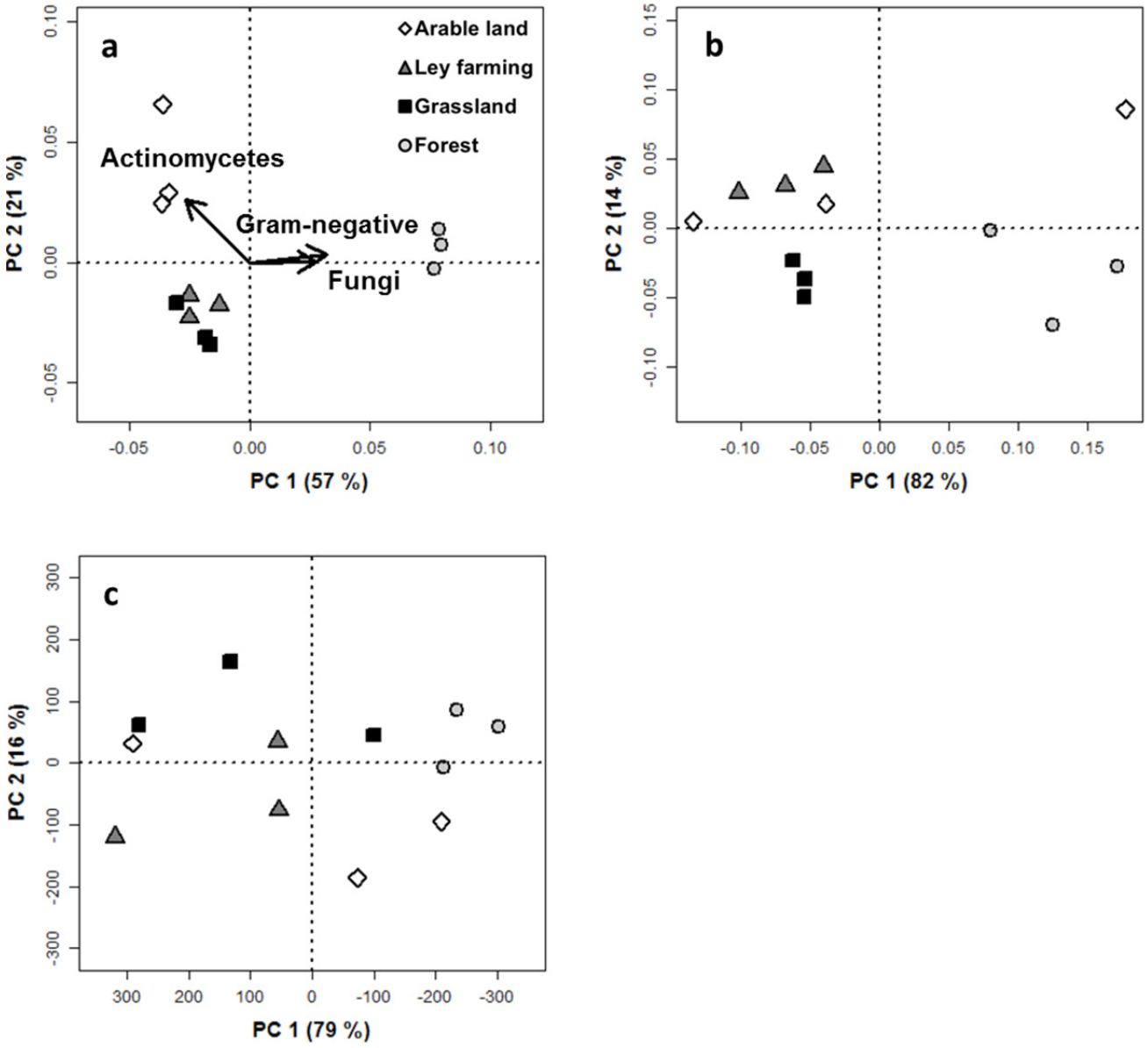
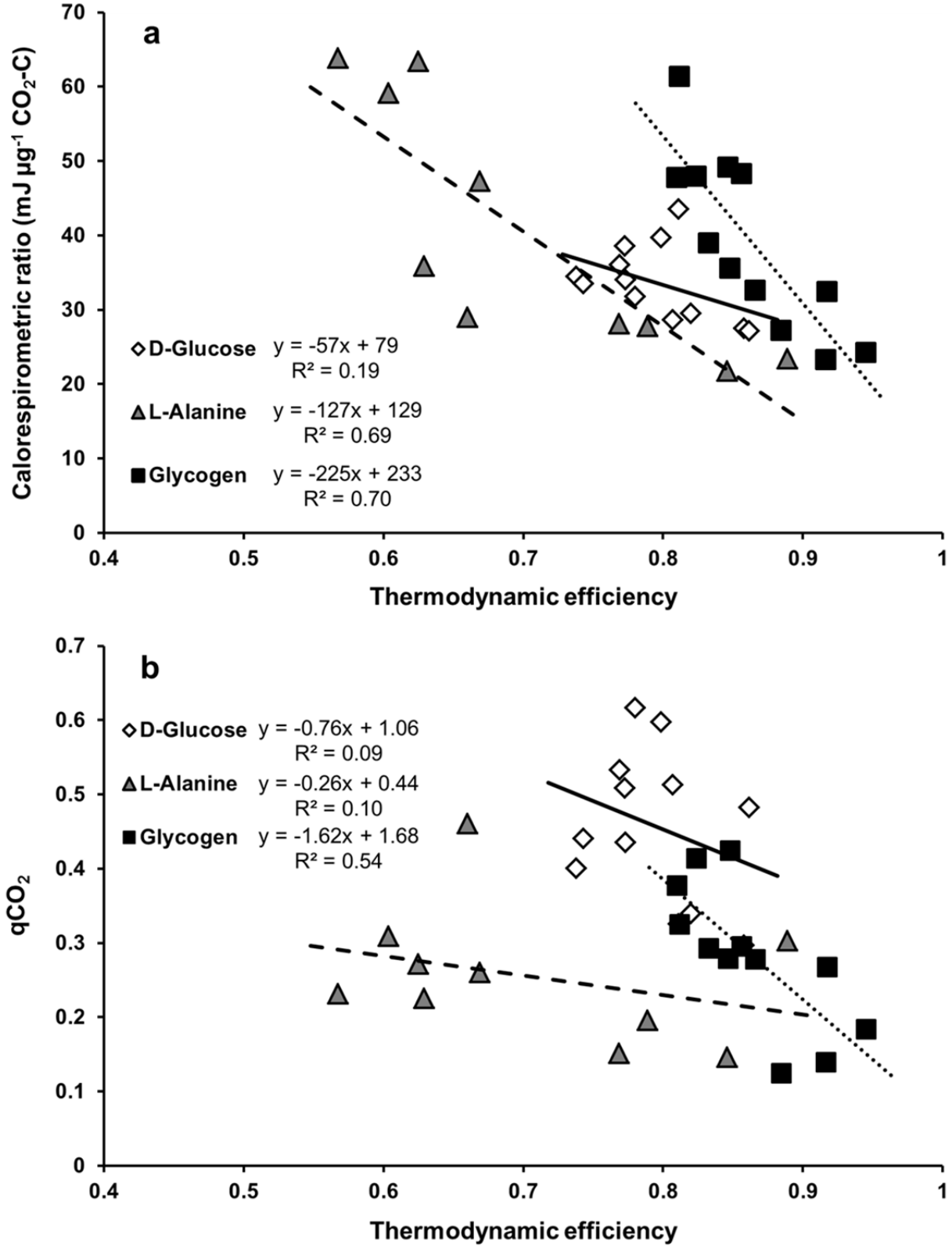

a
Arable land
Ley farming
Grassland
Forest
Actinomycetes
Gram-negative
Fungi
PC 2 (21 %)
PC 1 (57 %)
b
PC 2 (14 %)
PC 1 (82 %)
c
PC 2 (16 %)
PC 1 (79 %)

Fig. 5


a
Calorespirometric ratio (mJ µg-1 CO2-C)
70
60
50
40
30
20
10
0
0.4
0.5
0.6
0.7
0.8
0.9
1
Thermodynamic efficiency
D-Glucose y = -57x + 79
R² = 0.19
L-Alanine y = -127x + 129
R² = 0.69
Glycogen y = -225x + 233
R² = 0.70
b
qCO2
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.4
0.5
0.6
0.7
0.8
0.9
1
Thermodynamic efficiency
D-Glucose y = -0.76x + 1.06
R² = 0.09
L-Alanine y = -0.26x + 0.44
R² = 0.10
Glycogen y = -1.62x + 1.68
R² = 0.54

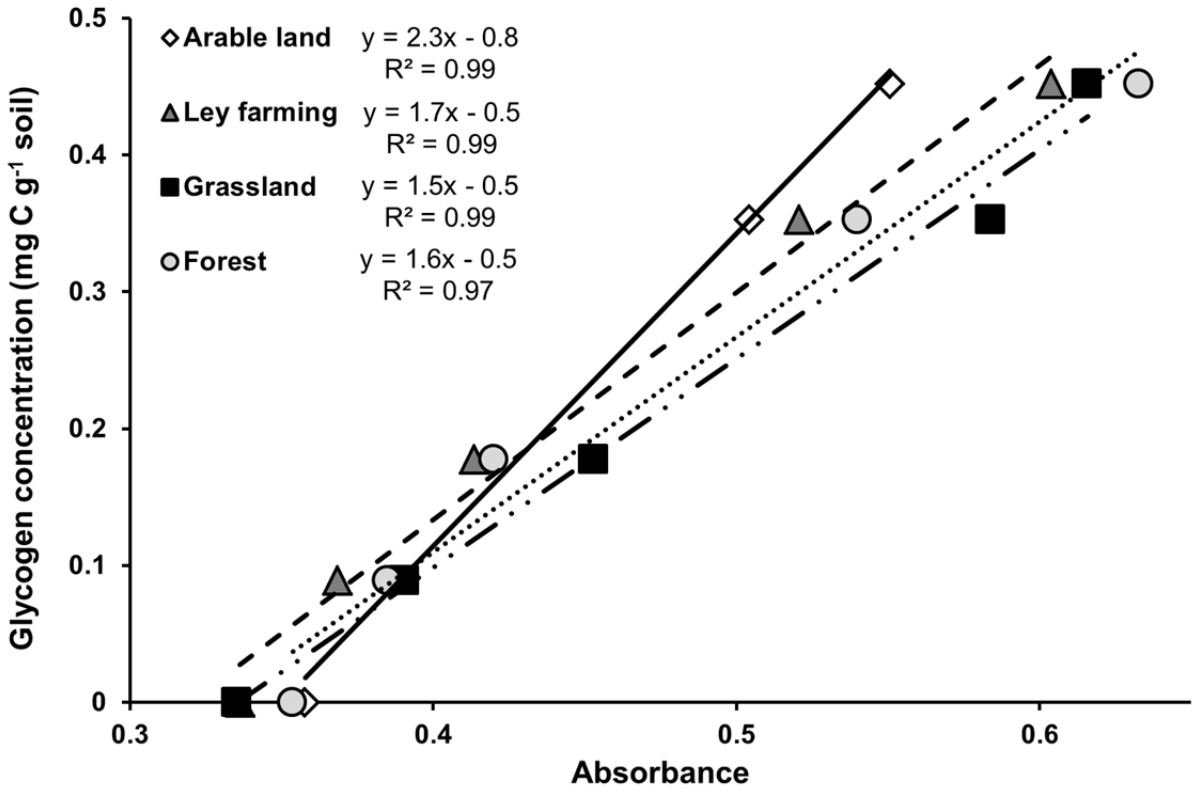
Supplementary Material: Differences in substrate use efficiency: Impacts of microbial community composition, land use management and substrate complexity

Tobias Bölscher[1], Lars Wadsö[2], Gunnar Börjesson[3], Anke M. Herrmann[1]

Table S1 Calibration curves for quantification of substrate in soil solutions: Concentrations of D-glucose, L-alanine and glycogen used for establishing substrate assays in soils

	Substrate concentration (μg C g^{-1} soil)				
D-Glucose	0	45	102	155	
L-Alanine	0	325	414	503	
Glycogen	0	89	177	353	452

Fig. S1 Calibration curves for glycogen and all four land use management regimes. Values display means (n = 4); whiskers fall within confines of symbols



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