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

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

3

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13 calorimetry

14

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16

17

18 **Abstract**

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

35

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## 41 **Introduction**

42 Soil microorganisms are key players in governing the terrestrial carbon (C) cycle (Schimel and Schaeffer 2012). But  
43 due to the complexity of soils, they are often not specifically incorporated into simplistic process-based models used  
44 to predict soil organic matter decomposition. For example, the Century-model (Parton et al. 1987), RothC model  
45 (Coleman and Jenkinson 2014) or Q-model (Ågren and Bosatta 1987) treat the microbial compartment system as a  
46 'black box'. During microbial decomposition, organic C is partitioned between respiratory energy production and  
47 substrate assimilated into microbial biomass and stabilized in soil organic matter. This partitioning is often referred  
48 to as substrate use efficiency or C use efficiency, and it is an important microbial physiological feature in  
49 determining the fate of C during organic matter decomposition in soils. This property is often assumed to be  
50 constant in process-based models, but research indicates that substrate use efficiency is (i) temperature dependent  
51 (Devêvre and Horwáth 2000; Steinweg et al. 2008; Wetterstedt and Ågren 2011; Frey et al. 2013; Tucker et al.  
52 2013) and (ii) varies among C substrates (Frey et al. 2013). Recent model frameworks emphasize that changes in  
53 microbial physiology i.e. varying substrate use efficiency have (i) significant impacts on global soil C stocks  
54 (Allison et al. 2010; Frey et al. 2013; Wieder et al. 2013) and (ii) their incorporation may improve future climate  
55 change model projections (Allison et al. 2010; Wieder et al. 2013). The underlying process mechanisms of substrate  
56 use efficiency are still unclear, but differences in efficiencies have been reported to reflect microbial community  
57 composition (Harris et al. 2012; Herrmann et al. 2014; Creamer et al. 2015).

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

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

74 Besides differences in substrate use efficiencies, soil fungi (e.g. wood decomposing fungi) have the ability  
75 to decompose complex soil organic matter through the production of extracellular enzymes (Bödeker et al. 2009;  
76 2014). The thermodynamic argument suggests that reactions metabolizing structurally complex, aromatic  
77 components have higher activation energies than reactions metabolizing structurally simpler, more labile  
78 components (Bosatta and Ågren 1999). Thus, utilization of complex substrates requires higher initial energy costs  
79 reducing net energy gain (Bradford 2013). Furthermore, metabolic pathways during decomposition are dependent on  
80 the nature of the utilized substrate and show varying respiration rates leading to variable substrate use efficiencies  
81 (Gommers et al. 1988; Manzoni et al. 2012). Simple carbohydrates such as glucose are primarily used when  
82 evaluating microbial substrate use efficiency (e.g. Dijkstra et al. 2011; Tucker et al. 2013; Blagodatskaya et al.  
83 2014). However, soil organic matter consists of heterogeneous organic material with inherent chemical energy  
84 stored below-ground which is exchanged within the soil system. Recent research has shown that the use of various  
85 substrates resulted in varying efficiencies (Frey et al. 2013) emphasizing the importance to evaluate several  
86 substrates when assessing microbial substrate use efficiencies.

87 Substrate induced respiration in combination with incorporation of C into the microbial biomass is often  
88 used to evaluate substrate use efficiency (e.g. Behera and Wagner 1974; Blagodatskaya et al. 2014). Recently,  
89 microbial energetics approaches such as thermodynamic efficiency of microbial communities (Harris et al. 2012)  
90 and calorespirometric ratio (Barros et al. 2010) have been tested for exploring microbial substrate use efficiencies in  
91 soil systems. Isothermal calorimetry is used to determine these indices and its main advantage is that it quantifies all  
92 metabolic processes not only those accounted for by CO<sub>2</sub> respiration measurements. Thus, it provides  
93 complementary information to the CO<sub>2</sub> respiratory approach (Herrmann et al. 2014). Thermodynamic efficiency is a  
94 dimensionless index of substrate use efficiency and high values of this index indicate that microbial metabolism is

95 efficient (Harris et al. 2012). The calorespirometric ratio is metabolic heat released in relation to CO<sub>2</sub> production  
96 (i.e. J mol<sup>-1</sup> CO<sub>2</sub> or mJ μg CO<sub>2</sub>-C). If the same organic material is undergoing decomposition, changes in  
97 calorespirometric ratios indicate differences in substrate use efficiencies with decreasing ratios indicating an  
98 increase in efficiencies (Hansen et al. 2004). Both indices are independent of the amount of microbial biomass  
99 residing in soils. Previously, Anderson and Domsch (1986, 1990, 2010) used the ratio of respiration to microbial  
100 biomass (i.e. μg CO<sub>2</sub>-C μg<sup>-1</sup> biomass-C), i.e. the microbial metabolic quotient, as an index for substrate use  
101 efficiency of microbial communities. This quotient has been previously criticized (Wardle and Ghani 1995;  
102 Nannipieri et al. 2003) and should be therefore used with care. However, the microbial biomass is an important soil  
103 property when using the metabolic quotient for assessment of substrate use efficiency. So far, these three indices  
104 have not been used in the same study and it is not known if they would lead to similar conclusions.

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

## 110 **Material and Methods**

### 111 *Soils*

112 In August 2012, we sampled soils from the agricultural long-term field experiment in Röbbäcksdalen (63°48'N,  
113 20°14'E) and the forest long-term nutrient fertilization experiment at Flakaliden (64°07'N, 19°27'E). Both research  
114 sites are closely located to the city of Umeå, Northern Sweden, and exposed to a boreal climate. Mean annual  
115 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  
116 field experiment in Röbbäcksdalen was established in 1965 on an *Eutric Cambisol* (FAO) (Bergkvist and Öborn  
117 2011), and we selected three land use management systems: (i) barley annually (*arable land*), (ii) barley for one year  
118 followed by a two-year period with green fallow (*ley farming*) and (iii) barley for one year followed by a five-year  
119 period with green fallow (*grassland*) (Bergkvist and Öborn 2011). At the time of soil sampling, the ley farming and  
120 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 were 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<sup>-1</sup> soil) were added drop-wise  
147 providing 500 µg C g<sup>-1</sup> 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  $\mu\text{g C g}^{-1}$  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<sub>2</sub> 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 18:2 $\omega$ 6 and 18:1 $\omega$ 9 (Federle 1986) and the sum of 11 bacterial PLFA biomarkers (i15:0, a15:0,  
168 15:0, i16:0, 16:1 $\omega$ 9, 16:1 $\omega$ 7t, i17:0, cy17:0, 17:0, 18:1 $\omega$ 7 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  $\mu$ l 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  $\mu$ m 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  $^{\circ}C$ . 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  $\mu$ l of the supernatant were then mixed with 30 %  
193 (w/v) 5-Sulfosalicylic acid and centrifuged for 30 minutes at 14 000 xg. 200  $\mu$ l 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/ $\mu$ l 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

201 glycogen assay. Glycogen in soil samples was extracted by shaking with 0.25 M K<sub>2</sub>SO<sub>4</sub> (see above), and glycogen  
202 levels were quantified as D-glucose equivalent after 7 M HCl hydrolysis (extractant:HCl ratio of 1:1; 1 hour in  
203 boiling water bath to accelerate hydrolysis), followed by neutralization with 3.5 M K<sub>2</sub>CO<sub>3</sub> (Geary et al. 1981). D-  
204 glucose concentrations were quantified on 0.5 ml of soil extract using the enzymatic D-glucose assay kit (see  
205 above). Recovery of substrate in soil solutions varied among land use management systems, and therefore individual  
206 calibration curves were established for each management system (supplementary Fig. S1).

#### 207 *Microbial substrate use efficiency indices*

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

210 Thermodynamic efficiency ( $\eta_{eff}$ ) was expressed by adapting the equations of Battley (1960; 1987) and Harris et al.  
211 (2012). In the present study, added substrate was not completely decomposed during the incubation period and  
212 therefore utilized substrate needs to be taken into account when calculating thermodynamic efficiency:

$$213 \quad \eta_{eff} = 1 - \left( \frac{Q}{\Delta H_{Initial} - \Delta H_{Residual}} \right) \quad (1)$$

214 where  $Q$  (J g<sup>-1</sup> soil) is heat produced from microbial metabolism,  $\Delta H_{Initial}$  (J g<sup>-1</sup> soil) and  $\Delta H_{Residual}$  (J g<sup>-1</sup> soil) is the  
215 combustion enthalpy of initial substrate in soil solution and residual substrate in soil solution at the end of the  
216 incubation period, respectively. The difference between  $\Delta H_{Initial}$  and  $\Delta H_{Residual}$  is the combustion enthalpy of the  
217 utilized substrate, i.e. it is the theoretically available energy for metabolic processes during the incubation period.  
218 The standard enthalpy of combustion  $\Delta H_c^\circ$  is 2,816.8 kJ mol<sup>-1</sup> for D-glucose, 1,626.1 kJ mol<sup>-1</sup> for L-alanine and  
219 2,841.3 kJ mol<sup>-1</sup> for the smallest repeating polymer unit of glycogen (Washburn 2003). These values were used to  
220 calculate  $\Delta H_{Initial}$  and  $\Delta H_{Residual}$ . In the present work, 19.5 J g<sup>-1</sup> soil for D-glucose, 22.6 J g<sup>-1</sup> soil for L-alanine and  
221 19.8 J g<sup>-1</sup> soil for glycogen were added to the soil samples.

222 The calorespirometric ratio  $\gamma$  (J mol<sup>-1</sup> CO<sub>2</sub> or mJ  $\mu$ g<sup>-1</sup> CO<sub>2</sub>-C) is the ratio of heat production and CO<sub>2</sub> production  
223 (Hansen et al. 2004):

$$224 \quad \gamma = \frac{Q}{CO_2} \quad (2)$$

225 where  $Q$  ( $\text{J g}^{-1}$  soil) and  $CO_2$  ( $\text{mol CO}_2 \text{ g}^{-1}$  soil or  $\mu\text{g}^{-1} \text{CO}_2\text{-C g}^{-1}$  soil) are the heat and  $CO_2$  production after substrate  
226 addition, respectively.

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

$$229 \quad qCO_2 = \frac{CO_2}{C_{mic}} \quad (3)$$

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

### 231 *Statistical analysis*

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

## 248 **Results**

249 In forest management systems, heat production rates were constant when soils were amended with glucose or  
250 glycogen (Fig. 2a and c) whereas in alanine amended samples heat production rates increased from ca.  $2 \mu\text{W g}^{-1}$  soil  
251 to ca.  $10 \mu\text{W g}^{-1}$  soil (Fig. 2b) during the 32-hours incubation period. The other three land use management systems  
252 increased slightly up to ca.  $45 \mu\text{W g}^{-1}$  soil during the incubation (Fig. 2), but based on heat flow data exponential  
253 microbial growth was not observed in any of the amended soil systems. Land use management systems and substrate  
254 type had significant effects on cumulative heat produced from microbial metabolism over the 32 hours incubation  
255 period (Table 2). Overall, heat production of soils from different land use managements decreased in the order ley  
256 farming > arable land/grassland > forest ecosystems ( $P < 0.01$ ; Table 2). Across all land uses, D-glucose amended  
257 management systems produced significantly more heat in comparison with systems amended with L-alanine or  
258 glycogen ( $P < 0.01$ , Table 2). These patterns were mirrored in  $\text{CO}_2$  production (Table 2), but utilized substrate  
259 ( $\Delta H_{\text{Initial}} - \Delta H_{\text{Residual}}$ ) resulted in a different pattern. Although we observed significant differences in heat production  
260 between management systems, substrate utilization was similar within each substrate class ( $P = 0.06$ , Table 2).  
261 Across all management systems, utilization of D-glucose and glycogen were significantly higher than L-alanine  
262 utilization ( $P < 0.01$ , Table 2). The largest total heat production was observed in the ley farming system amended  
263 with D-glucose (Table 2). Taking this heat production, Thornton's rule, and the ideal gas equation into account,  $\text{O}_2$ -  
264 concentration decreased from 21 to 17 % at the most indicating that sufficient  $\text{O}_2$  was present in the reaction vials  
265 throughout the entire incubation period (data not shown).

266 Taking the heat output and utilized substrate into account, thermodynamic efficiency of soil microbial  
267 communities (Eq. 1) ranged between 0.63 and 0.92 with significant differences among land use management  
268 systems as well as applied C substrate (Fig. 3a). Generally, microbial communities residing in forest soils used  
269 substrates most efficiently and microorganisms in ley farming systems were least efficient in substrate use among  
270 the four management systems ( $P < 0.05$ , Fig. 3a). Thermodynamic efficiencies of microbial communities residing in  
271 arable land and grassland systems were in between but not significantly different to the ley farming system (Fig. 3a).  
272 Glycogen was used most efficiently by soil microorganisms followed by glucose and the efficiency of microbial L-  
273 alanine use was the lowest among the three substrates ( $P < 0.01$ , Fig. 3a). Mean calorespirometric ratios (Eq. 2)  
274 ranged from 22 to  $59 \text{ mJ } \mu\text{g}^{-1} \text{ CO}_2\text{-C}$  (Fig. 3b). Here, only land use management had a significant effect on  
275 calorespirometric ratios. Forest soils revealed on average the lowest ratio of  $28 \text{ mJ } \mu\text{g}^{-1} \text{ CO}_2\text{-C}$  indicating highest  
276 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  $44 \text{ mJ } \mu\text{g}^{-1} \text{ CO}_2\text{-C}$ ), 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  $40 \text{ mJ } \mu\text{g}^{-1}$   
279  $\text{CO}_2\text{-C}$ ) 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 (18:1 $\omega$ 7c; Frostegård et al. 1993a) and fungi (18:1 $\omega$ 9c; 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

304 *Substrate use efficiencies across land use management systems*

305 Our initial hypothesis that substrate use efficiencies increase along a successional gradient due to changes in  
306 microbial community composition was partially confirmed. Irrespectively of efficiency indices, microbial  
307 communities in forest soils were most efficient in using substrates and separated clearly from arable, grassland and  
308 ley farming management systems (Fig. 3). Forest soils had a higher abundance of fungi and Gram-negative bacteria  
309 as well as higher F:B and Gram-negative:Gram-positive ratios in comparison with the other three management  
310 systems (Table 3). Our results therefore support the common assumption that fungi are more efficient in utilizing  
311 soil organic matter in comparison to bacteria (Holland and Coleman 1987; Herrmann et al. 2014), and that the  
312 abundance of Gram-negative bacteria may be of importance for differences in substrate use efficiencies (Harris et al.  
313 2012; Creamer et al. 2015). The Mantel tests for dissimilarities support the hypothesis that there may be a link  
314 between the composition of the microbial community and substrate use efficiency. Differences in microbial  
315 substrate use efficiencies among arable land, grassland and ley farming management systems were not significant,  
316 but grassland systems tend to have higher efficiencies than ley farming and arable systems (Fig. 3). This observation  
317 supports our initial hypothesis that substrate use efficiencies increase with maturing ecosystems along a successional  
318 gradient. These three management systems were located at the same field site in Röbbäcksdalen. Here, microbial  
319 community composition was similar except that arable land use management differed from ley farming and  
320 grassland systems due to a higher abundance of actinomycetes (Table 3; Fig. 4). However, there was no relationship  
321 between actinomycetes and microbial substrate use efficiencies ( $R^2 = 0.26$  for thermodynamic efficiency), and we  
322 therefore conclude that actinomycetes are unlikely an important biomarker that could be made responsible for  
323 differences in substrate use efficiency. We expected that 47 years of various soil management systems at  
324 Röbbäcksdalen would result in significant differences in soil microbial communities as observed in various land use  
325 management systems (e.g. Bossio et al. 2005; Cookson et al. 2007; Drenovsky et al. 2010). Although, along two  
326 successional gradients at the Kellogg Biological Station (Michigan State University, USA), Jangid et al. (2011)  
327 showed that legacy effects of past management still have an influence on soil microbial community composition,  
328 particularly in early succession sites. Our grassland management system consists of a five-year period with green  
329 fallow followed by one year barley and therefore, it represents a management system in early succession. Thus, the  
330 timeframe and/or type of land use managements at Röbbäcksdalen were not sufficient to alter soil microbial

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

333 Differences in microbial substrate use efficiencies between the sites at Röbbäcksdalen and Flakaliden (forest  
334 system) could also be due to differences in general soil characteristics such as soil texture and pH (Table 1)  
335 (Blagodatskaya and Anderson 1998; Bååth and Anderson 2003; Rousk and Bååth 2011; Manzoni et al. 2012), and  
336 we cannot conclude firmly that differences in microbial community composition are the driver for changes in  
337 microbial substrate use efficiencies. To test Odum's (1969) and Addiscott's (1995) theory of increasing substrate use  
338 efficiency along a successional gradient, future studies could consider (i) the use of selective inhibition of microbial  
339 groups (Rousk et al. 2008) or (ii) gamma radiation following re-inoculation with microbial communities of different  
340 complexities obtained through a combination of soil fumigation, dilution and filtering techniques (Griffiths et al.  
341 2004). Such studies would avoid confounding effects of major soil properties such as soil texture and/or pH  
342 (Table 1; Delmont et al. 2014), and they should provide unequivocal evidence on the relationship between microbial  
343 community composition and microbial substrate use efficiency along a successional gradient.

#### 344 *Carbon chemistry and substrate use efficiency*

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

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

### 360 *Implications for soil organic matter modelling*

361 Research has implied that substrate use efficiency is temperature dependent (Devêvre and Horváth 2000; Steinweg  
362 et al. 2008; Wetterstedt and Ågren 2011; Tucker et al. 2013; Frey et al. 2013), and assuming varying substrate use  
363 efficiencies in modelling frameworks have significant consequences for projections of global soil C stocks (Allison  
364 et al. 2010; Frey et al. 2013; Wieder et al. 2013). Temperature dependency of substrate use efficiencies is therefore  
365 of major concern when modelling terrestrial C cycling. In our study, thermodynamic efficiencies varied up to 32 %  
366 across various land use management systems and C substrates (mean values varied between 0.63 and 0.92). Within  
367 the same substrate amendment, thermodynamic efficiencies varied between 10 and 20 % across land use  
368 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-  
369 alanine or glycogen, respectively; Fig. 3a). Similarly, within the same land use management system but across  
370 various substrates, thermodynamic efficiencies varied between 10 and 30 % (mean values varied between 0.71 to  
371 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).  
372 These variations are in a similar range as temperature induced changes in microbial substrate use efficiencies when  
373 temperature changes by 10 °C (Devêvre and Horváth 2000; Steinweg et al. 2008; Tucker et al. 2013; Frey et al.  
374 2013). A recent model framework showed that relative alteration in substrate use efficiency by 10 to 30 % could  
375 result in a change of 1 to 4 % of total organic C stored below-ground within 98 years, corresponding to approx. 90 to  
376 270 g C m<sup>-2</sup> in a forest system (Frey et al. 2013). Thus, our results emphasize that variation in substrate use  
377 efficiency across land use management systems and various substrates are equally important as temperature induced  
378 changes in efficiencies. The composition of soil organic matter changes significantly along a successional gradient  
379 (Quideau et al. 2001; Garnier et al. 2004; Merilä et al. 2010), and future research should focus on evaluating  
380 potential proxies for microbial substrate use efficiencies that are applicable in modeling approaches of soil organic  
381 matter dynamics.

382 Soil C models commonly assume values of substrate use efficiency  $\leq 0.55$  (Parton et al. 1987; Ågren and  
383 Bosatta 1987; Coleman and Jenkinson 2014). In comparison, our thermodynamic efficiency values are above this  
384 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 (Devèvre and Horwath 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<sub>2</sub> 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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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  $mJ \mu g^{-1} CO_2-C$  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

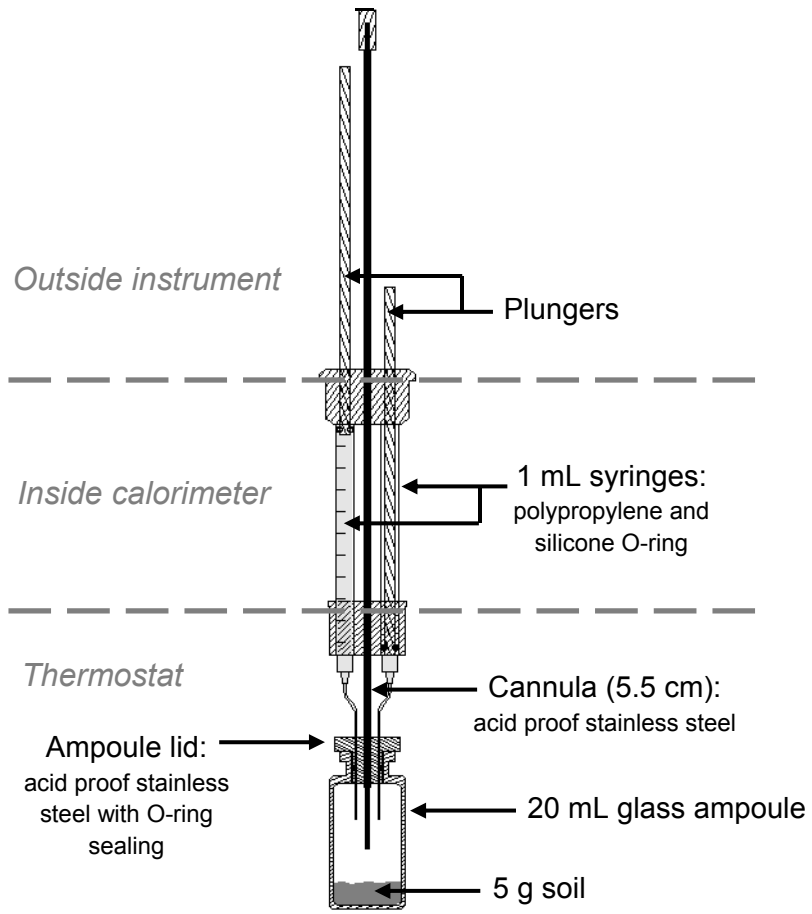


Fig. 2

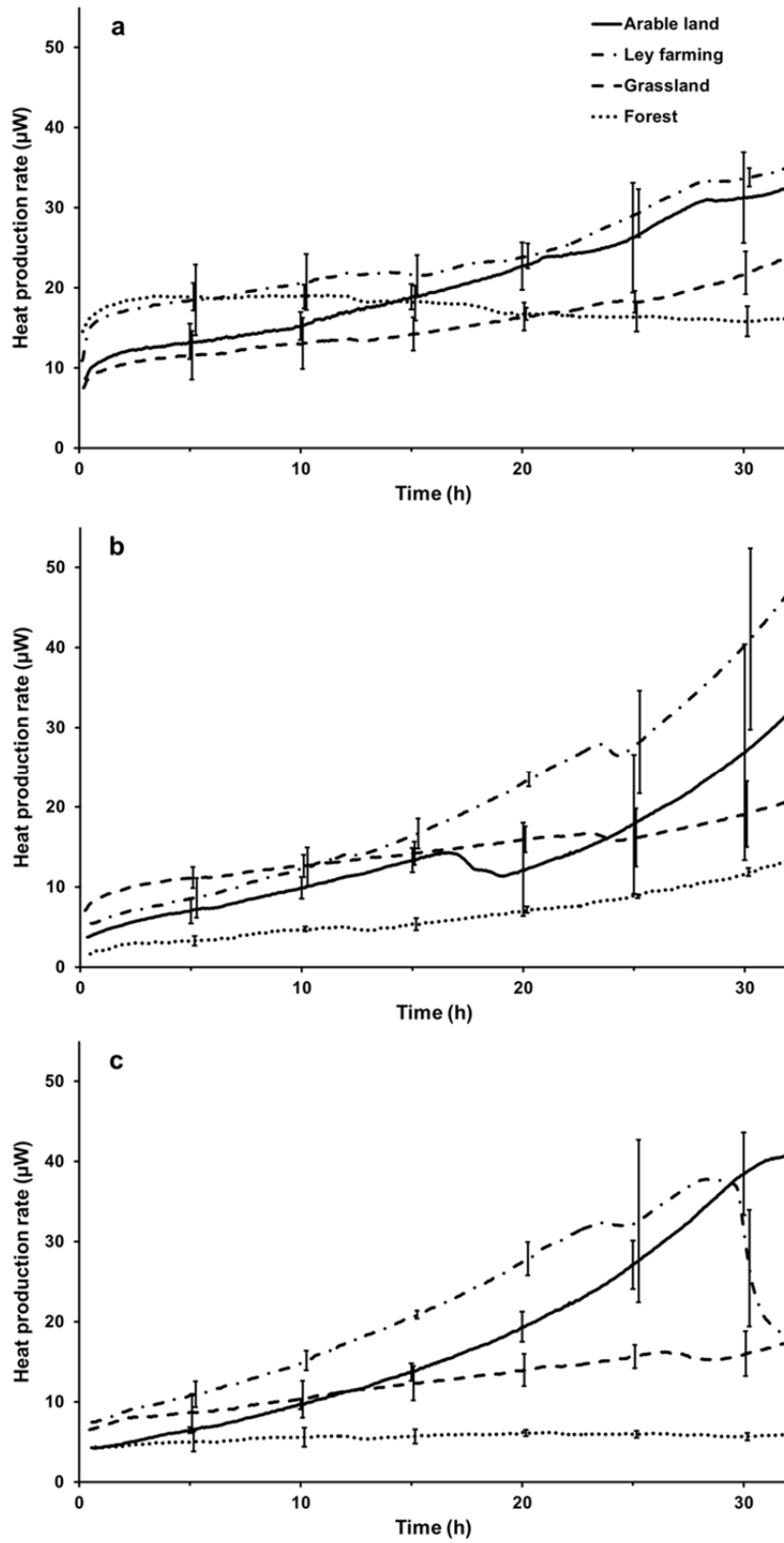


Fig. 3

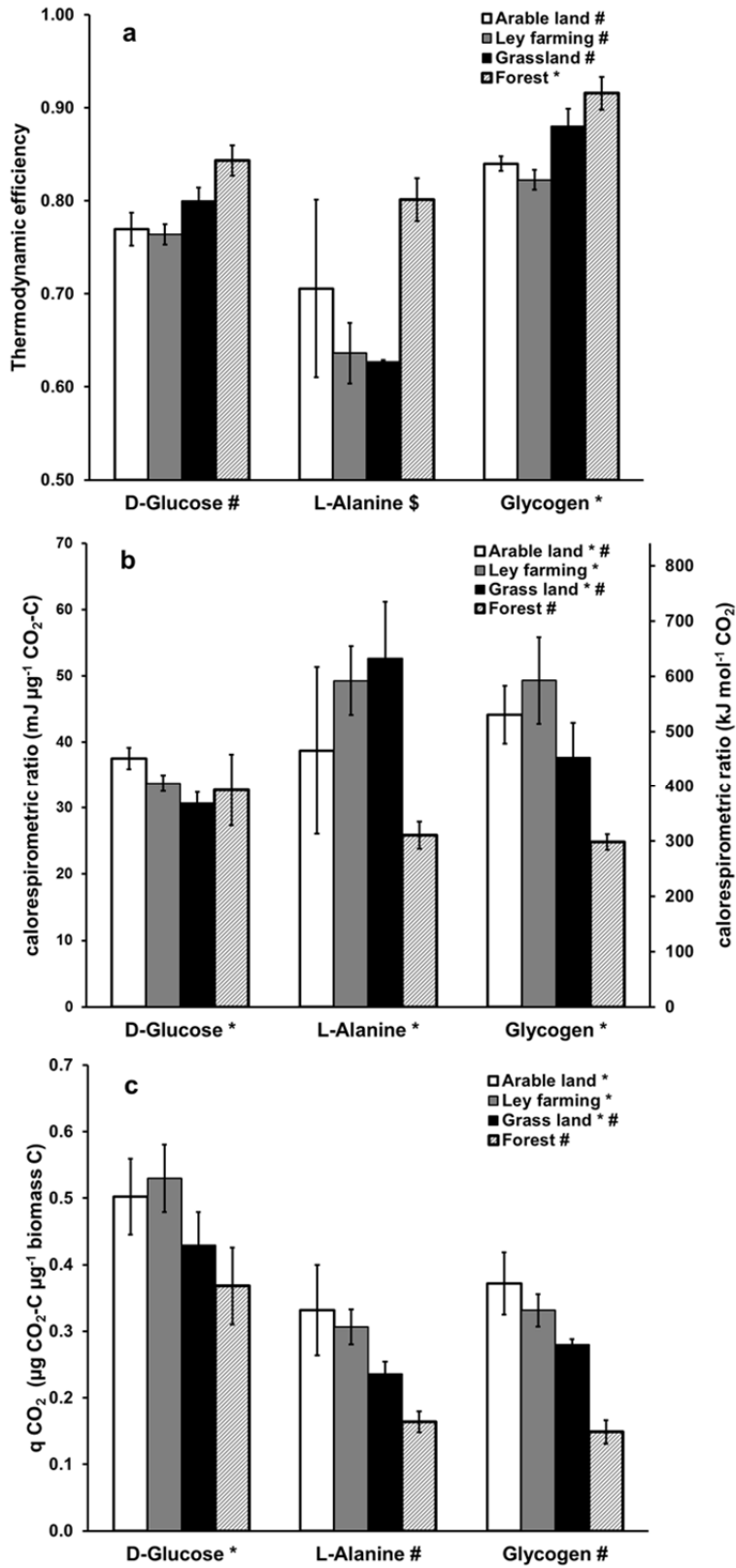


Fig. 4

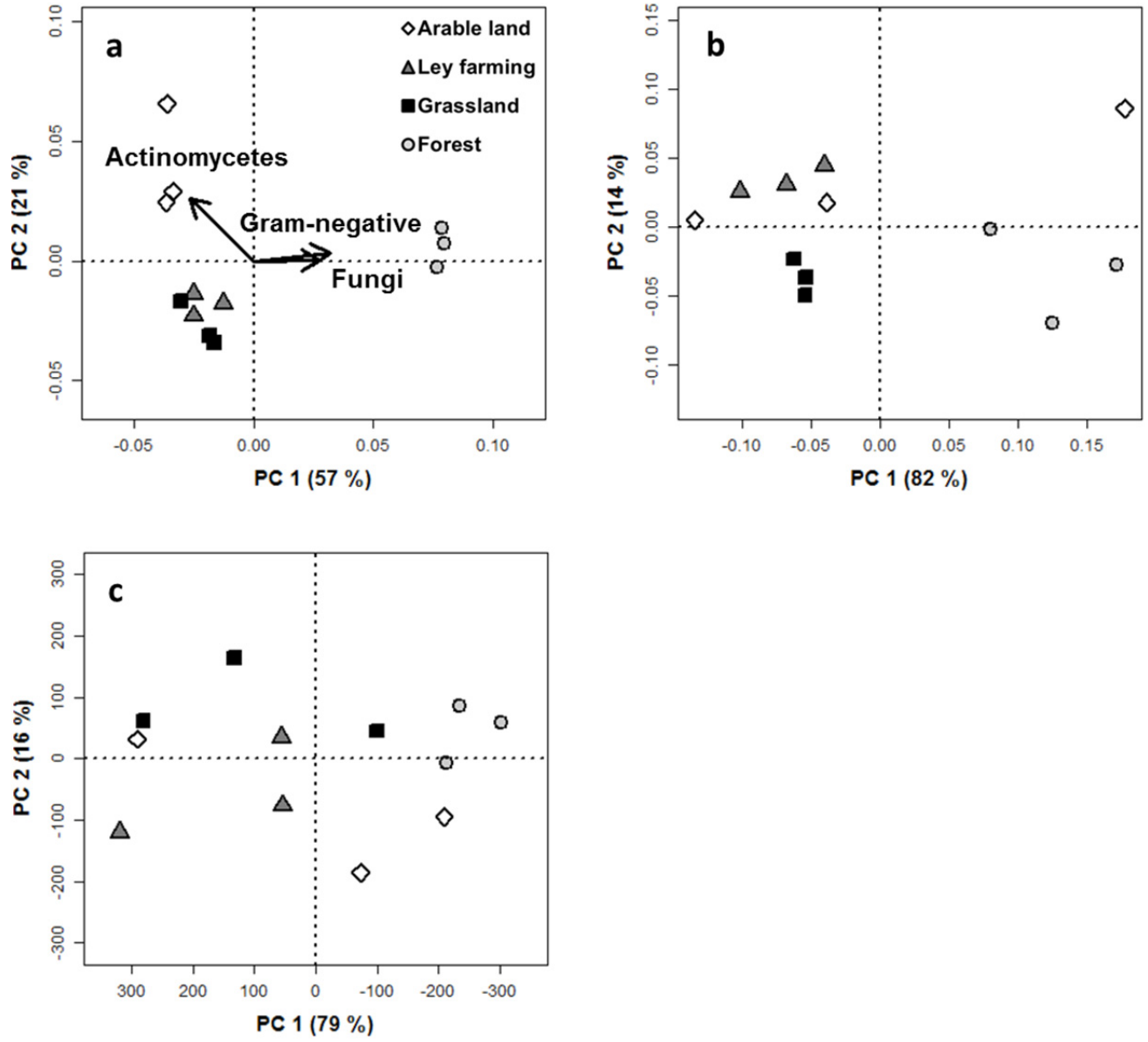
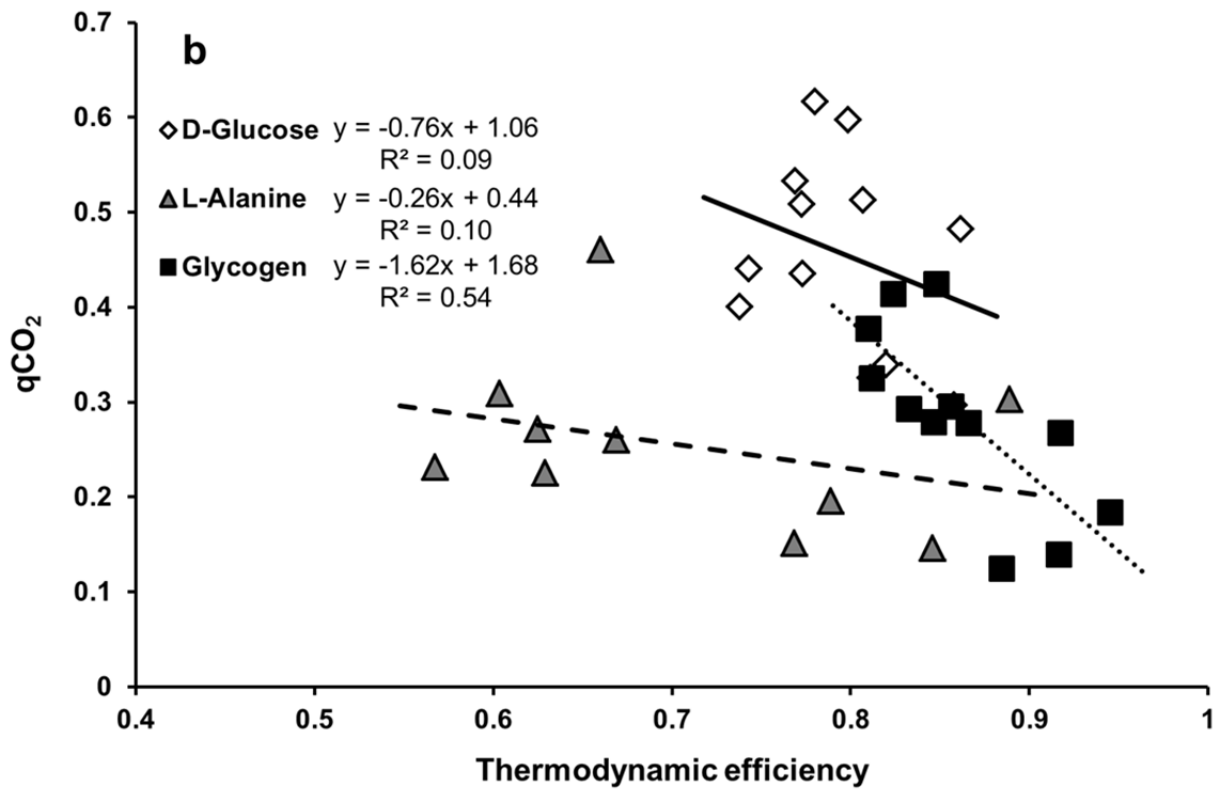
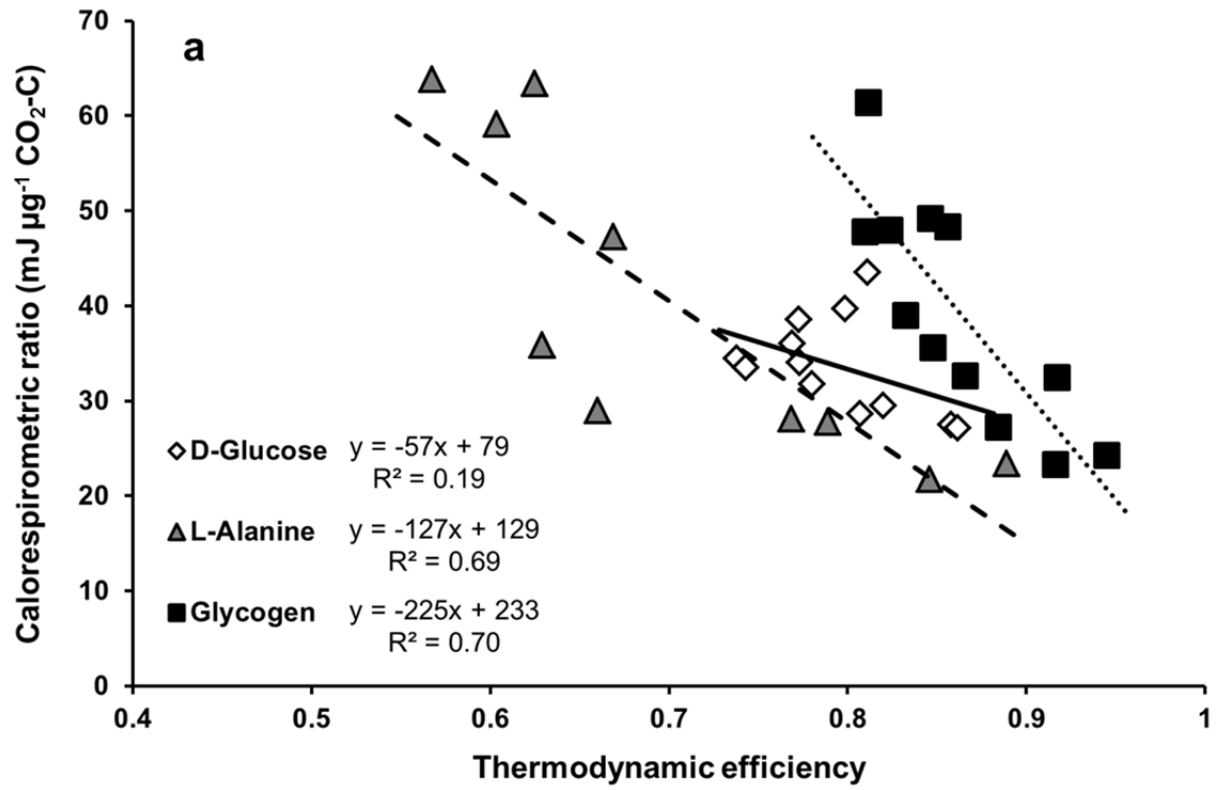


Fig. 5



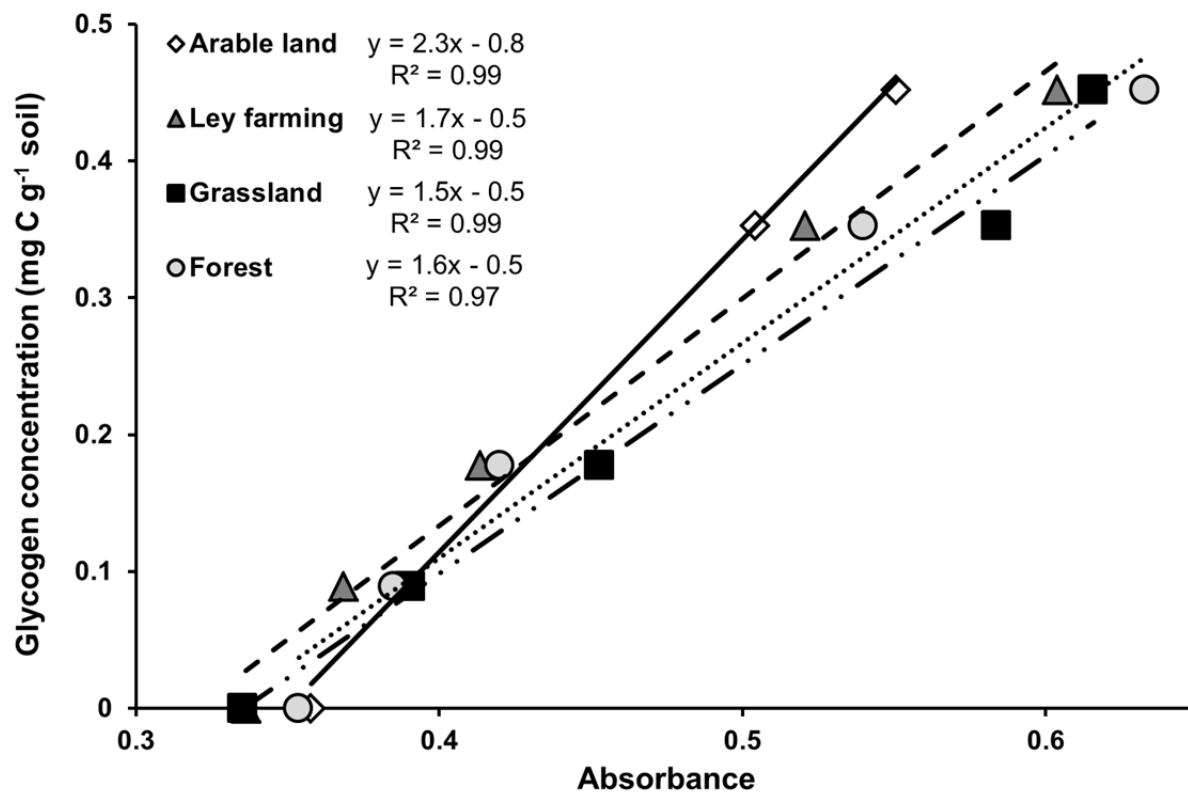
## Supplementary Material: Differences in substrate use efficiency: Impacts of microbial community composition, land use management and substrate complexity

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**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 ( $\mu\text{g C g}^{-1}$ 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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