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1 Isothermal microcalorimetry provides new insight  
2 into terrestrial carbon cycling

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11 KEYWORDS

12 soil carbon | Energy | microbial community | use efficiency | isothermal microcalorimetry

13

## 14 ABSTRACT

15 Energy is continuously transformed in environmental systems through the metabolic activities  
16 of living organisms, but little is known about the relationship between the two. In this study, we  
17 tested the hypothesis that microbial energetics are controlled by microbial community  
18 composition in terrestrial ecosystems. We determined the functional diversity profiles of the soil  
19 biota (i.e. multiple substrate-induced respiration and microbial energetics) in soils from an arable  
20 ecosystem with contrasting long-term management regimes (54 y). These two functional  
21 profiling methods were then related to the soils' microbial community composition. Using  
22 isothermal microcalorimetry, we show that direct measures of energetics provide a functional  
23 link between energy flows and the composition of belowground microbial communities at a high  
24 taxonomic level (Mantel R = 0.4602, P = 0.006). In contrast, this link was not apparent when  
25 carbon dioxide (CO<sub>2</sub>) was used as an aggregate measure of microbial metabolism (Mantel R =  
26 0.2291, P = 0.11). Our work advocates that the microbial energetics approach provides  
27 complementary information to soil respiration for investigating the involvement of microbial  
28 communities in belowground carbon dynamics. Empirical data of our proposed microbial  
29 energetics approach can feed into carbon-climate based ecosystem feedback modeling with the  
30 suggested conceptual ecological model as a base.

31

32 INTRODUCTION

33 Life above- and belowground has evolved complex and diverse communities and a key issue in  
34 ecology is to explore the functional significance of community composition. Despite the central  
35 role of soil microorganisms in the Earth's biogeochemical cycles, the importance of microbial  
36 diversity in ecosystem functioning is still debated<sup>1</sup>. The regulation of our climate and the carbon  
37 cycle is an important ecosystem service and function. Soil organic matter is the largest carbon  
38 pool in terrestrial ecosystems and soils are therefore major players in the global carbon cycle<sup>2</sup>.  
39 Organic matter contains energy-rich bonds and is the primary energy source for the abundant and  
40 diverse soil biological communities. Through metabolic activities, heterotrophic microorganisms  
41 utilize energy stored in organic matter and exchange it within the biosphere and with the  
42 atmosphere.

43 According to the second law of thermodynamics, high order energy (exergy) dissipates as low  
44 order energy from a system over time and this process is irreversible. From an energy point of  
45 view, soil ecosystems can be characterized as open systems of non-equilibrium thermodynamics  
46 with the decomposition of soil organic matter to carbon dioxide (CO<sub>2</sub>) as a dissipative process  
47 that increases entropy<sup>3,4</sup>. Microbial metabolism is divided into two categories: catabolic reactions  
48 that release energy and anabolic reactions that demand energy. An example of catabolic reactions  
49 in soils is the breakdown of organic material into smaller compounds which releases energy  
50 necessary for anabolic biosynthetic reactions. Energy not required for anabolic processes is  
51 dissipated as heat and CO<sub>2</sub> is released from the soil system into the atmosphere. However, we do  
52 not grasp in detail how life belowground abides by the second law of thermodynamics<sup>5,6</sup>.

53 Isothermal microcalorimetry provides information on heat flows of all processes with very  
54 high precision<sup>7</sup>. It is of particular interest for studying microbial involvement in soil carbon  
55 dynamics as it quantifies all microbial metabolic processes (i.e. the net outcome of catabolic and  
56 anabolic processes) not only accounted for by CO<sub>2</sub> measurements. As such, it is an alternative,  
57 yet complementary, approach to CO<sub>2</sub> production for exploring microbial activity and carbon  
58 dynamics in soil systems. Further information on the use, advantages and challenges of  
59 isothermal microcalorimetry in soil and environmental sciences can be found in comprehensive  
60 reviews<sup>7-9</sup>. The calorespirometric ratio (ratio of heat-to-CO<sub>2</sub>-C) has been used to evaluate  
61 metabolism and metabolic efficiency in soil systems<sup>10,11</sup>, and this ratio appears to vary among  
62 soil systems with different land uses<sup>11,12</sup>. Recently, Harris and co-workers<sup>13</sup> proposed a  
63 dimensionless index of microbial thermodynamic efficiency determined using isothermal  
64 microcalorimetry. The index is based on the ratio of energy output in relation to energy input.  
65 Small values of this index indicate that microbial energetics are efficient; in other words that the  
66 biota has the ability to minimize energy dissipation from a system whilst maintaining  
67 metabolism. Although it is known that soil organisms require both energy and carbon to drive  
68 belowground processes, little is known about how energy flows are linked to the carbon cycle  
69 and if there is a relation between microbial energetics and microbial community composition in  
70 the soil. A better understanding of the relationship between the two is likely to help evaluate the  
71 efficiency of carbon allocation in soil ecosystems and the consequences of the different  
72 efficiencies.

73 Soil organisms have developed diverse life strategies to assimilate carbon and energy for  
74 maintenance, growth and reproduction<sup>14</sup>, and they can rapidly adapt to changes in external  
75 environmental conditions<sup>15</sup> through alternative biochemical pathways<sup>16</sup>. Although the

76 allochthonous *r*- versus zymogenous K-selection concept<sup>17</sup> has been criticized as being an  
77 oversimplified view of the processes of natural selection in ecology<sup>18</sup>, it is still consistent with  
78 modern interpretation of community type and soil microbial functioning<sup>14</sup>. In general,  
79 allochthonous *r*-strategists are adapted to rapidly acquiring resources when abundant and  
80 maximizing their growth rate. These organisms generally release a larger fraction of organic  
81 material to the atmosphere as CO<sub>2</sub>. In comparison, zymogenous K-strategists have developed a  
82 suite of extracellular enzymes<sup>19-21</sup> to break down complex organic material and they are therefore  
83 adapted to competing and surviving when resources are limited. In ecosystems dominated by K-  
84 strategists, it is assumed that more of the organic material is sequestered in soils through carbon  
85 allocation to microbial cell maintenance and synthesis of extracellular components such as  
86 enzymes, polysaccharides, metabolites, proteins etc.<sup>22</sup>. Consequently, different soil microbial  
87 communities are likely to call upon different biochemical pathways resulting in different carbon  
88 and energy flows through the communities and ecosystems. Under this scenario there may be  
89 divergences between CO<sub>2</sub> production and energy utilization among microbial communities with  
90 different makeups in the short-term and potentially long-term consequences for the carbon cycle  
91 in soil.

92 Here, we tested the hypothesis that the composition of microbial communities in soils and  
93 their functioning controls energy flows as soil organisms have developed diverse biochemical  
94 pathways and life strategies. The general assumption is that measurements of microbial  
95 energetics provide a more subtle description of microbial processes related to the carbon cycle  
96 than do measurements of microbial CO<sub>2</sub> production. Soils from an arable ecosystem which differ  
97 only in their contrasting long-term organic matter inputs were chosen<sup>23</sup> to avoid the confounding  
98 effects of major soil properties such as soil texture or pH (SI Table 1). For illustration purposes,

99 we also proposed a conceptual ecological model of microbial energetics in terrestrial ecosystems  
100 in which the different energy flows are explicitly described (Scheme 1).

101 EXPERIMENTAL SECTION

102 We established a laboratory experiment in which we added a range of carbon substrates to  
103 soils from an arable ecosystem in order to test the hypothesis. Seven substrates (see SI Table 1  
104 for details on all substrates used) or Milli-Q water as control were added separately to either non-  
105 sterile or gamma-irradiated sterile soil samples. The release of heat or CO<sub>2</sub> after substrate and  
106 water additions to gamma-irradiated soil was also included in order to account for abiotic  
107 processes (abiotic CO<sub>2</sub> evolution<sup>24</sup> or substrate interactions with soil matrix<sup>25</sup>). We then  
108 measured substrate-induced CO<sub>2</sub> production<sup>26</sup> and energy flow profiles and determined the  
109 strength of the relationship between these profiles and microbial community composition<sup>27</sup>.

110 **Site and Sample Collection.** Soils were sampled from the Ultuna Long-Term Soil Organic  
111 Matter Experiment (Uppsala, Sweden; 60°N, 17°E)<sup>23</sup>. The experiment was started in 1956 on a  
112 postglacial clay loam classified as an Eutric Cambisol. In this experiment, soils (2 x 2 m blocks)  
113 have been treated with mineral nitrogen fertilizers (80 kg N ha<sup>-1</sup> y<sup>-1</sup>; applied annually as either  
114 Ca(NO<sub>3</sub>)<sub>2</sub> or (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) or organic amendments (biennial addition at 8 Mg ash-free organic  
115 matter ha<sup>-1</sup> y<sup>-1</sup>). The treatments are replicated in four blocks, but one of the four blocks does not  
116 have randomly distributed treatments and was therefore omitted from the present study. At the  
117 end of May 2010, four treatments were selected, *viz.* (i) Green Manure, (ii) Straw+calcium  
118 nitrate, (iii) Farmyard Manure and (iv) Peat+calcium nitrate (approx. 6 months after the last  
119 application of organic manure). Eight sub-samples from 0-7 cm depth were taken from each  
120 replicate block, sieved < 2mm, composited and mixed per replicate block and stored frozen until

121 spring 2012. Soils were then adjusted to 45% of their water holding capacity (WHC) and pre-  
122 incubated for two weeks at 25°C to allow any disturbance due to sieving to subside.

123       **Substrate-Induced Respiration.** The use of multiple substrate-induced respiration  
124 (MicroResp™ approach<sup>26</sup>) is often used to evaluate the functional diversity status of the soil  
125 biota and to investigate carbon dynamics in soils. The correct use of this approach requires that  
126 sufficient substrate is provided to saturate the microbial respiratory metabolism. For this study,  
127 seven substrates and recommended carbon concentrations<sup>26</sup> were selected:  $\gamma$ -amino butyric acid,  
128 D-glucose, citric acid and  $\alpha$ -ketoglutaric acid were prepared so that 30 mg of C substrate per mL  
129 soil water were supplied to each well; substrates that did not readily dissolve in water (i.e. *N*-  
130 acetyl glucosamine, L-alanine and  $\alpha$ -cyclodextrin) were supplied at a concentration of 7.5 mg C  
131 mL<sup>-1</sup> soil water. These substrates are commonly used in functional diversity profiling and they  
132 have shown to discriminate between different soil microbial communities.<sup>26,28</sup> For each soil  
133 treatment, soil samples (300  $\mu$ L total volume per well, approx. 0.5 g dry soil) were added to a 96-  
134 well microtiter deep well plates and then 30  $\mu$ L of each substrate was dispensed to each deep  
135 well (four replicate wells per substrate plus four Milli-Q water controls). The substrate addition  
136 brought the water content to 65% of WHC and soils were incubated at 25° for eight hours. After  
137 2 hours, the gel detector plates were mounted onto the microtiter plate system and substrate  
138 induced respiration was measured between 2-8 h. The gel detector plates were then read in a  
139 plate reader (Multiskan RC, Labsystem Finland). A calibration curve of absorbance (x) versus  
140 headspace equilibrium CO<sub>2</sub> concentration (y) was measured independently and absorbance data  
141 from microtiter deep well plates were fitted to a power decay model ( $R^2 = 0.976$ ) as follows:  $y =$   
142  $0.0499x^{-2.702}$ .

143       **Microbial Energetics.** For each soil treatment, eight aliquots of soil (5 g) were placed into 20  
144 mL glass reaction vessels and each vessel was sealed with an admix ampoule set up consisting of  
145 two 1 ml syringes (SI Fig. 1). Each admix ampoule contained either one of the seven substrates  
146 mentioned above or Milli-Q water as control (SI Table 1). The samples were then introduced  
147 into a TAM Air isothermal micro-calorimeter (TA Instruments Sollentuna, Sweden) with the  
148 thermostat set to 25°C. The calorimeter was then sealed and the samples were allowed to  
149 equilibrate for 3 hours. After equilibration, the plungers of the two syringes were slowly pressed  
150 down to add the C substrates and Mill-Q water control drop wise (60 µL per gram of soil  
151 corresponding to the same volume as for substrate-induced respiration described above) and heat  
152 flows were determined over 8 hours after substrate addition.

153       **Assessment of Abiotic Processes.** For each soil treatment, (i) one set of soil samples (300 µL  
154 total volume per well) were added to a 96-well microtiter deep well plates to assess CO<sub>2</sub>  
155 evolution due to abiotic processes<sup>24</sup>, and (ii) eight aliquots of each soil treatment (5 g soil) were  
156 weighed into 20 mL glass reaction vessels to evaluate substrate interactions with soil physical  
157 properties. The plates and reaction vessels were covered with aluminum foil and samples were  
158 then gamma-irradiated to sterilize them (CODAN Steritex APS, Espergaerde, Denmark) at a  
159 minimum of 25 kGy. Samples were then kept in a laminar flow cabinet for 36 hours to avoid  
160 contamination. To ensure complete sterilization, gamma-irradiation was repeated and samples  
161 were then allowed to settle for four weeks. Seven C substrates (see above for substrate selection  
162 and concentrations) and Milli-Q water control were filter sterilized with a DMSO Safe  
163 Acrodisc® Syringe Filter (0.2 µm Nylon Membrane, 25 mm). For substrate-induced respiration,  
164 30 µL of each filter sterilized substrate or Milli-Q water controls had been dispensed to each  
165 deep well and samples have been treated as described above. For microbial energetics, the admix

166 ampoules (SI Fig. 1) were thoroughly cleaned with ethanol and rinsed repeatedly with filter  
167 sterilized Milli-Q water prior addition of one of the C substrates or MilliQ water as control. The  
168 samples were then introduced into a TAM Air isothermal microcalorimeter and heat flows were  
169 determined as described above.

170 **Microbial Community Profiles.** Phospholipid fatty acid (PLFA) profiling was used to  
171 assess the composition of the microbial communities using the method of Frostegård et al.<sup>27</sup>.  
172 This analysis was used to determine which of the two functional diversity profiling methods, i.e.  
173 substrate-induced CO<sub>2</sub> respiration or microbial energetics, was best related to microbial  
174 community composition. Phospholipids were extracted from approximately 7-g fresh soil using  
175 chloroform, methanol and citrate buffer to the ratio of 1:2:0.8 (v/v/v), fractionated by solid phase  
176 extraction, depolymerized and then derivatized by mild alkaline methanolysis. The resultant fatty  
177 acid methyl esters were analyzed by gas chromatography (Agilent/HP model 5890N, Santa  
178 Clara, California, USA). Mono-unsaturated and cyclopropyl fatty acids were taken as gram-  
179 negative bacteria (G-) biomarkers<sup>29</sup>, iso- and anteiso-fatty acids as grampositive bacteria (G+)  
180 biomarkers<sup>30</sup>, C18:2(9,12) as a fungal biomarker<sup>27</sup> and carboxylic acids with a methyl function  
181 on the carbon chain as biomarkers for actinobacteria<sup>31</sup>.

182 **Statistical Analysis.** All statistical analyses were performed in R version 2.15.1<sup>32</sup> using the  
183 ‘Vegan: Community Ecology Package’<sup>33</sup>. The resultant data was analyzed by one-way analysis  
184 of variance (ANOVA) and homogeneous groups of means established using Duncan’s multiple  
185 range test. Levene’s test was used to evaluate variance homogeneity and, where necessary, data  
186 were log-transformed prior further statistical analysis. PLFA and functional diversity profiling of  
187 the soils were examined with principal component analysis (PCA) using normalized covariance  
188 of %mol of PLFA data, substrate-induced respiration or substrate-induced heat flow data,

189 respectively. Significant differences between soil treatments along ordination axes were analyzed  
190 by post-hoc one-way ANOVA followed by Bartlett's test and Tukey multiple pair test  
191 comparison on PC scores. The association between the substrate-induced respiration, heat release  
192 and PLFA data was determined by comparing the dissimilarity matrices of each of the datasets  
193 using the Mantel test based on the Pearson product-moment correlation coefficient (999  
194 permutations). Pearson correlation analysis was used to evaluate linear regression between PLFA  
195 biomarkers of fungal-bacterial ratio data (X-axis) and respiration as well as microbial energetics  
196 data (Y-axis).

197 **Model description and parameterization.** A conceptual ecological model of microbial  
198 energetics (catabolic and anabolic processes) in terrestrial soil ecosystems under aerobic, dark  
199 conditions was devised and it is presented in Scheme 1. As such, reactions requiring light (e.g.  
200 autotrophy) are not included in the model which only considers oxygen as a terminal electron  
201 acceptor because nitrate and sulfate reduction are negligible in aerobic systems.

202 Water amended control soils show significant specific heat flows ( $Q_{Control}$ ) with respect to  
203 basal metabolism<sup>13</sup>. It is therefore essential to correct the heat output of each substrate-amended  
204 soil in order to obtain heat produced from substrate addition only ( $Q_{Substrate}$ ):

205 
$$Q_{Substrate} = Q_{Total} - Q_{Control} \quad (1)$$

206 where  $Q_{Total}$  ( $\text{mJ g}^{-1} \text{soil h}^{-1}$ ) and  $Q_{Control}$  ( $\text{mJ g}^{-1} \text{soil h}^{-1}$ ) are the heat flow of each substrate-  
207 amended and water amended control soil, respectively.

208 Heat dissipated from abiotic processes was also removed in order to obtain heat flows due to  
209 metabolic activity of microbial substrate decomposition only ( $Q_{Metabolism}$ ,  $\text{mJ g}^{-1} \text{soil h}^{-1}$ ).

210 Assuming that the abiotic processes that occur in sterile soils and in non-sterile soils generate  
211 equal heat flows,  $Q_{\text{Metabolism}}$  can then be obtained by subtracting the heat flow of substrate-  
212 amended sterile soils ( $Q_{\text{Abiotic}}$ ):

213 
$$Q_{\text{Metabolism}} = Q_{\text{Substrate}} - Q_{\text{Abiotic}} \quad (2)$$

214 When there are no abiotic processes then heat produced from substrate addition only ( $Q_{\text{Substrate}}$ ) is  
215 equal to heat flow due to soil biological activity ( $Q_{\text{Metabolism}}$ ).

216 The heat signal  $Q_{\text{Metabolism}}$  is heat dissipated from the soil system and it corresponds to the net  
217 outcome of catabolic (energy releasing) and anabolic (energy demanding) processes. It is the  
218 sum of energy conversions associated with (i) complete biological oxidation of the added  
219 substrate to  $\text{CO}_2$  ( $Q_{\text{CO}_2}$ ); and (ii) the sum of incomplete decomposition and anabolic soil  
220 processes ( $Q_{\text{Net soil}}$ ). Incomplete decomposition processes result in intermediate products  
221 (intermediary catabolism with  $\text{CO}_2$  not being the decomposition end product; Scheme 1 red  
222 arrow in  $Q_{\text{Net soil}}$ ) and anabolic soil processes include microbial growth and maintenance,  
223 production of secondary metabolites, synthesis of extracellular enzymes, extracellular  
224 polysaccharides and so forth (biosynthetic anabolism; Scheme 1 green arrows in  $Q_{\text{Net soil}}$ ).

225 The maximum theoretical available energy that becomes dissipated as heat during metabolism  
226 is associated with the complete oxidation of the added substrate carbon to  $\text{CO}_2$ . In this case, no  
227 energy is conserved within the system. The heat dissipated during the complete oxidation to  $\text{CO}_2$   
228 ( $Q_{\text{CO}_2}$ ) is derived from the following equation assuming that heat production from possible  
229 priming effects of native soil organic matter is negligible in comparison with decomposition of  
230 the added substrate:

231      
$$Q_{\text{CO}_2} = \Delta H_c^o \text{ (kJ mol}^{-1}\text{)} \times \frac{[n(\text{CO}_2)_{\text{substrate}} - n(\text{CO}_2)_{\text{control}}]}{N_C} \quad (3)$$

232      where  $\Delta H_c^o$  (kJ mol<sup>-1</sup>) is the standard molar enthalpy of combustion of the added substrates  
233      (SI Table 1);  $n(\text{CO}_2)_{\text{substrate}}$  and  $n(\text{CO}_2)_{\text{control}}$  is the amount of CO<sub>2</sub> mineralized (mol) in the  
234      substrate-amended and water-amended control soils, respectively, and  $N_C$  is the number of  
235      carbon atoms in the substrate.

236      All intermediary catabolic processes release less heat than the heat associated with the  
237      complete oxidation to CO<sub>2</sub> ( $Q_{\text{CO}_2}$ ). The net outcome between intermediary processes and  
238      biosynthetic anabolic reactions ( $Q_{\text{Net soil}}$ ) can be calculated by the difference between heat  
239      dissipated from overall metabolic activity ( $Q_{\text{Metabolism}}$ ) and  $Q_{\text{CO}_2}$ :

240      
$$Q_{\text{Net soil}} = Q_{\text{Metabolism}} - Q_{\text{CO}_2} \quad (4)$$

241      The carbon involved in transformations associated with the net outcome of  $Q_{\text{Net soil}}$  remains in  
242      the soil system, but CO<sub>2</sub> is lost to the atmosphere.

243      RESULTS AND DISCUSSIONS

244      **Assessment of Abiotic Processes.** The addition of carboxylic acids to the sterile soils induced  
245      significant heat signals with the shape of the curve resembling that of non-sterile soils but of  
246      lower magnitude (SI Fig. 2a and b). In contrast to heat production, abiotic CO<sub>2</sub> production was  
247      negligible (*cf.* Fig. 1 and SI Fig. 2; SI Table 2). The other substrates and water amended control  
248      soils did not result in any measurable abiotic CO<sub>2</sub> production (SI Table 2) or heat flow apart  
249      from an initial (less than 30 minutes) small wetting enthalpy peak when adding the substrates to  
250      the sterile soils (SI Fig. 2c). The absence of any significant heat signal in water amended sterile

251 control soils beyond 30 minutes indicates that possible enzymes or metabolites released from  
252 microbial cells into soil solution during gamma-sterilization had no discernible effect on energy  
253 flows. In contrast with the sterile samples, adding the substrates and water to the non-sterile  
254 samples resulted in a significant substrate or water-induced heat release (SI Fig. 2d).

255 The origin of the abiotic heat signals upon carboxylic acid addition (SI Fig. 2a and b) is not  
256 known, but neutralization reactions and ligand binding of weak acids onto organic material are  
257 known to cause substantial exothermic reactions<sup>25,34</sup>. In non-sterile soil it is, however, uncertain  
258 if abiotic and biotic reactions have similar strengths or if one of them is a stronger sink for  
259 breakdown of carboxylic acids. Sensitivity analysis was therefore required to validate if our  
260 assumption of equal abiotic heat flows in sterile and non-sterile soils was violated (see below in  
261 the following section). Because the first initial immediate reaction was no longer apparent after  
262 two hours (SI Fig. 2a and b), we opted for the use of the 2-8 hour incubation period to evaluate  
263 the relationship between microbial community composition and functional diversity profiles.

264 **Relationship between microbial community and functional diversity profiles.** The  
265 principal component analysis (PCA) of the microbial energetics data ( $Q_{\text{Metabolism}}$ ) revealed a clear  
266 separation among soil treatments ( $P < 0.01$ , Fig. 2a), but only green manure and straw+calcium  
267 nitrate amended soils were separated along PC1 in the respiration data ( $P = 0.034$ ; Fig. 2b).  
268 Furthermore, microbial community composition was also significantly different among soil  
269 treatments with actinobacteria (10Me-C18:0)<sup>31</sup>, Gram-negative bacteria/fungal (C18:1 $\omega$ 9c)<sup>35,36</sup>  
270 and fungal biomarkers (C18:2 $\omega$ 6,9)<sup>27</sup> being the main variables responsible for the separation of  
271 the different soil management regimes ( $P < 0.001$ , Fig. 2c). Pairwise comparison of dissimilarity  
272 matrices between overall microbial metabolic heat profiles and microbial community profiles  
273 revealed a significant similarity between the two data sets (Mantel R = 0.4602, P = 0.006, cf. Fig.

274 2a and c), but no such similarity was detected between respiration and community profiles  
275 (Mantel R = 0.2291, P = 0.11; *cf.* Fig. 2b and c). These data clearly show that the composition of  
276 the microbial community was related to the metabolic processes that occurred in the samples and  
277 that this relationship was not apparent when CO<sub>2</sub> evolution was used as an aggregate measure of  
278 microbial metabolism. Microbial metabolism in soils consists of a plethora of processes  
279 including reactions that do not produce CO<sub>2</sub> as an end-product.<sup>11</sup> Isothermal microcalorimetry  
280 quantifies all metabolic processes and therefore accounts for the different processes that occur  
281 within different microbial communities, regardless of the different life strategies of soil  
282 organisms<sup>14</sup>. This is not always the case with respiration measurements.

283 Moreover, the mantel test for dissimilarity matrices indicated that overall microbial heat flow  
284 ( $Q_{\text{Metabolism}}$ ) and CO<sub>2</sub> data provided different information, i.e. that there are divergences between  
285 the two. This was independent of whether the analysis was based on pairwise comparison  
286 between metabolic heat profiles and respiration profiles of all seven substrates (Mantel R =  
287 0.2173, P = 0.112; *cf.* Fig. 2a and b) or when the two carboxylic acids, which generated  
288 significant heat flows in sterile soils, were excluded from the analysis (Mantel R = 0.03488, P =  
289 0.426). The overall microbial heat flows ( $Q_{\text{Metabolism}}$ ) were based on the assumption that the  
290 abiotic processes that occur in sterile soils generate equal heat flows in non-sterile soils. This is a  
291 challenging assumption to validate however. Sensitivity analysis was done on microbial heat  
292 flows assuming (i)  $Q_{\text{Substrate}} = Q_{\text{Metabolism}}$ , i.e. there was no abiotic heat release upon carbon  
293 substrate addition or (ii) that abiotic heat release was 50% of that determined in sterile soils. The  
294 analysis resulted in the same conclusion, namely that there were divergences between heat  
295 profiles and CO<sub>2</sub> data ((i) Mantel R = 0.2887, P = 0.059; *cf.* Fig. 2b and SI Fig. 3a; (ii) Mantel R  
296 = 0.2266, P = 0.114; *cf.* Fig. 2b and SI Fig. 3b). Thus, potential violations of this assumption are

297 unlikely to affect overall conclusion drawn from this experiment. In contrast, Currie<sup>37</sup> found that  
298 heat flows and CO<sub>2</sub> were closely related when combining energy balance with a model that was  
299 parameterized through bomb calorimetric analysis, i.e. measurements of stored energy in organic  
300 material. However, the two studies are not directly comparable, as different approaches were  
301 used. Nevertheless, they warrant further investigation into the relation between energy and  
302 carbon cycling in terrestrial ecosystems.

303 Long-term organic inputs of peat+calcium nitrate resulted in the greatest fungal-to-bacterial  
304 ratio among the different long-term management regimes (Table 1). The other management  
305 regimes had lower ratios and were ranked in the order green manure > farmyard manure >  
306 straw+calcium nitrate amended soils (Table 1). Soils amended with peat+calcium nitrate  
307 dissipated the least heat ( $Q_{\text{Substrate}}$  and  $Q_{\text{Metabolism}}$ ) (Fig. 1a and b), and the net outcome of heat  
308 dissipated between anabolic and intermediary catabolic reactions ( $Q_{\text{Net soil}}$ ) was lowest in  
309 peat+calcium nitrate or green manure amended soils (Fig. 1a and c). Conversely, green manure  
310 amended soils showed the highest CO<sub>2</sub> production among the four soil systems (Fig. 1a and d).  
311 Such differences in respiration and heat flows strongly suggest that carbon and energy allocation  
312 varied among the four soil management systems. All soils received the same amount of energy  
313 ( $\Sigma$  energy input carbon substrates: 1.29 kJ g<sup>-1</sup> soil; SI Table 1). The fact that less heat was  
314 dissipated in green manure and peat+calcium nitrate systems may be merely due to overall lower  
315 metabolic activities. However, lower calorespirometric ratios, i.e. heat output  $Q_{\text{Metabolism}}$  per unit  
316 CO<sub>2</sub> (Table 1) indicate that the green manure and peat+calcium nitrate systems, with higher  
317 relative abundances of fungi, may have a more efficient microbial metabolism. Consequently,  
318 more energy is retained within soil systems that contain higher proportions of fungi. Specifically,  
319 Pearson correlation analysis based on all field replicates (n = 12) revealed a negative linear

320 relationship between the fungal-bacterial ratio and the sum of all energy heat flows (Fig. 3a;  $P <$   
321 0.001), but there was no relationship between the fungal-bacterial ratio and overall respiratory  
322 activity (Fig. 3b;  $P = 0.66$ ). Normalizing each substrate by the sum of overall heat release  
323 response revealed a negative relationship between the fungal-bacterial ratio and substrate-  
324 induced heat release of *N*-acetyl glucosamine additions ( $Q_{\text{Metabolism}}: X = -5.17, r^2 = 0.73$  or  $Q_{\text{Net}}$   
325 soil:  $X = -6.90, r^2 = 0.83$ ). Fungal cell walls contain chitin which is a long-chain polymer of *N*-  
326 acetyl glucosamine<sup>38</sup>. Hence, *N*-acetyl glucosamine was used in anabolic processes and therefore  
327 less heat was dissipated into the atmosphere from soil systems that contain relatively more fungi  
328 than bacteria. Overall, our results are in line with a long-standing paradigm in microbial ecology  
329 that microbial communities dominated by fungi are more efficient in carbon assimilation<sup>39</sup> and  
330 nutrient resource retention<sup>40</sup> than bacterial-dominated communities.

331 **Implications for carbon cycling in terrestrial ecosystems.** Our findings demonstrate that  
332 the composition of microbial communities in soil and their functioning are related to energy  
333 flows. These findings provide an indication that microbial communities may not be functionally  
334 redundant with respect to carbon cycling as hitherto thought. If this were to be confirmed, we  
335 would therefore have to re-evaluate the concept of functional redundancy in soil microbial  
336 ecology. In the present study, microbial energetics were related to microbial communities at a  
337 high taxonomic level and described under optimal, saturated microbial metabolism. Although  
338 PLFA profiles only provide a description of microbial community composition at a high  
339 taxonomic level, recent research syntheses<sup>41,42</sup> accentuate that this level may matter for  
340 ecosystem function. In the future it will be necessary to evaluate (i) if the taxonomic level of  
341 diversity matters, i.e. different taxonomic levels of diversity for example at the species level may  
342 result in a different relationship with energy flows, (ii) if microbial energetics are similar under

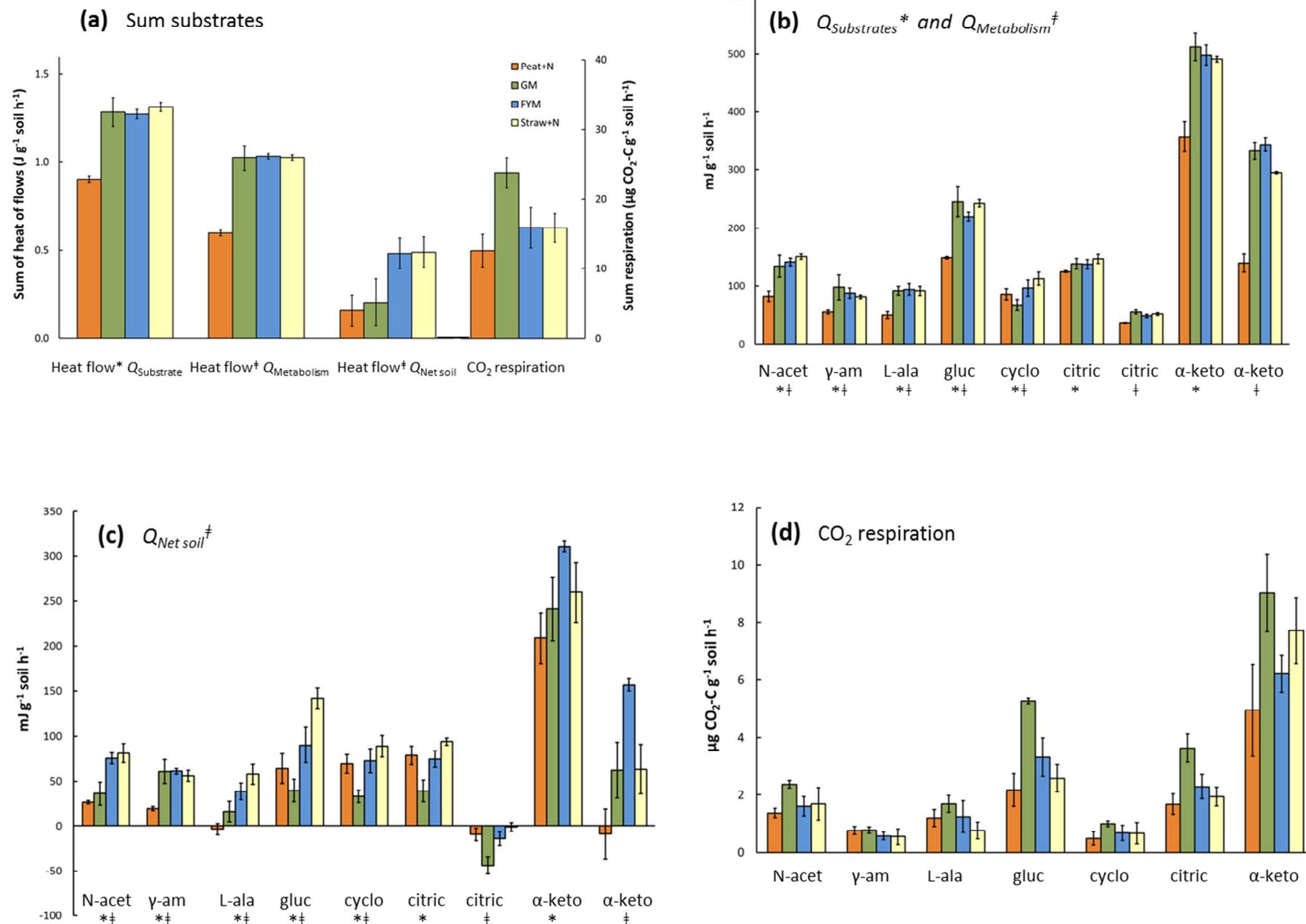
343 ecologically relevant substrate levels, i.e. poorer carbon conditions and (iii) if microbial  
344 communities with different energy flows respond differently to external forces such as flooding,  
345 heat or cold stress and so forth.

346 Our results have significant implications for carbon cycling in terrestrial ecosystems and  
347 support the emerging view of carbon sequestration. The classical view that carbon sequestration  
348 belowground is mainly due to the molecular property of residing organic matter is increasingly  
349 considered obsolete. It is replaced by a conceptual model which describes carbon stabilization as  
350 an ecosystem property<sup>43</sup> with soil microorganisms as important facilitators<sup>41</sup>. Data from the  
351 present study furthermore confirm that soil systems that contain relatively more fungi may have  
352 the ability to sequester more carbon belowground in comparison with systems with relatively  
353 more bacteria. Allison and co-workers<sup>44</sup> have suggested that changes in microbial metabolism,  
354 resulting in a decrease in the fraction of assimilated carbon allocated to growth, can explain the  
355 apparent acclimation to warming that is often observed for soil respiration. Subtle changes in  
356 metabolism, not apparent when aggregate measures such as soil respiration are used as an  
357 indicator of community activity, may thus potentially have significant consequences for  
358 ecosystem-scale function. Such metabolic changes may therefore have to be accounted for to  
359 fully understand terrestrial climate change feedback mechanisms. It is therefore imperative to  
360 develop our knowledge of soil microbial community functioning using a microbial energetics  
361 approach, if we are to construct a complete understanding of carbon dynamics in soils. The work  
362 presented here provides empirical data that can feed into emerging microbial-enzyme carbon-  
363 climate based feedback models<sup>44,45</sup>, and the proposed ecological model of microbial energetics in  
364 soil ecosystems can be used as a start.

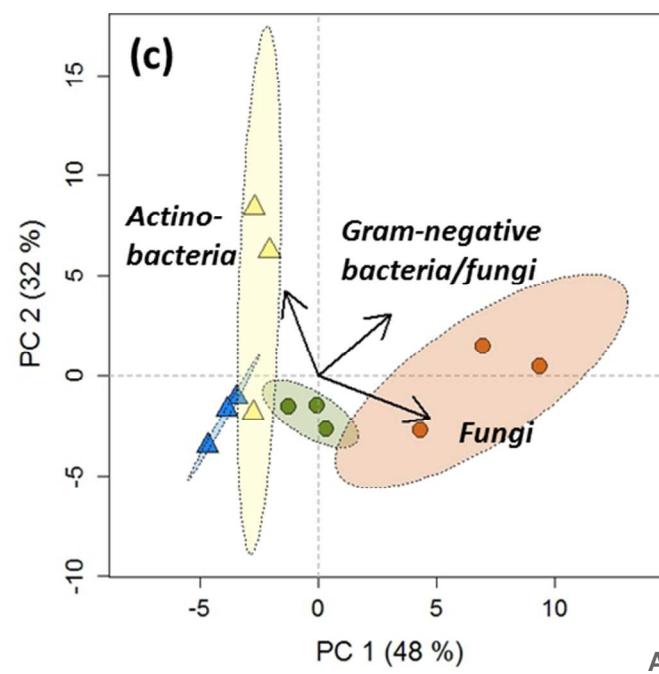
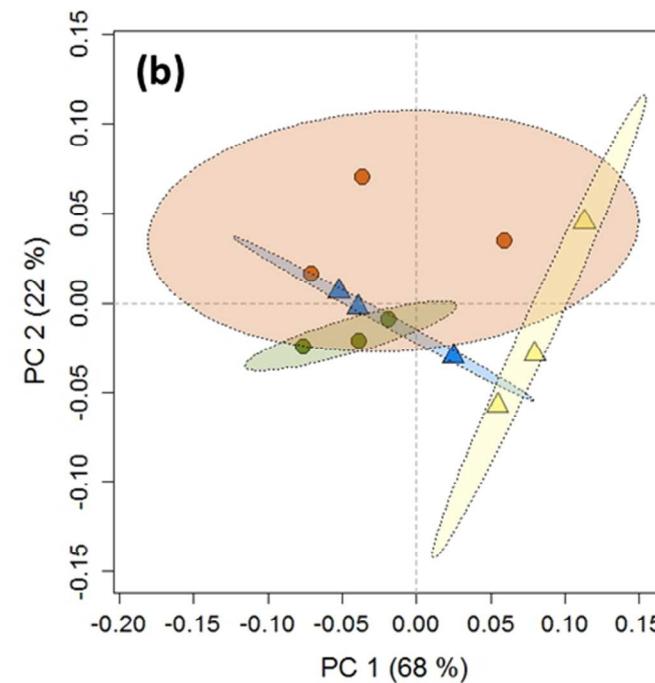
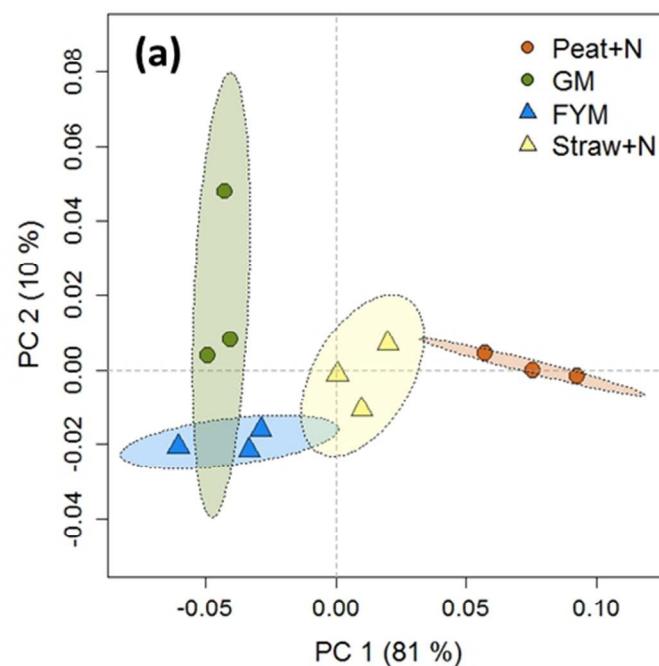
365 In the last century, theoretical ecological frameworks of ecosystem bioenergetics have been  
366 proposed<sup>46,47</sup> and energy budget of organic forest floors<sup>48</sup> were established. Currie<sup>37</sup> evaluated  
367 the relation between carbon and energy and our proposed work on microbial energetics in  
368 terrestrial soil ecosystems further develops the area of ecosystem bioenergetics. However, the  
369 ecological model is still in its infancy within soil science and there is a clear scope for further  
370 development. Soils are structurally heterogeneous and external environmental conditions do not  
371 have a uniform effect throughout the soils, resulting in a large diversity of micro-habitats. Future  
372 studies could examine microbial energetics under various environmental conditions. Here, soils  
373 could be exposed to photoperiods, flooded conditions or oxygen-free atmosphere to estimate the  
374 importance of e.g. autotroph<sup>49</sup>, methanogen<sup>50</sup>, sulfate- or nitrate<sup>51</sup> reducing microorganisms on  
375 microbial energetics. In a broader perspective, the microbial energetics approach has the  
376 potential to provide further information when employing ecological theory into microbial  
377 ecology to better understand microbial systems. In particular, it provides new insights into the  
378 relation between biodiversity and land use extensification<sup>52</sup>, ecosystem development<sup>53,54</sup> as well  
379 as key ecosystem functioning such as carbon sequestration<sup>43</sup> and nutrient retention<sup>52</sup>. By taking  
380 an energetic view of soil microbial metabolism, we may improve our understanding of the  
381 significance of microbial biodiversity on ecosystem function and thus improve prediction of  
382 microbial feedback mechanisms and ecosystem responses to climate change.

## FIGURES

**Figure 1.** Substrate-induced heat flows and respiration. (a) Overall responses of four soils from the Ultuna Long-Term Field Experiment. Mean values represent sum of responses to all seven substrates. (b-d) Responses of each carbon substrate separately (N-acet = N-acetyl glucosamine;  $\gamma$ -am =  $\gamma$ -amino butyric acid; L-ala = L-alanine; gluc = D-glucose; cyclo =  $\alpha$ -cyclodextrin; citric = citric acid and  $\alpha$ -keto =  $\alpha$ -ketoglutaric acid): (b) Heat flows  $Q_{\text{Substrate}}$  and  $Q_{\text{Metabolism}}$ , (c)  $Q_{\text{Net soil}}$  and (d) respiration. Heat flows and respiration were determined by isothermal microcalorimetry or MicroResp, respectively; for explanation of heat flow abbreviations see Scheme 1. The error bars indicate standard deviation ( $n=3$ ). Peat+N = peat+Ca(NO<sub>3</sub>)<sub>2</sub>; GM = Green manure; FYM = farmyard manure; Straw+N = straw+Ca(NO<sub>3</sub>)<sub>2</sub>.  $Q_{\text{Substrate}} = Q_{\text{Metabolism}}$  when there are no abiotic substrate interactions with physical properties and these substrates are suffixed with \*□. Substrates suffixed with \* are  $Q_{\text{Substrate}}$  and substrates suffixed with □ are  $Q_{\text{Metabolism}}$ . In the latter,  $Q_{\text{Substrate}}$  were corrected for heat outputs derived from sterile soils (Table S2, equation (2)) on the assumption that abiotic substrates interactions with soil matrix are occurring in the same order of magnitude in non-sterile and sterile soils.

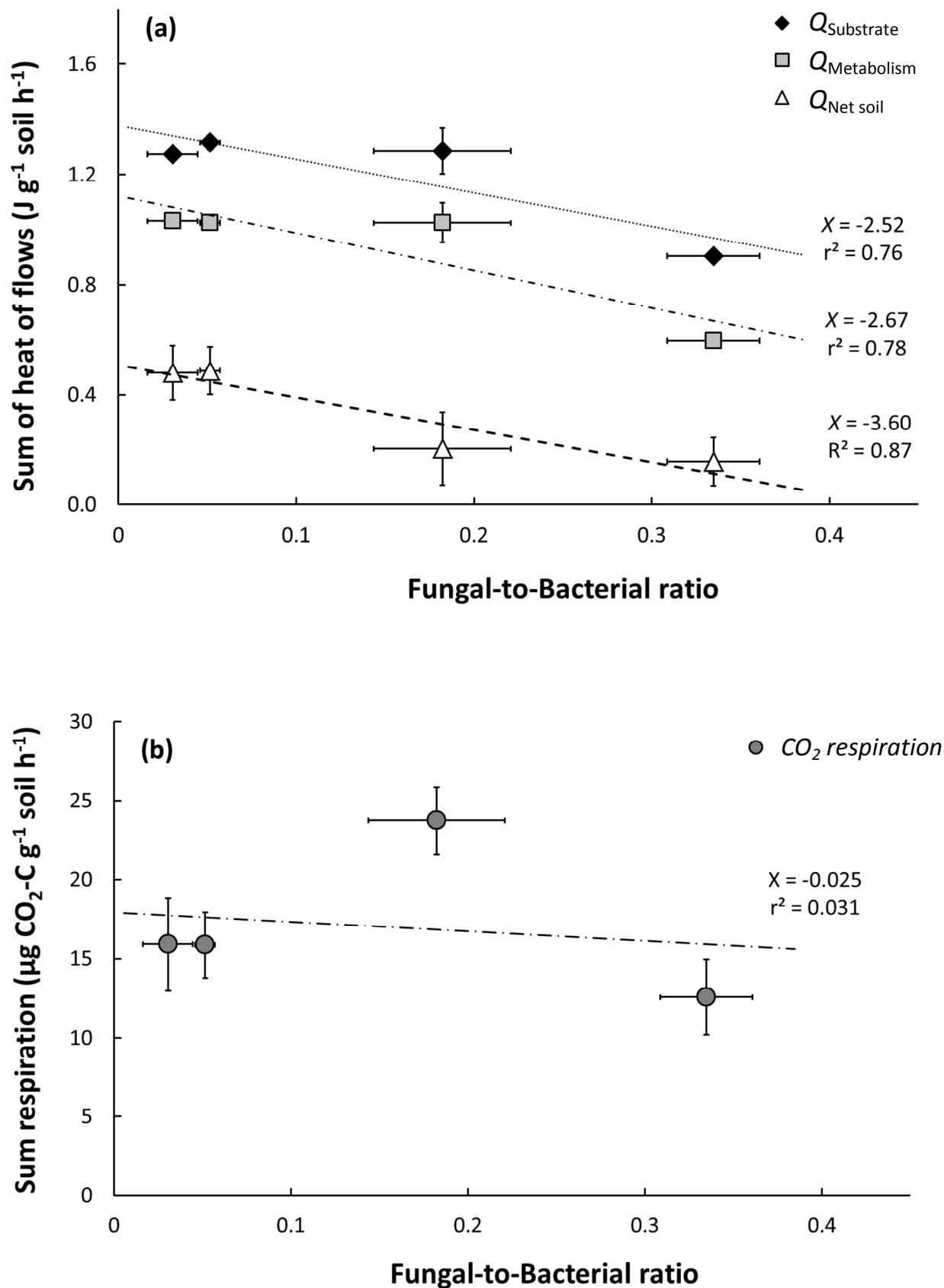


**Figure 2.** Functional diversity profiling and composition of soil microbial communities. Principal component analysis representing the effect of contrasting long-term organic matter inputs on (i) the functional diversity profiling of the soil biota based on utilization of 7 different substrates via (a) overall microbial metabolic activity ( $Q_{\text{Metabolism}}$ ); and (b) CO<sub>2</sub> respiration and (ii) (c) the composition of the soil microbial communities by PLFA. Values in parentheses on axis labels denote % variation accounted for by the respective components, and 95% confidence ellipses are provided for each soil treatment. Peat+N = peat+ Ca(NO<sub>3</sub>)<sub>2</sub>; GM = Green manure; FYM = farmyard manure; Straw+N = straw+Ca(NO<sub>3</sub>)<sub>2</sub>.



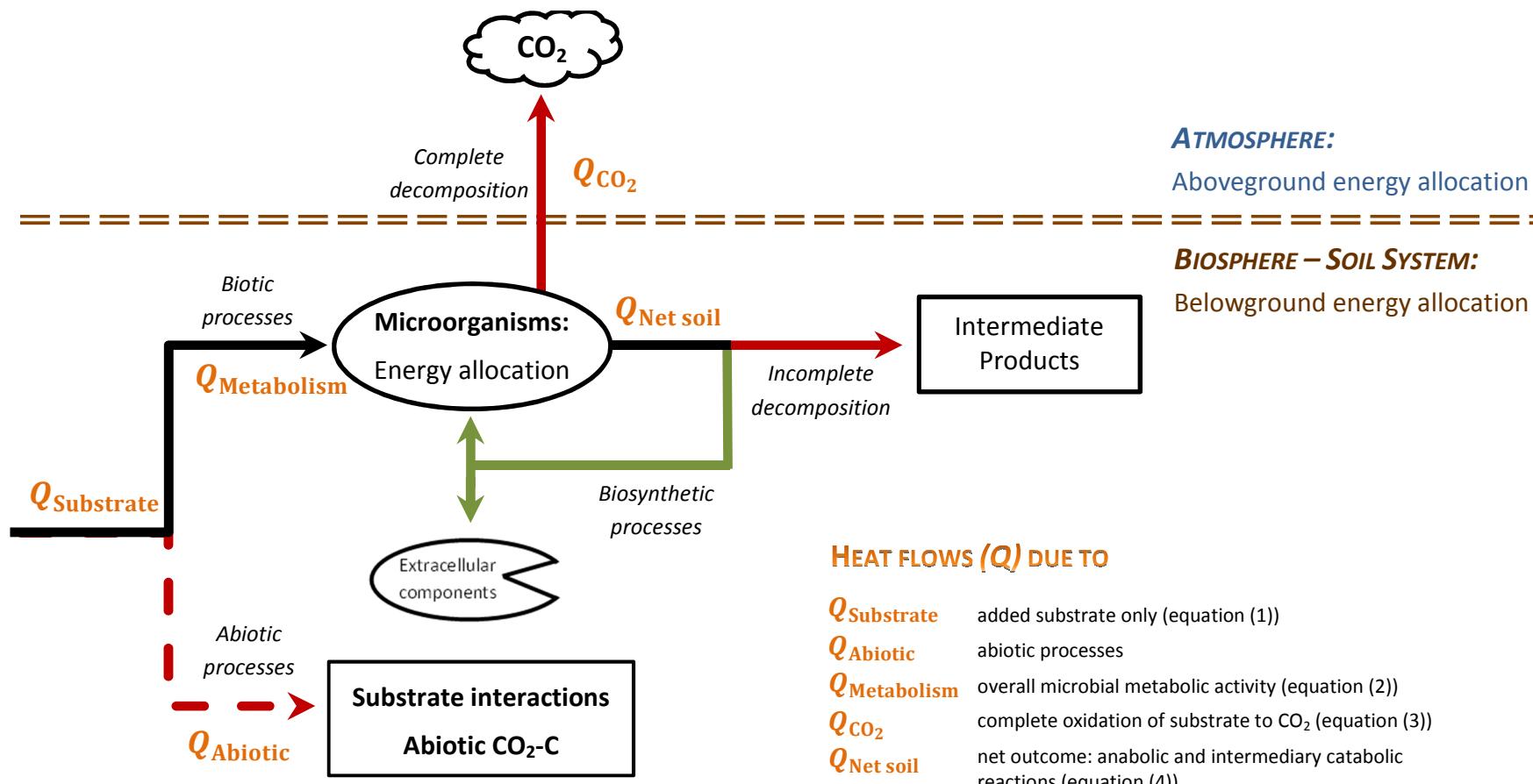
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**Figure 3.** Pearson correlation analysis. Linear correlation analysis between fungal-to-bacterial ratio (x-axis) and (a) heat flows and (b) CO<sub>2</sub> respiration (n = 12).



## SCHEMES

**Scheme 1.** Conceptual model of microbial energetics of metabolism in aerobic soils. Red and green arrows represent catabolic and anabolic processes, respectively. Heat flows ( $Q_{\text{Subscript}}$ ) are represented in orange. Solid lines indicate dominant processes whereas dashed lines represent minor processes.



## TABLES.

**Table 1.** Basic characteristics including fungal-to-bacterial ratios (F:B ratio) and calorespirometric ratio (heat output  $Q_{\text{Metabolism}}$  per unit  $\text{CO}_2$ ;  $\text{mJ } \mu\text{g}^{-1} \text{ CO}_2\text{-C}$ ) of soils used in study. Mean values ( $n = 3$ ); common letters show homogenous means using Duncan's multiple range test at 1% significance level.

Treatment	C (%)	N (%)	C-to-N ratio	Microbial biomass ( $\mu\text{g C g}^{-1}$ soil)	pH (H <sub>2</sub> O)	F:B ratio*	calorespirometric ratio $\text{mJ } \mu\text{g}^{-1} \text{ CO}_2\text{-C}$
Green Manure	1.7 A	0.19 A	9.7 A	205 A	5.9 A	0.18 A	44 A
Straw+Ca(NO <sub>3</sub> ) <sub>2</sub>	2.0 B	0.17 B	10.7 B	254 B	6.4 B	0.03 B	65 B
Farmyard Manure	2.3 C	0.23 C	10.1 C	298 C	6.4 B	0.05 B	66 B
Peat+Ca(NO <sub>3</sub> ) <sub>2</sub>	3.9 D	0.22 C	17.6 D	186 A	5.8 A	0.33 C	49 A

\*Fungal-to-bacterial ratio (F:B ratio) was based on the abundance of the fungal PLFA biomarker 18:2 (9, 12)<sup>27</sup> and the sum of 8 bacterial PLFA biomarkers.

## ASSOCIATED CONTENT

**Supporting Information.** Additional information noted in the text is available. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS

$\text{CO}_2$ , carbon dioxide;  $Q_{\text{Total}}$ , heat flows of substrate-amended soils;  $Q_{\text{Substrate}}$ , heat flows from added substrate;  $Q_{\text{Control}}$ , heat flows from water amended soils/basal metabolism,  $Q_{\text{Metabolism}}$ , heat flows from overall microbial metabolic acitivity;  $Q_{\text{Abiotic}}$ , heat flows in sterile soils/abiotic processes;  $Q_{\text{Net soil}}$ , net outcome: heat flows of anabolic and intermediary catabolic reactions;  $(Q_{\text{CO}_2})$ , heat dissipated during complete biological oxidation of the added substrate;  $H_c^o$ , standard molar enthalpy;  $n(\text{CO}_2)_{\text{substrate}}$  and  $n(\text{CO}_2)_{\text{control}}$ ,  $\text{CO}_2$  mineralized (mol) in substrate-amended and water-amended control soils, respectively;  $N_C$ , the number of carbon atoms in substrate added.

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