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Role of substrate supply on microbial carbon use efficiency and its role in interpreting soil microbial community-level physiological profiles (CLPP)

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11th April, 2018

Dear Soil Biology & Biochemistry,

Please find attached the revised version of the manuscript "**Carbon use efficiency at different geographical scales and its role in interpreting soil microbial community-level physiological profiles (CLPP)**" by David L. Jones, Paul W. Hill, Andrew R. Smith, Mark Farrell, Tida Ge, Natasha C. Banning and Daniel V. Murphy which we wish to submit to Soil Biology & Biochemistry. We thank you for considering this manuscript and look forward to hearing from you soon.

Yours sincerely

Davey Jones
(on behalf of all the authors)

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Response to the reviewers comments

Ref.: Ms. No. SBB13301
Title: Role of substrate supply on microbial carbon use efficiency and its role in interpreting soil microbial community-level physiological profiles (CLPP)

We thank the two reviewers for their very positive comments on our manuscript. They have raised some valid points and identified some minor errors that somehow eluded us during the finalization on the manuscript. We have now addressed their comments as detailed below (reviewer comments in bold with our responses placed underneath).

Reviewer #1:

1. **Line 25: It is important to state that these CUE estimates are substrate specific, rather than general measures of community carbon use efficiency. CUE estimates based on turnover of specific substrates are also dependent on the duration of the measurements. These limitations need to be clearly stated. The comparability of CUE estimates obtained from various methods is an active topic of debate.**
    Yes, this is a valid point. We have now clarified that this is the average across all substrates (line 25). We have also clarified on line 21 that we are interested in the variability of substrate CUE.

2. **Line 27: "actively conserved" over what time interval?**
    The timescale has now been added to the Abstract (line 27). It was 72 h.

3. **Line 29: meaning not clear. If you are trying to say the CLPP results did not correlate with substrate-specific CUE estimates, say so directly and provide the statistics. Also not clear whether the comparisons were pairwise by substrate or aggregrated.**
    This was not what we meant so clearly the sentence was poorly written in the original manuscript. We have now reworded this sentence accordingly (lines 29-34). We hope it is now much clearer.

4. **Line 31: what is meant by "little difference in interpretation"? What was your interpretation?**
    This has now been rewritten as part of (3) above.

5. **Line 34: not clear how the results presented are related to "variation in ecosystem scale CUE" which is affected by many things that appear extrinsic to this study.**
    Yes, point taken. I don't think it was our intention to suggest that differences in ecosystem CUE were solely due to shifts in substrate supply. However, we do still think that the type of substrate supply is an important modulator of CUE. We have therefore clarified the final line of the abstract as follows (underlined text): "In conclusion, we present new mechanistic evidence to support the paradigm that variation in ecosystem CUE may in part reflect differences in the types of C supplied to the microbial biomass."

6. **The Introduction is more informative than the abstract. However, the methodological dependence of CUE estimates merits further discussion. A good general reference is Geyer et al. 2016 (Biogeochemistry (2016) 127:173-188). The main point is that substrate-specific, substrate-independent and stoichiometric methods do not necessarily yield comparable CUE values. In particular, substrate specific mineralization/ immobilization ratios are time dependent.**
    This is an excellent paper and it was amiss of us not to include it in the original manuscript. We have now added a new section to the Introduction (lines 69-77) to cover this aspect. The reference has now also been used to support the statement made on Line 261 in the Discussion. The Geyer et al. (2016) reference has been added to the reference list at the end of the manuscript.

7. **Line 134: Soil samples differed in moisture. Was the effective concentration of substrate standardized to dry mass or soil organic carbon, for example?**
There is always a dilemma in soil science experiments about whether to normalize water content across samples (e.g. to 70% water holding capacity or to -100 kPa) or to just use the soils in their natural field state. Clearly, there are pros and cons of each approach. As in previous studies published in SBB, we took the executive decision to use the soils at their intrinsic moisture content as this better reflects the natural conditions of the microbial community. The average (±SEM) percentage moisture content of the soils in the field, regional and continental scale studies was 18.0 ± 0.9, 31.0 ± 6.1 and 31.1 ± 3.3, respectively (so not that dissimilar). Overall, we don't think our use of soils in their intrinsic moisture state compromises the findings of our study in any significant way. Normalizing for soil organic carbon was not considered as this would be really difficult to interpret given the wide range in SOC contents used in the study (1-30% SOC) and differences in SOC quality. This would be a good idea to consider in future studies alongside normalizing the addition rates for microbial biomass-C.

8. **Lines 250-260: Some of this qualification about CUE methods and limitations should go in the abstract and introduction to improve clarity and impact of the presentation.**
We require some editorial guidance here. The abstract is quite long as it stands. Our feeling was that these points made in lines 250-260 were derived from our results and therefore are best placed in the Discussion, rather than the Introduction. We did try putting some of the text in the abstract but it just didn't sound right and detracted from the main messages in our view. We can modify this, however, if the editor wishes us too.

Reviewer #2:

1. **In the end of the abstract, the author concluded that "In conclusion, we present new mechanistic evidence to support the paradigm that variation in ecosystem CUE reflects differences in the types of C supplied to the microbial biomass". I don't think this statement is well supported by the results. As the authors mentioned in line 246-250, CUE is influenced by many factors other than substrate. Ecosystems with different microbial community would have different CUE values even they were given the sample types of C supply.**
See point 5 for reviewer 1 above. We have now clarified this.

2. **Several places of typos: line 42, leader; line 59, top; line 284, map.**
Thanks for highlighting these. We have now corrected these typographical mistakes.

RESEARCH HIGHLIGHTS

- Microbial CUE varied greatly between C substrates

- CUE was highly conserved across soils for some C substrates, but not for others

- CUE does not need to be accounted for to separate soils based on CLPP

- CUE does need to be accounted for when interpreting C use in CLPP

- Microbial CUE varied greatly between C substrates

- CUE was highly conserved across soils for some C substrates, but not for others

1   **Role of substrate supply on microbial carbon use efficiency and its role in**

2   **interpreting soil microbial community-level physiological profiles (CLPP)**

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ABSTRACT

14

15   Carbon use efficiency (CUE) describes the relative partitioning of carbon (C)

16   between anabolic and catabolic processes within the soil microbial community. Further, it

17   represents a major factor regulating the amount of C cascading through the trophic levels

18   of the soil food web. How CUE relates to C supply, however, remains poorly understood.

19   The primary aim of this study was to determine how CUE varies across a range of spatial

20   scales as a function of C substrate supply. Our secondary aim was to understand how

21   variations in substrate CUE influences the interpretation of community level

22   physiological profiles (CLPP). Using 16 different ^{14}C-labelled substrates (including

23   amino acids, sugars, organic acids and amino sugars) and soils collected at the field,

24   regional and continental scale, we measured the rate of substrate uptake and

25   mineralization from which we calculated CUE. Across all soils ($n = 114$) and substrates

26   ($n = 16$), the average CUE for the microbial community was 0.568 ± 0.004 (range 0.492

27   to 0.794). While the partitioning of substrate-C within the biomass

28   (immobilization/mineralization) over 72 h was highly conserved for some substrates (e.g.

29   glucose), others showed a wide variability in CUE across the samples (e.g. valine). In the

30   context of the CLPP methodology, we showed that individual sites could be statistically

31   separated from each other, irrespective of whether the statistical analysis was based on

32   microbial substrate uptake rate or mineralization rate. However, our results do suggest

33   that caution is needed when ascribing observed CLPP differences to the importance of

34   individual C pathways operating in soil due to the wide variation of CUE between

35   substrates. In conclusion, we present new mechanistic evidence to support the paradigm

that variation in ecosystem CUE may in part reflect differences in the types of C supplied to the microbial biomass.

Keywords: Carbon sequestration; Metabolic profiling; Organic matter cycling; Substrate induced respiration; Soil quality indicator.

1. Introduction

The carbon use efficiency (CUE; i.e. mineralization-to-immobilization ratio) of individual organisms within the soil community regulates the relative amount of carbon (C) that flows through each trophic level within the decomposer food web. Knowledge of the factors regulating CUE is therefore important for predicting the conditions that promote C retention and loss from soils and may also aid the design of management interventions to promote enhanced C sequestration. Indeed, the CUE term is central to many terrestrial C models, and thus understanding variability in CUE can lead to more accurate models and calculation of uncertainty in their predictions of C sequestration and loss. Microbial CUE has been shown to be dependent upon the structure of the microbial community as well being responsive to changes in a range of abiotic soil properties (e.g. temperature, nutrient availability; Spohn et al., 2016a; Maynard et al., 2017) and to the presence of plants (Blagodatskaya et al., 2014). Further, CUE is generally lowered under stress conditions due to the need to expend more energy on repair and defence mechanisms (e.g. Rath et al., 2016). It is therefore not surprising that CUE varies between land uses (Spohn et al., 2016b). In the most comprehensive analysis to date, Sinsabaugh et al. (2017) compared microbial CUE across a broad range of ecosystems and found that, although CUE is responsive to a range of factors, it has a relatively narrow range. The

59　variability in CUE between soils may be partially explained by the different spectrum of

60　C compounds flowing through the soil and their relative use in catabolic

61　reactions/processes leading to mineralization of C to CO_2. This is supported by studies

62　showing that the immobilization-mineralization potential of individual C substrates varies

63　greatly depending on their molecular weight and oxidation state (Gunina et al., 2017;

64　Oquist et al., 2017). Our knowledge of how substrate CUE varies with C supply,

65　however, remains limited (Liang et al., 2011). As CUE is a key regulator of microbial

66　biomass turnover and soil C sequestration (Sinsabaugh et al., 2013, 2017ab), there is an

67　increasing need to include it alongside microbial diversity within global ecosystem

68　models to better predict ecosystem feedbacks to anthropogenic perturbation (e.g. climate

69　warming; Li et al., 2014; Graham et al., 2016; Sinsabaugh et al., 2017). Fundamental to

70　this is a knowledge of how CUE varies at a range of spatial scales. It should also be noted

71　that CUE can be defined in different ways depending on the nature of the study (Geyer et

72　al., 2016). For example, studies have looked at C partitioning and CUE in soil microbial

73　communities supplied with C substrates over short time scales (hours→days), while

74　others have explored CUE from a food web and ecosystem perspectives (months→years

75　timescale). This makes the standardisation of CUE problematic as it is highly dependent

76　on the spatial and temporal scale over which the measurement are made (Geyer et al.,

77　2016; Glanville et al., 2016). In this study we are focusing on community-scale efficiency

78　of microbial biomass synthesis (Geyer et al., 2016).

79　　　Community-level physiological profiling (CLPP) is a commonly used approach for

80　assessing shifts in microbial community function in soil (Garland, 1997; Macdonald et

81　al., 2015; Siles et al., 2017). Since its inception in the 1990s, both the underlying method

82 and subsequent data analysis techniques have been progressively refined (Mayr et al.,

83 1999; Garland et al., 2001; Classen et al., 2003; Calabrix et al., 2005; San Miguel et al.,

84 2007; Swallow and Quideau, 2015). One of the biggest step-changes in methodology

85 when applied to soils was the move away from the need to pre-extract organisms prior to

86 performing CLPP using Biolog® microplates to a direct approach using soil-filled

87 MicroResp® plates (Campbell et al., 2003). The MicroResp® CLPP approach frequently

88 reveals differences in catabolic substrate use between soils under different management

89 regimes and has been shown to be better at discriminating between samples than other

90 profiling techniques (Lalor et al., 2007). In most cases, inferences are made from the

91 substrate use profiles about C availability and microbial processing rates in soil under

92 different management regimes (Artz et al., 2006). Apart from the potential artefacts

93 introduced by soil preparation (Swallow and Quideau, 2015), caution is also required

94 when interpreting the catabolic profiles. For example, CO_2 release rate is likely

95 dependent upon a range of soil factors (e.g. moisture content, carbonate content). While

96 this does not change the substrate use profile for a particular soil it can prevent direct

97 comparison of CO_2 evolution rates between samples obtained from different geographical

98 locations. In addition, differential sorption of charged substrates (e.g. amino acids,

99 organic acids) between soils can have a major impact on substrate availability and

100 therefore CO_2 output. While this is likely to have minimal effect for neutral or univalent

101 solutes (e.g. amino acids; Fischer et al., 2010) it is likely to have a major impact on

102 di/trivalent C substrates (e.g. citrate; Jones and Edwards, 1998). Lastly, differences in

103 CUE both between substrates and soils may lead to over- or under-estimation of the

104 importance in the use of some substrates.

105   The aim of this study was to investigate the variability in CUE for a wide range of

106   C substrates across a range of geographical scales. We hypothesized: (i) that substrates

107   would vary widely in their CUE but that these values would be conservative with

108   geographical scale due to commonality in microbial metabolic pathways operating in soil,

109   and (ii) that ignoring substrate CUE may lead to bias in the functional interpretation of

110   CLPP results.

111

112   **2. Materials and methods**

113   *2.1. Selection of soils*

114   Soil samples were collected at three spatial scales: (1) field scale ($n = 48$), (2)

115   regional scale ($n = 24$), and (3) continental scale ($n = 42$). Samples for the field-scale

116   evaluation were collected from a replicated field experiment located at Abergwyngregyn,

117   UK (53°14′N, 4°01′W). The experiment consisted of three tree species (*Alnus glutinosa*,

118   *Betula pendula* and *Fagus sylvatica*) grown either in monoculture or together in

119   polyculture either at ambient (380 ppm) or elevated CO_2 (580 ppm) in the BangorFACE

120   free-air CO_2 enrichment facility (for full details see Smith et al. (2013ab) and Table S1).

121   Samples of soil (Eutric Cambisol) were taken with a 5 cm diameter stainless steel corer

122   from three individual depths (0-10, 10-20 or 20-30 cm) in each of the 4 forest treatments

123   under each CO_2 regime (i.e. 24 treatments in total with $n = 4$ replication).

124   Samples for the regional-scale evaluation were chosen to incorporate 8 different

125   soil type/agricultural land use combinations along an altitudinal gradient (2-500 m asl) in

126   North Wales (for full details see Farrell et al. (2014a) and Table S2). Independent

127   samples along the gradient were collected with a spade from a depth of 0-10 cm (8

128 treatments in total with *n* = 3 replication). The gradient has commonality with the field-

129 scale measurement site, albeit on a different land use (forestry versus grassland).

130   Samples for the continental-scale evaluation were obtained from 42 locations across

131 Europe and were chosen to incorporate a range of contrasting soil and land use types. A

132 summary of the soil and associated land used are provided in Table S3. These were

133 essentially treated as single replicates.

134

135 *2.2. Community level physiological profiling and CUE*

136   Sixteen low molecular weight (MW) C substrates were chosen based upon their

137 widespread use within Biolog® and MicroResp® CLPP assays. These included (1) six

138 amino acids; arginine, aspartic acid, glycine, lysine, phenylalanine and valine; (2) four

139 sugars; fructose, glucose, starch and sucrose, (3) one amino sugar; glucosamine, and (4)

140 five carboxylic acids; oxalic acid, salicylic acid, succinic acid, acetic acid and malic acid.

141 The substrates were also chosen as they represent common metabolites found in soil and

142 in organic materials entering soil (e.g. plant litter, rhizodeposits, manure etc.) and their

143 known dominance in soil plant and microbial metabolism. For the CLPP analysis, 5 g of

144 field-moist soil was placed in individual 50 cm^3 polypropylene tubes. 500 µl (10 mM) of

145 each ^{14}C-labelled substrate was pipetted onto separate soil samples (one substrate per soil

146 sample). This level of C substrate addition was chosen to reflect the likely concentration

147 in soil following the lysis of a root cell and that typically used in CLPP assays (Jones et

148 al., 2004). A polypropylene scintillation vial containing 1 M NaOH (1 cm^3) was

149 immediately suspended above the soil to trap the respired ^{14}CO$_2$, and the tubes sealed.

150 The soils were then incubated at 20 °C for 4 h after which the NaOH trap was recovered

151   and replaced. The short incubation time was chosen to ensure that the substrate was not

152   fully depleted and is consistent with previous CLPP methodologies (Table S4). After 72

153   h, when most of the substrate was assumed to have been taken up by the microbial

154   community and partitioned into anabolic and catabolic pathways (Table S4), the second

155   NaOH trap was recovered. This time was selected based on many previous studies

156   measuring the dynamics of low MW C turnover by the microbial biomass which show

157   that partitioning is quasi-complete after 72 h (Glanville et al., 2016). After trap removal at

158   72 h, the amount of available ^{14}C remaining in the soil was quantified by extracting the

159   soil with 25 ml of 0.5 M K_2SO_4 (Joergensen and Brooks, 1990; Glanville et al., 2016).

160   ^{14}C in the NaOH traps and K_2SO_4 was determined by liquid scintillation counting with

161   Optiphase 3 scintillation fluid (Wallac EG&G, Milton Keynes, UK) and a Wallac 1404

162   scintillation counter with automated quench correction (Wallac EG&G). All samples

163   were corrected for the presence of ^{40}K.

164   Microbial immobilization of the ^{14}C-substrate ($^{14}C_{imm}$) after 72 h was estimated as

165   follows:

166    $$^{14}C_{imm} = {}^{14}C_{tot} - {}^{14}C_{K2SO4} - {}^{14}CO_{2\text{-}72h}$$ (Eqn. 1)

167   where $^{14}C_{tot}$ is the total amount of ^{14}C-substrate added to the soil at time (t) = 0, $^{14}C_{K2SO4}$

168   is the amount of ^{14}C recovered in the 0.5 M K_2SO_4 extract (Table S4) and $^{14}CO_{2\text{-}72h}$ is the

169   total amount of ^{14}C recovered as $^{14}CO_2$ after 72 h. Microbial CUE for each substrate was

170   then estimated as follows:

171    $$CUE = {}^{14}C_{imm} / ({}^{14}C_{imm} + {}^{14}CO_{2\text{-}72h})$$ (Eqn. 2)

172 following the method of Jones et al. (2018). Based on the amount of $^{14}CO_2$ produced after

173 4 h ($^{14}CO_{2\text{-}4h}$), the corresponding microbial substrate uptake rate ($^{14}C_{uptake}$) was estimated

174 as follows:

175 $$^{14}C_{uptake} = {}^{14}C_{CO2\text{-}4h} \times (1\text{-}CUE)^{-1}$$ (Eqn. 3)

176

177 *2.3. Statistical analysis*

178 Following the CLPP approach of Lalor et al. (2007), the RELATE routine in

179 PRIMER v6 (Quest Research Ltd., Auckland, New Zealand) was used to test whether

180 there was any difference in the interpretation of CLPP data (in soil groupings or treatment

181 structures) between using substrate mineralization (i.e. $^{14}CO_{2\text{-}4h}$) versus the use of

182 substrate uptake data ($^{14}C_{uptake}$). RELATE measures how closely related two sets of

183 multivariate data (substrate mineralization vs uptake) are by calculating a rank correlation

184 coefficient (Spearman's ρ) between all elements of their respective (dis)similarity

185 matrices. If among-sample relationships agree in exactly the same way in both data sets,

186 then the rank correlation (ρ) =1, is a perfect match. Under the null hypothesis that there is

187 no relation between the two similarity matrices, ρ will be approximately zero.

188 Other differences between substrate group behaviour were evaluated by ANOVA

189 with Tukey post-hoc pairwise comparison using $P < 0.05$ as the cut-off value to indicate

190 statistical significance (Minitab v16; Minitab Inc., State College, PA). Linear regressions

191 and principle component analysis (PCA) were performed with Minitab v16.

192

193 **3. Results**

194 *3.1. Microbial substrate removal from soil*

195   Extracting the soils with 0.5 M K_2SO_4 at the end of the 72 h incubation period

196   indicated that the majority of the ^{14}C-labelled substrate had been taken up from the soil

197   by the microbial biomass. Across all the different C substrates, on average $12.6 \pm 2.6\%$ of

198   the substrate ^{14}C could be recovered from the soil after 72 h (Table S4). This tended to be

199   higher for the amino acids in comparison to the sugars and organic acids ($P = 0.02$).

200

201   *3.2. Microbial C use efficiency for individual substrates*

202   Across all the 16 substrates and 114 samples used in this study ($n = 1824$) we

203   calculated the average CUE value for the soil microbial community to be 0.568 ± 0.004

204   (Fig. 1). This ranged from 0.492 to 0.794 across all the soils used in the study. As

205   expected, the variation in CUE increased from the field scale (0.548 ± 0.002; CV% 3.1)

206   to the regional scale (0.574 ± 0.007; CV% 6.1) and again to the continental scale ($0.600 \pm$

207   0.011; CV% 11.5; Fig. S1). Across all samples, the CUE for sugars (0.677 ± 0.004) was

208   higher than for amino sugars (0.601 ± 0.014; $P < 0.001$). Further, these were both higher

209   than for amino acids (0.551 ± 0.007; $P < 0.001$) which proved to be higher than for

210   organic acids (0.498 ± 0.007; $P < 0.001$). Overall, the CUE values for the different sugars

211   were similar with few differences observed between the regional and continental scale. In

212   contrast, the CUE values for the individual amino acids were different and followed the

213   series: ASP = LYS > PHE > VAL > ARG > GLY ($P < 0.001$; Fig. 1). In addition,

214   differences in CUE for the amino acids were apparent at the three spatial scales. The

215   greatest variability in CUE was seen between the individual organic acids ($P < 0.001$),

216   with large differences in CUE seen for some organic acids at the different spatial scales.

217

218   *3.3. Substrate uptake and mineralization rate*

219   Overall, there were major differences in the rate of mineralization of the

220   individual substrates when added to soil ($P < 0.001$; Fig. 2). Across all samples, the

221   highest rate of mineralization was observed for aspartate (61 ± 2 µmol kg^{-1} h^{-1}) while the

222   lowest rate was observed for salicylic acid (1.3 ± 0.1 µmol kg^{-1} h^{-1}). At the field scale, the

223   rate of substrate mineralization was not greatly affected by treatment (i.e. depth, elevated

224   CO_2 or forest type) with the same general pattern in CO_2 evolution seen across all

225   samples. While some substrates were used at similar rates independent of field treatment

226   (e.g. sucrose, phenylalanine), other substrates showed increased variability between

227   samples (e.g. valine, salicylate). In contrast to the field scale, greater variability in the

228   overall profile of substrate mineralization was seen at the regional and continental scale,

229   although the patterns were broadly similar to those observed at the field plot level.

230   After taking into account the proportion of substrate-C immobilized in the

231   microbial biomass (i.e. CUE), the rate of microbial substrate uptake was calculated. This

232   showed that the rate of ^{14}C uptake was approximately three-fold higher than accounted

233   for by $^{14}CO_2$ alone. Overall, there was a close linear correlation between substrate uptake

234   rate and subsequent mineralization across all 16 substrates ($r^2 = 0.920$; Fig. S2).

235   Consequently, the broad patterns of microbial substrate uptake were similar to those

236   observed for substrate mineralization across all spatial scales (Fig. 2). Although little

237   variation was seen in substrate uptake rate at the field scale, large differences were seen

238   between the different land uses and soil types in the regional and continental samples.

239   With the exception of a few substrates, the coefficient of variability (CV%) across the

240   samples was similar if substrate use was calculated based on either the rate of depletion

241   from soil or its mineralization (Fig. S3).

242

243   *3.4. Sample similarity*

244   There was considerable similarity between the individual substrates that best

245   separate soil groupings and treatment structures for substrate mineralization and substrate

246   uptake (Table S5). Ordinations based on substrate mineralization and uptake were

247   significantly related to each other at the field, regional and continental scales (Table 1)

248   indicating no major difference in interpretation of data by either approach (Fig.S7-S9).

249

250   **Discussion**

251   *4.1. Substrate C use efficiency*

252   Our soils displayed a wide range of CUE values for the 16 different C substrates

253   tested here (Fig. 1). This variability in CUE reflects the use of substrate-C within a

254   diverse array of metabolic pathways present within the microbial community, some of

255   which preferentially feed key anabolic processes (e.g. cell wall production, protein

256   synthesis) while others are predominantly used for energy production. The differences

257   may also partially reflect differences in microbial community composition (e.g. fungal-

258   to-bacterial or copiotroph-to-oligotroph ratios), or the degree of competition/stress being

259   experienced by the community and therefore the relative abundance of specific metabolic

260   pathways operating in the soil (Rath et al., 2016; Maynard et al., 2017). The results do,

261   however, clearly indicate the adaptability of the community to split the C derived from

262   these common substrates into both anabolic and catabolic use pathways. The range of

263   CUE values reported here (0.28-0.78; Table S6) are consistent with previously published

264   studies on individual or limited ranges of C substrates (Steinweg et al., 2008; Dijkstra et

265   al., 2011; Frey et al., 2013; Bölscher et al., 2016; Geyer et al., 2016). Although we

266   present a mean CUE value averaged across all 16 substrates, this only provides a

267   reflection of the limited number of substrates used here and may not reflect the many

268   thousands of compounds that microbes may be exposed to in soil (Swenson et al., 2015).

269   It also does not account for the use of C from other sources which may 'subsidise'

270   metabolism of the labelled substrates. Despite this, the mean CUE values for the diverse

271   collections of soils (regional and continental scale) were 0.574 and 0.600 respectively,

272   similar to the maximum CUE values reported by Sinsabaugh et al. (2013).

273   We deliberately chose substrates commonly found in soil which are likely to

274   dominate organic matter inputs (Stevenson, 1994; Schulten and Schnitzer, 1997;

275   Glanville et al., 2012) and for which membrane transporters are broadly encoded across

276   the microbial community (Jennings, 1995; Padan, 2009). It is currently unclear, however,

277   to what extent the mixture and relative concentration of compounds influences the overall

278   CUE of the community. Previous experiments with individual substrates have indicated

279   that CUE remains independent of concentration at low substrate addition rates but then

280   reduces as more of the same substrate is added (Vinolas et al., 2001; Roberts et al., 2007).

281   In addition, CUE may be expected to change as the population becomes more active,

282   starts growing, and on the availability of other nutrients required for growth (e.g. N and

283   P; Roberts and Jones, 2102; Sinsabaugh et al., 2013; Creamer et al., 2014; Spohn et al.,

284   2016). In this study, we normalised substrate addition on a molar basis and chose a

285   representative substrate concentration to reflect a pulse addition of C into the soil (e.g.

286   when a root cell bursts). Overall, the amounts of C added to the soil were therefore quite

287   low (ca. 0.06 mg C g^{-1}) relative to the average organic C content of the soils (49 ± 5 mg C

288   g^{-1}). The CUE values reported here therefore do not reflect rapid growth as observed in

289   conventional substrate-induced respiration assays (Kaiser et al., 1992; Lin and Brooks,

290   1999; Wutzler et al., 2012). In this study, we only investigated the CUE of substrates

291   added in isolation. We therefore cannot discount the potential that the CUE for each

292   substrate might change when added within a cocktail of other C compounds. The co-

293   addition of other similar C sources may be expected to repress substrate uptake at the

294   transporter level and may also alter C partitioning through internal feedbacks on

295   metabolic pathways (Roberts and Jones, 2012; Farrell et al., 2014b). Further work is

296   clearly required to confirm the extent of this phenomenon in soil microbial communities.

297   In the case of sugars, the CUE values showed little variability across the wide

298   range of soil types and land management regimes investigated here, suggesting they are

299   processed similarly within the community. Interestingly, polymeric glucose (starch) had a

300   significantly higher CUE than monomeric glucose. The most likely explanation for this is

301   that the intermediates of starch breakdown (e.g. linear and branched glucans, maltose,

302   maltodextrin) are partitioned differently to glucose once they have entered the cell (Boos

303   and Shuman, 1988; Farrell et al., 2014b). Here we assume that the enzymes required to

304   degrade starch extracellularly were already abundant in the soil as otherwise CUE would

305   be expected to drop in response to the extra energetic drain needed to synthesize and

306   secrete α and β amylases (Bölscher et al., 2016). Alternatively, the difference in the CUE

307   could be attributable to the preferential utilization of starch by a specific group of

308   microorganisms exhibiting strong α/β amylase activity and a different pattern of

309   intracellular C partitioning.

310   The accurate calculation of substrate CUE is dependent on the recovery of unused

311   substrate at the end of the experiment. Here we used 0.5 M K_2SO_4 as an extractant due to

312   its proven ability to recover simple low MW substrates from soil (e.g. amino acids,

313   sugars, amino sugars; Joergensen, 1996), whilst minimising damage to microbial cells

314   (Rousk and Jones, 2010). For uncharged or weakly charged substrates a complete

315   recovery with 0.5 M K_2SO_4 is expected. However, we acknowledge that it may be less

316   efficient at recovering some organic acids from soil. Our experience shows that 0.5 M

317   K_2SO_4 is excellent at recovering monocarboxylic organic acids (e.g. acetate) from soil

318   and largely effective at recovering divalent organic acids which do not precipitate (e.g.

319   succinate). However, some divalent organic acids, such as oxalate, readily precipitate in

320   the presence of Ca^{2+}, possibly preventing complete recovery. This would lead to an

321   overestimate of CUE. In all the samples investigated here, no relationship was apparent

322   between exchangeable Ca^{2+} and the CUE for oxalate ($r^2 = 0.003$; $P = 0.140$) suggesting

323   that this is not a major influence (Fig. S6). Further, the recovery of oxalate with K_2SO_4

324   was not correlated with exchangeable Ca^{2+}. As most common tricarboxylic acids can

325   become fixed or strongly sorbed to the solid phase and are difficult to fully recover (e.g.

326   citrate, aconitate; Jones and Edwards, 1998; Rasamimanana et al., 2017), this substrate

327   group was not included in this CLPP study.

328

329   *4.2. Method of analysing CLPP data*

330   Overall, our data showed a reasonably consistent pattern of CUE across the

331   diverse range of samples for the different C substrates. Despite this, however, significant

332   variation in CUE existed for some individual organic and amino acids (Table S6). This

333   suggests that a universal set of constants cannot be applied to CLPP mineralization data

334   for individual substrates to routinely convert them back to actual C uptake values (e.g. as

335   only mineralization is measured in typical non-isotopically labelled CLPP studies).

336   Despite this caveat, and the obvious underestimation of rates of substrate use within

337   conventional CLPP assays, statistical analysis revealed that accounting for CUE did not

338   greatly alter the separation pattern between samples. The group of compounds largely

339   responsible for driving the statistical separation between the isotopic-based (uptake) and

340   conventional (mineralization) CLPP approaches were slightly different (Table S5, Fig.

341   S7). Consequently, care is needed when inferring dominant processes occurring in soil

342   from conventional CLPP profiles.

343   To ensure comparability across the samples, we standardized the climatic

344   conditions for the study, however, mean annual temperature was known to vary both at

345   the local and continental scale. Based on previous studies, where CUE was relatively

346   insensitive to temperature, we expect this to have had a minor outcome on the CLPP

347   profiles (Roberts and Jones, 2012; Oquist et al., 2017), however, further work is required

348   to confirm this. Following convention, our CLPP study was performed in the laboratory

349   in the absence of plants and associated mycorrhizas and consequently this may have

350   affected our CLPP profiles (Blagodatskaya et al., 2014). Although CLPP has largely been

351   confined to the laboratory, the approach has also been adapted for use in the field

352   (Glanville et al., 2012; Lehman et al., 2013). The benefits of *in situ* field measurements

353 include the lack of perturbation of microbial activity caused by soil preparation (e.g.

354 sieving) and the inclusion of intact rhizosphere communities. While these effects can be

355 minimized in the laboratory (Swallow and Quideau, 2015), it has been demonstrated that

356 substrate C partitioning does vary slightly between laboratory and field samples (Oburger

357 and Jones, 2009). In spite of this Glanville et al. (2012), suggested that substrate use

358 profiles were more likely to vary inter-annually in the field relative to differences

359 between the laboratory and the field. In conclusion, our results suggest that conventional

360 CLPP profiling represents a good way of distinguishing between communities with

361 different abilities to assimilate labile C, however, some caution is required in ascribing

362 these differences to processes occurring in the field.

363

370

371 **References**

372 Artz, R.R.E., Chapman, S.J., Campbell, C.D., 2006. Substrate utilisation profiles of

373 microbial communities in peat are depth dependent and correlate with whole soil

374 FTIR profiles. Soil Biology & Biochemistry 38, 2958-2962.

375   Blagodatskaya, E., Blagodatsky, S., Anderson, T.H., Kuzyakov, Y., 2014. Microbial

376   growth and carbon use efficiency in the rhizosphere and root-free soil. Plos One 9,

377   e93282.

378   Boos, W., Shuman, H., 1998. Maltose/maltodextrin system of *Escherichia coli*:

379   Transport, metabolism, and regulation. Microbiology and Molecular Biology

380   Reviews 62, 204-228.

381   Calbrix, R., Laval, K., Barray, S., 2005. Analysis of the potential functional diversity of

382   the bacterial community in soil: a reproducible procedure using sole-carbon-source

383   utilization profiles. European Journal of Soil Biology 41, 11-20.

384   Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A

385   rapid microtiter plate method to measure carbon dioxide evolved from carbon

386   substrate amendments so as to determine the physiological profiles of soil microbial

387   communities by using whole soil. Applied and Environmental Microbiology 69,

388   3593-3599.

389   Classen, A.T., Boyle, S.I., Haskins, K.E., Overby, S.T., Hart, S.C., 2003. Community-

390   level physiological profiles of bacteria and fungi: plate type and incubation

391   temperature influences on contrasting soils. FEMS Microbiology Ecology 44, 319-

392   328.

393   Creamer, C.A., Jones, D.L., Baldock, J.A., Farrell, M.F., 2014. Stoichiometric controls

394   upon low molecular weight carbon decomposition. Soil Biology & Biochemistry

395   79, 50-56.

396 Garland, J.L., Mills, A.L., Young, J.S., 2001. Relative effectiveness of kinetic analysis vs

397   single point readings for classifying environmental samples based on community-

398   level physiological profiles (CLPP). Soil Biology & Biochemistry 33, 1059-1066.

399 Farrell, M., Macdonald, L.M., Hill, P.W., Wanniarachchi, S.D., Farrar, J., Bardgett, R.D.,

400   Jones, D.L., 2014a. Amino acid dynamics across a grassland altitudinal gradient.

401   Soil Biology & Biochemistry 76, 179-182.

402 Farrell, M., Prendergast-Miller, M., Jones, D.L., Hill, P.W., Condron, L.M., 2014b. Soil

403   microbial organic nitrogen uptake is regulated by carbon availability. Soil Biology

404   & Biochemistry 77, 261-267.

405 Fischer, H., Ingwersen, J., Kuzyakov, Y., 2010. Microbial uptake of low-molecular-

406   weight organic substances out-competes sorption in soil. European Journal of Soil

407   Science 61, 504-513.

408 Geyer, K.M., Kyker-Snowman, E., Grandy, A.S., Frey, S.D., 2016. Microbial carbon use

409   efficiency: accounting for population, community, and ecosystem-scale controls

410   over the fate of metabolized organic matter. Biogeochemistry 127, 173-188.

411 Glanville, H., Rousk, J., Golyshin, P., Jones, D.L., 2012. Mineralization of low molecular

412   weight carbon substrates in soil solution under laboratory and field conditions. Soil

413   Biology & Biochemistry 48, 88-95.

414 Glanville, H.C., Hill, P.W., Schnepf, A., Oburger, E., Jones, D.L., 2016. Combined use of

415   empirical data and mathematical modelling to better estimate the microbial turnover

416   of isotopically labelled carbon substrates in soil. Soil Biology & Biochemistry 94,

417   154-168.

418   Graham, E.B., Knelman, J.E., Schindlbacher, A., 2016.   Microbes as engines of

419   ecosystem function: when does community structure enhance predictions of

420   ecosystem processes? Frontiers in Microbiology 7, 214.

421   Gunina, A., Smith, A.R., Kuzyakov, Y., Jones, D.L., 2017. Microbial uptake and

422   utilization of low molecular weight organic substrates in soil depend on carbon

423   oxidation state. Biogeochemistry 133, 89-100.

424   Jennings, D.H., 1995. The Physiology of Fungal Nutrition. Cambridge University Press,

425   Cambridge, UK.

426   Joergensen, R.G., 1996. Quantification of the microbial biomass by determining

427   ninhydrin-reactive N. Soil Biology & Biochemistry 28, 301-306.

428   Joergensen, R.G., Brookes, P.C., 1990. Ninhydrin-reactive nitrogen measurements of

429   microbial biomass in 0.5 M K_2SO_4 soil extracts. Soil Biology & Biochemistry 22,

430   1023-1027.

431   Jones, D.L., Edwards, A.C., 1998. Influence of sorption on the biological utilization of

432   two simple carbon substrates. Soil Biology & Biochemistry 30, 1895-1902.

433   Jones, D.L., Olivera-Ardid, S., Klumpp, E., Knief, C., Hill, P.W., Lehndorff, E., Bol, R.,

434   2018. Moisture activation and carbon use efficiency of soil microbial communities

435   along an aridity gradient in the Atacama Desert. Soil Biology & Biochemistry 117,

436   68-71.

437   Kaiser, E.A., Mueller, T., Joergensen, R.G., Insam, H., Heinemeyer, O., 1992. Evaluation

438   of methods to estimate the soil microbial biomass and the relationship with soil

439   texture and organic-matter. Soil Biology & Biochemistry 24, 675-683

440 Lalor, B.M., Cookson, W.R., Murphy, D.V., 2007. Comparison of two methods that
441   assess soil community level physiological profiles in a forest ecosystem. Soil
442   Biology & Biochemistry 39, 454-462.

443 Lehman, R.M., Garland, J.L., Osborne, S.L., 2013. Applying an oxygen-based respiratory
444   assay to assess soil microbial responses to substrate and N availability. Applied Soil
445   Ecology 64, 127-134.

446 Li, J.W., Wang, G.S., Allison, S.D., Mayes, M.A., Luo, Y.Q., 2014. Soil carbon
447   sensitivity to temperature and carbon use efficiency compared across microbial-
448   ecosystem models of varying complexity. Biogeochemistry 119, 67-84.

449 Liang, C., Cheng, G., Wixon, D.L., Balser, T.C., 2011. An Absorbing Markov Chain
450   approach to understanding the microbial role in soil carbon stabilization.
451   Biogeochemistry 106, 303-309.

452 Lin, Q., Brookes, P.C., 1999. An evaluation of the substrate-induced respiration method.
453   Soil Biology & Biochemistry 31, 1969-1983.

454 Macdonald, C.A., Crawley, M.J., Wright, D.J., Kuczynski, J., Robinson, L., Knight, R.,
455   Abu Al-Soud, W., Sorensen, S.J., Deng, Y., Zhou, J.Z., Singh, B.K., 2015.
456   Identifying qualitative effects of different grazing types on below-ground
457   communities and function in a long-term field experiment. Environmental
458   Microbiology 17, 841-854.

459 Maynard, D.S., Crowther, T.W., Bradford, M.A., 2017. Fungal interactions reduce carbon
460   use efficiency. Ecology Letters 20, 1034-1042.

461   Mayr, C., Winding, A., Hendriksen, N.B., 1999. Community level physiological profile

462   of soil bacteria unaffected by extraction method. Journal of Microbiological

463   Methods 36, 29-33.

464   Oburger, E., Jones, D.L., 2007. Substrate mineralization studies in the laboratory show

465   different microbial C partitioning dynamics than in the field. Soil Biology &

466   Biochemistry 41, 1951-1956.

467   Oquist, M.G., Erhagen, B., Haei, M., Sparrman, T., Ilstedt, U., Schleucher, J., Nilsson,

468   M.B., 2017. The effect of temperature and substrate quality on the carbon use

469   efficiency of saprotrophic decomposition. Plant and Soil 414, 113-125.

470   Padan, E., 2009. Bacterial Membrane Transport: Superfamilies of Transport Proteins.

471   John Wiley & Sons, Chichester, UK.

472   Rasamimanana, S., Lefevre, G., Dagnelie, R.V.H., 2017. Various causes behind the

473   desorption hysteresis of carboxylic acids on mudstones. Chemosphere 168, 559-

474   567.

475   Rath, K.M., Maheshwari, A., Bengtson, P., Rousk, J., 2016. Comparative toxicities of

476   salts on microbial processes in soil. Applied and Environmental Microbiology 82,

477   2012-2020.

478   Roberts, P., Jones, D.L., 2012. Microbial and plant uptake of free amino sugars in

479   grassland soils. Soil Biology & Biochemistry 49, 139-149.

480   Rousk, J., Jones, D.L., 2012. Loss of low molecular weight dissolved organic carbon

481   (DOC) and nitrogen (DON) in H_2O and 0.5 M K_2SO_4 soil extracts. Soil Biology &

482   Biochemistry 42, 2331-2335.

483 San Miguel, C., Dulinski, M., Tate, R.L., 2007. Direct comparison of individual substrate

484   utilization from a CLPP study: A new analysis for metabolic diversity data. Soil

485   Biology & Biochemistry 39, 1870-1877.

486 Schulten, H.R., Schnitzer, M., 1997. The chemistry of soil organic nitrogen: a review.

487   Biology and Fertility of Soils 26, 1-15.

488 Siles, J.A., Cajthaml, T., Filipova, A., Minerbi, S., Margesin, R., 2017. Altitudinal,

489   seasonal and interannual shifts in microbial communities and chemical composition

490   of soil organic matter in Alpine forest soils. Soil Biology & Biochemistry 112, 1-

491   13.

492 Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency

493   of microbial communities: stoichiometry, methodology and modelling. Ecology

494   Letters 16, 930e939.

495 Sinsabaugh, R.L., Moorhead, D.L., Xu, X.F., Litvak, M.E., 2017. Plant, microbial and

496   ecosystem carbon use efficiencies interact to stabilize microbial growth as a

497   fraction of gross primary production. New Phytologist 214, 1518-1526.

498 Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R.,

499   Shah, J.J.F., 2017. Stoichiometry of microbial carbon use efficiency in soils.

500   Ecological Monographs 86, 172-189.

501 Smith, A.R., Lukac, M., Bambrick, M., Miglietta, F., Godbold, D.L., 2013a. Tree species

502   diversity interacts with elevated CO_2 to induce a greater root system response.

503   Global Change Biology 19, 217-228.

504   Smith, A.R., Lukac, M., Hood, R., Healey, J.R., Miglietta, F., Godbold, D.L., 2013b.

505   Elevated CO_2 enrichment induces a differential biomass response in a mixed

506   species temperate forest plantation. New Phytologist 198, 156-168.

507   Söderberg, K.H., Probanza, A., Jumpponen, A., Bååth, E., 2004. The microbial

508   community in the rhizosphere determined by community-level physiological

509   profiles (CLPP) and direct soil- and cfu-PLFA techniques. Applied Soil Ecology

510   25, 135-145.

511   Spohn, M., Potsch, E.M., Eichorst, S.A., Woebken, D., Wanek, W., Richter, A., 2016.

512   Soil microbial carbon use efficiency and biomass turnover in a long-term

513   fertilization experiment in a temperate grassland. Soil Biology & Biochemistry 97,

514   168-175.

515   Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016. Microbial carbon use efficiency and

516   biomass turnover times depending on soil depth - Implications for carbon cycling.

517   Soil Biology & Biochemistry 96, 74-81.

518   Stevenson, F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions, 2nd Edition.

519   John Wiley & Sons, New York.

520   Swallow, M.J.B., Quideau, S.A., 2015. A method for determining community level

521   physiological profiles of organic soil horizons. Soil Science Society of America

522   Journal 79, 535-542.

523   Swenson, T.L., Jenkins, S., Bowen, B.P., Northen, T.R., 2015. Untargeted soil

524   metabolomics methods for analysis of extractable organic matter. Soil Biology &

525   Biochemistry 80, 189-198.

526   Winding, A., Hendriksen, N.B., 2007. Comparison of CLPP and enzyme activity assay

527   for functional characterization of bacterial soil communities. Journal of Soils and

528   Sediments 7, 411-417.

529   Wutzler, T., Blagodatsky, S.A., Blagodatskaya, E., Kuzyakov, Y., 2012. Soil microbial

530   biomass and its activity estimated by kinetic respiration analysis - Statistical

531   guidelines. Soil Biology & Biochemistry 45, 102-112.

Figure legends

533 **Fig. 1.** Box plots showing microbial carbon substrate use efficiency (CUE) for a range of

534 different carbon substrates in soils collected at three contrasting spatial scales (field scale

535 ($n = 48$), regional scale ($n = 24$) or continental scale ($n = 42$)). Different capital letters at

536 the top of each substrate box indicate significant differences ($P < 0.05$) in CUE between

537 the three sampling scales while different letters at the bottom indicate significant

538 differences in CUE between substrates ($P < 0.05$). The boundary of the box closest to

539 zero indicates the 25th percentile, a line within the box marks the median, and the

540 boundary of the box farthest from zero indicates the 75th percentile. Error bars indicate

541 the 90th and 10th percentiles and the dots the 95th and 5th percentiles.

542

543 **Fig. 2.** Box plots showing substrate uptake rate microbial carbon use efficiency (CUE)

544 for a range of different carbon substrates in soils collected at three contrasting spatial

545 scales (field scale ($n = 48$), regional scale ($n = 24$) or continental scale ($n = 42$)).

546 Different capital letters at the top of each substrate box indicate significant differences (P

547 < 0.05) in CUE between the three sampling scales. The boundary of the box closest to

548 zero indicates the 25th percentile, a line within the box marks the median, and the

549 boundary of the box farthest from zero indicates the 75th percentile. Error bars indicate

550 the 90th and 10th percentiles and the dots the 95th and 5th percentiles. AS indicates amino

551 sugars.

Table 1

Comparison of resemblance matrices of substrate mineralization and uptake data based on Manhattan distances of standardised data.

	Sample statistic (Spearman's ρ)	Significance level	Number of permutations
Field-scale	0.760	0.1%	999
Regional-scale	0.806	0.1%	999
Continental-scale	0.857	0.1%	999

Fig. 1. Box plots showing microbial carbon use efficiency (CUE) for a range of different carbon substrates in soils collected at three contrasting spatial scales (field scale ($n = 48$), regional scale ($n = 24$) or continental scale ($n = 42$)). Different capital letters at the top of each substrate box indicate significant differences ($P < 0.05$) in CUE between the three sampling scales while different letters at the bottom indicate significant differences in CUE between substrates ($P < 0.05$). The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars indicate the 90th and 10th percentiles and the dots the 95th and 5th percentiles.
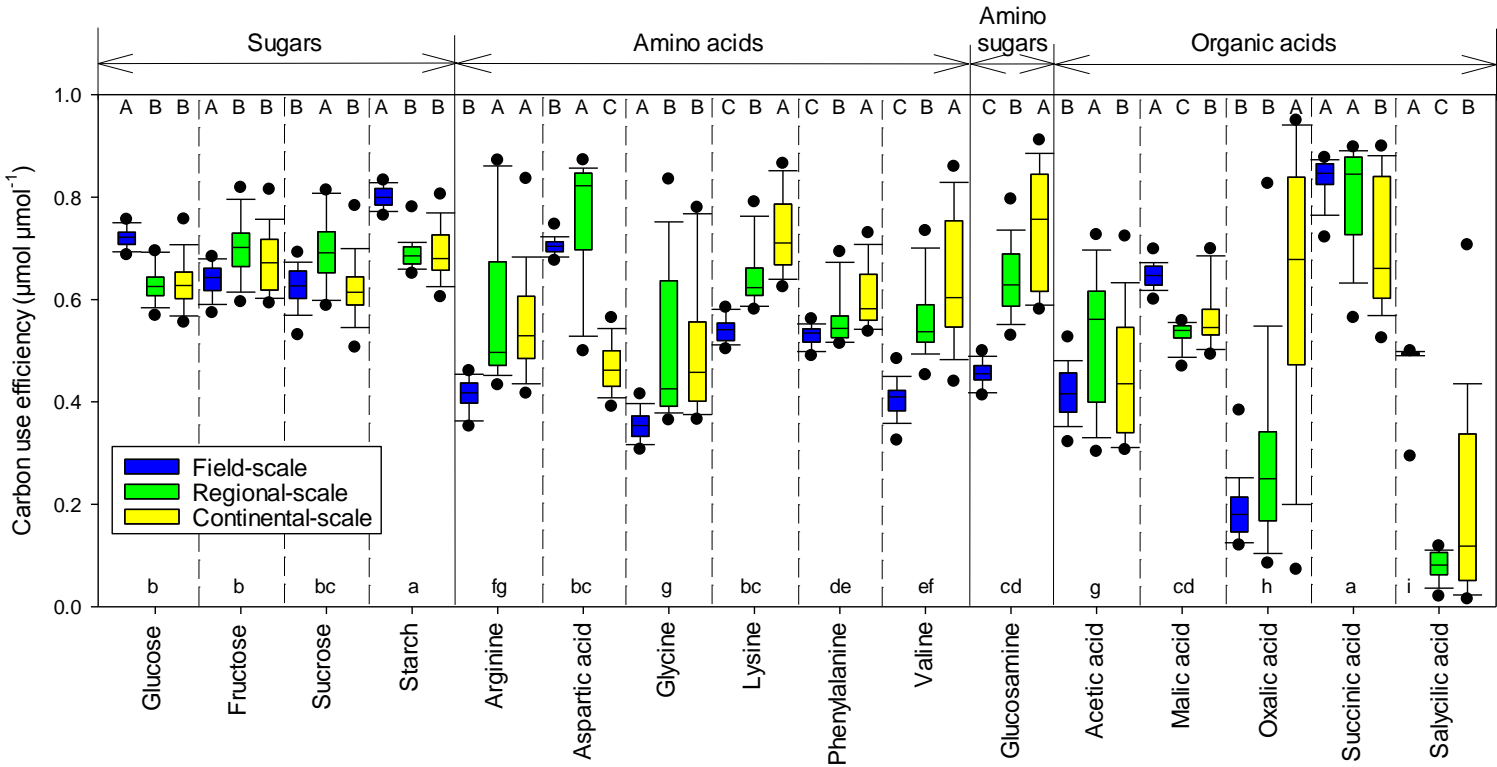
Fig. 2. Box plots showing substrate uptake rate (lower panels) and mineralization rate (upper panels) for a range of different carbon substrates in soils collected at three contrasting spatial scales (field scale ($n = 48$), regional scale ($n = 24$) or continental scale ($n = 42$)). The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars indicate the 90th and 10th percentiles and the dots the 95th and 5th percentiles. AS indicates amino sugars.
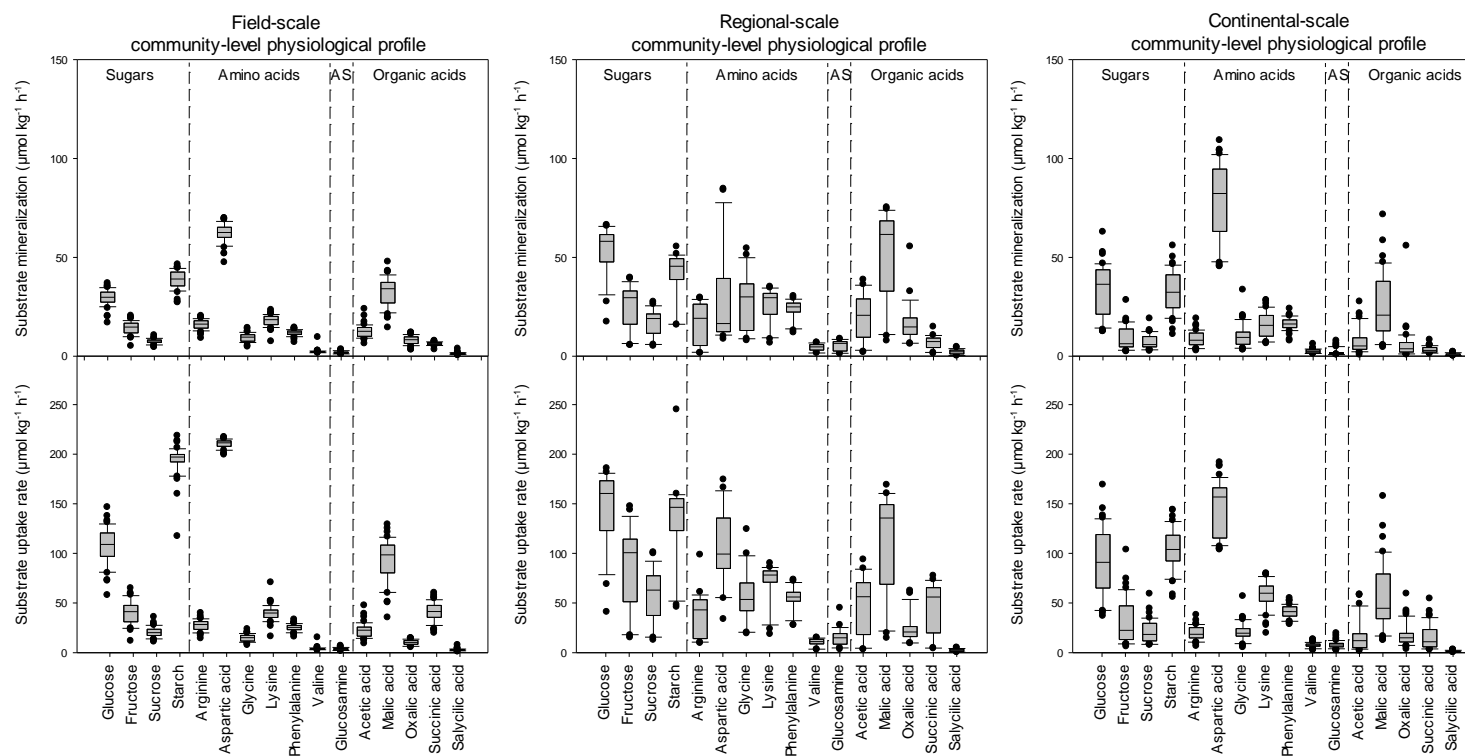
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