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

Jones, Davey L.; Hill, Paul; Smith, Andrew; Farrell, Mark; Ge, T.; Murphy, Daniel V.

Soil Biology and Biochemistry

DOI:
10.1016/j.soilbio.2018.04.014

Published: 01/08/2018

Peer reviewed version

Elsevier Editorial System(tm) for Soil Biology and Biochemistry

Manuscript Draft

Manuscript Number: SBB13301R1

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

Article Type: Research Paper

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

Corresponding Author: Professor David Leonard Jones, PhD

Corresponding Author's Institution: Bangor University

First Author: David Leonard Jones, PhD

Order of Authors: David Leonard Jones, PhD; Paul W Hill, PhD; Andrew R Smith, PhD; Mark Farrell, PhD; Tida Ge, PhD; Natasha C Banning, PhD; Daniel V Murphy, PhD

Manuscript Region of Origin: UNITED KINGDOM

Ysgol Amgylchedd, Adnoddau Naturiol a Daearyddiaeth
Prifysgol Bangor

Bangor
Gwynedd LL57 2UW, UK
Ffôn: +44 (01248) 382281
Ffacs: +44 (01248) 354997
http://www.bangor.ac.uk/senrgy/

M. McDonald, BSc., Ph.D
Pennaeth yr Ysgol
Rhif uniongyrchol: +44 (01248) 388076
E-bost: m.mcdonald@bangor.ac.uk

PRIFYSGOL BANGOR UNIVERSITY

School of Environment, Natural Resources & Geography
Bangor University

Bangor
Gwynedd LL57 2UW, UK
Tel: +44 (01248) 382281
Fax: +44 (01248) 354997
http://www.bangor.ac.uk/senrgy/

M. McDonald, BSc, Ph.D
Head of School
Direct line: +44 (01248) 388076
E-mail: m.mcdonald@bangor.ac.uk

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)

J.R. Healey, BSc, D.Phil.
Athro Gwyddorau Coed
Professor of Forest Sciences
Rhif uniongyrchol / Direct Line: +44 (01248) 383703
E-bost / E-mail: j.healey@bangor.ac.uk

D. Chadwick, BSc,PhD
Athro Amaethyddiaeth ac Astudiaethau Tir
Professor of Agriculture and Land Use Studies
Rhif uniongyrchol / Direct Line: +44 (01248) 383569
E-bost / E-mail: d.chadwick@bangor.ac.uk

D.L. Jones, BSc, D.Phil
Athro Gwyddorau Pridd a'r Amgylchedd
Professor of Soil & Environmental Science
Rhif uniongyrchol / Direct Line: +44 (01248) 382579
E-bost / E-mail: d.jones@bangor.ac.uk

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 <u>may in part</u> 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

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

D.L. Jones[a,*], P.W. Hill[a], A.R. Smith[a], M. Farrell[b], T. Ge[c], N.C. Banning[d], D.V. Murphy[d]

[a] *School of Environment, Natural Resources & Geography, Bangor University, Gwynedd LL57 2UW, UK*

[b] *CSIRO Agriculture & Food, Locked bag 2, Glen Osmond, SA 5054, Australia*

[c] *Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan 410125, China*

[d] *SoilsWest, UWA School of Agriculture and Environment, Faculty of Science, The University of Western Australia, Crawley 6009, Western Australia, Australia*

*Corresponding author. Tel.: +44 1248 382579; fax: +44 1248 354997.

E-mail address: d.jones@bangor.ac.uk (D.L. Jones).

ABSTRACT

Carbon use efficiency (CUE) describes the relative partitioning of carbon (C) between anabolic and catabolic processes within the soil microbial community. Further, it represents a major factor regulating the amount of C cascading through the trophic levels of the soil food web. How CUE relates to C supply, however, remains poorly understood. The primary aim of this study was to determine how CUE varies across a range of spatial scales as a function of C substrate supply. Our secondary aim was to understand how variations in substrate CUE influences the interpretation of community level physiological profiles (CLPP). Using 16 different ^{14}C-labelled substrates (including amino acids, sugars, organic acids and amino sugars) and soils collected at the field, regional and continental scale, we measured the rate of substrate uptake and mineralization from which we calculated CUE. Across all soils (n = 114) and substrates (n = 16), the average CUE for the microbial community was 0.568 ± 0.004 (range 0.492 to 0.794). While the partitioning of substrate-C within the biomass (immobilization/mineralization) over 72 h was highly conserved for some substrates (e.g. glucose), others showed a wide variability in CUE across the samples (e.g. valine). In the context of the CLPP methodology, we showed that individual sites could be statistically separated from each other, irrespective of whether the statistical analysis was based on microbial substrate uptake rate or mineralization rate. However, our results do suggest that caution is needed when ascribing observed CLPP differences to the importance of individual C pathways operating in soil due to the wide variation of CUE between 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

variability in CUE between soils may be partially explained by the different spectrum of C compounds flowing through the soil and their relative use in catabolic reactions/processes leading to mineralization of C to CO_2. This is supported by studies showing that the immobilization-mineralization potential of individual C substrates varies greatly depending on their molecular weight and oxidation state (Gunina et al., 2017; Oquist et al., 2017). Our knowledge of how substrate CUE varies with C supply, however, remains limited (Liang et al., 2011). As CUE is a key regulator of microbial biomass turnover and soil C sequestration (Sinsabaugh et al., 2013, 2017ab), there is an increasing need to include it alongside microbial diversity within global ecosystem models to better predict ecosystem feedbacks to anthropogenic perturbation (e.g. climate warming; Li et al., 2014; Graham et al., 2016; Sinsabaugh et al., 2017). Fundamental to this is a knowledge of how CUE varies at a range of spatial scales. It should also be noted that CUE can be defined in different ways depending on the nature of the study (Geyer et al., 2016). For example, studies have looked at C partitioning and CUE in soil microbial communities supplied with C substrates over short time scales (hours→days), while others have explored CUE from a food web and ecosystem perspectives (months→years timescale). This makes the standardisation of CUE problematic as it is highly dependent on the spatial and temporal scale over which the measurement are made (Geyer et al., 2016; Glanville et al., 2016). In this study we are focusing on community-scale efficiency of microbial biomass synthesis (Geyer et al., 2016).

Community-level physiological profiling (CLPP) is a commonly used approach for assessing shifts in microbial community function in soil (Garland, 1997; Macdonald et al., 2015; Siles et al., 2017). Since its inception in the 1990s, both the underlying method

and subsequent data analysis techniques have been progressively refined (Mayr et al., 1999; Garland et al., 2001; Classen et al., 2003; Calabrix et al., 2005; San Miguel et al., 2007; Swallow and Quideau, 2015). One of the biggest step-changes in methodology when applied to soils was the move away from the need to pre-extract organisms prior to performing CLPP using Biolog® microplates to a direct approach using soil-filled MicroResp® plates (Campbell et al., 2003). The MicroResp® CLPP approach frequently reveals differences in catabolic substrate use between soils under different management regimes and has been shown to be better at discriminating between samples than other profiling techniques (Lalor et al., 2007). In most cases, inferences are made from the substrate use profiles about C availability and microbial processing rates in soil under different management regimes (Artz et al., 2006). Apart from the potential artefacts introduced by soil preparation (Swallow and Quideau, 2015), caution is also required when interpreting the catabolic profiles. For example, CO_2 release rate is likely dependent upon a range of soil factors (e.g. moisture content, carbonate content). While this does not change the substrate use profile for a particular soil it can prevent direct comparison of CO_2 evolution rates between samples obtained from different geographical locations. In addition, differential sorption of charged substrates (e.g. amino acids, organic acids) between soils can have a major impact on substrate availability and therefore CO_2 output. While this is likely to have minimal effect for neutral or univalent solutes (e.g. amino acids; Fischer et al., 2010) it is likely to have a major impact on di/trivalent C substrates (e.g. citrate; Jones and Edwards, 1998). Lastly, differences in CUE both between substrates and soils may lead to over- or under-estimation of the 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

and replaced. The short incubation time was chosen to ensure that the substrate was not fully depleted and is consistent with previous CLPP methodologies (Table S4). After 72 h, when most of the substrate was assumed to have been taken up by the microbial community and partitioned into anabolic and catabolic pathways (Table S4), the second NaOH trap was recovered. This time was selected based on many previous studies measuring the dynamics of low MW C turnover by the microbial biomass which show that partitioning is quasi-complete after 72 h (Glanville et al., 2016). After trap removal at 72 h, the amount of available ^{14}C remaining in the soil was quantified by extracting the soil with 25 ml of 0.5 M K_2SO_4 (Joergensen and Brooks, 1990; Glanville et al., 2016). ^{14}C in the NaOH traps and K_2SO_4 was determined by liquid scintillation counting with Optiphase 3 scintillation fluid (Wallac EG&G, Milton Keynes, UK) and a Wallac 1404 scintillation counter with automated quench correction (Wallac EG&G). All samples were corrected for the presence of ^{40}K.

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

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

where $^{14}C_{tot}$ is the total amount of ^{14}C-substrate added to the soil at time (t) = 0, $^{14}C_{K2SO4}$ 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 total amount of ^{14}C recovered as $^{14}CO_2$ after 72 h. Microbial CUE for each substrate was then estimated as follows:

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

following the method of Jones et al. (2018). Based on the amount of $^{14}CO_2$ produced after 4 h ($^{14}CO_{2\text{-}4h}$), the corresponding microbial substrate uptake rate ($^{14}C_{uptake}$) was estimated as follows:

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

2.3. Statistical analysis

Following the CLPP approach of Lalor et al. (2007), the RELATE routine in PRIMER v6 (Quest Research Ltd., Auckland, New Zealand) was used to test whether there was any difference in the interpretation of CLPP data (in soil groupings or treatment structures) between using substrate mineralization (i.e. $^{14}CO_{2\text{-}4h}$) versus the use of substrate uptake data ($^{14}C_{uptake}$). RELATE measures how closely related two sets of multivariate data (substrate mineralization vs uptake) are by calculating a rank correlation coefficient (Spearman's ρ) between all elements of their respective (dis)similarity matrices. If among-sample relationships agree in exactly the same way in both data sets, then the rank correlation (ρ) =1, is a perfect match. Under the null hypothesis that there is no relation between the two similarity matrices, ρ will be approximately zero.

Other differences between substrate group behaviour were evaluated by ANOVA with Tukey post-hoc pairwise comparison using $P < 0.05$ as the cut-off value to indicate statistical significance (Minitab v16; Minitab Inc., State College, PA). Linear regressions and principle component analysis (PCA) were performed with Minitab v16.

3. Results

3.1. Microbial substrate removal from soil

Extracting the soils with 0.5 M K_2SO_4 at the end of the 72 h incubation period indicated that the majority of the ^{14}C-labelled substrate had been taken up from the soil by the microbial biomass. Across all the different C substrates, on average 12.6 ± 2.6% of the substrate ^{14}C could be recovered from the soil after 72 h (Table S4). This tended to be higher for the amino acids in comparison to the sugars and organic acids (P = 0.02).

3.2. Microbial C use efficiency for individual substrates

Across all the 16 substrates and 114 samples used in this study (n = 1824) we calculated the average CUE value for the soil microbial community to be 0.568 ± 0.004 (Fig. 1). This ranged from 0.492 to 0.794 across all the soils used in the study. As expected, the variation in CUE increased from the field scale (0.548 ± 0.002; CV% 3.1) to the regional scale (0.574 ± 0.007; CV% 6.1) and again to the continental scale (0.600 ± 0.011; CV% 11.5; Fig. S1). Across all samples, the CUE for sugars (0.677 ± 0.004) was higher than for amino sugars (0.601 ± 0.014; P < 0.001). Further, these were both higher than for amino acids (0.551 ± 0.007; P < 0.001) which proved to be higher than for organic acids (0.498 ± 0.007; P < 0.001). Overall, the CUE values for the different sugars were similar with few differences observed between the regional and continental scale. In contrast, the CUE values for the individual amino acids were different and followed the series: ASP = LYS > PHE > VAL > ARG > GLY (P < 0.001; Fig. 1). In addition, differences in CUE for the amino acids were apparent at the three spatial scales. The greatest variability in CUE was seen between the individual organic acids (P < 0.001), with large differences in CUE seen for some organic acids at the different spatial scales.

3.3. Substrate uptake and mineralization rate

Overall, there were major differences in the rate of mineralization of the individual substrates when added to soil ($P < 0.001$; Fig. 2). Across all samples, the highest rate of mineralization was observed for aspartate (61 ± 2 μmol kg^{-1} h^{-1}) while the lowest rate was observed for salicylic acid (1.3 ± 0.1 μmol kg^{-1} h^{-1}). At the field scale, the rate of substrate mineralization was not greatly affected by treatment (i.e. depth, elevated CO_2 or forest type) with the same general pattern in CO_2 evolution seen across all samples. While some substrates were used at similar rates independent of field treatment (e.g. sucrose, phenylalanine), other substrates showed increased variability between samples (e.g. valine, salicylate). In contrast to the field scale, greater variability in the overall profile of substrate mineralization was seen at the regional and continental scale, although the patterns were broadly similar to those observed at the field plot level.

After taking into account the proportion of substrate-C immobilized in the microbial biomass (i.e. CUE), the rate of microbial substrate uptake was calculated. This showed that the rate of ^{14}C uptake was approximately three-fold higher than accounted for by $^{14}CO_2$ alone. Overall, there was a close linear correlation between substrate uptake rate and subsequent mineralization across all 16 substrates ($r^2 = 0.920$; Fig. S2). Consequently, the broad patterns of microbial substrate uptake were similar to those observed for substrate mineralization across all spatial scales (Fig. 2). Although little variation was seen in substrate uptake rate at the field scale, large differences were seen between the different land uses and soil types in the regional and continental samples. With the exception of a few substrates, the coefficient of variability (CV%) across the

samples was similar if substrate use was calculated based on either the rate of depletion from soil or its mineralization (Fig. S3).

3.4. Sample similarity

There was considerable similarity between the individual substrates that best separate soil groupings and treatment structures for substrate mineralization and substrate uptake (Table S5). Ordinations based on substrate mineralization and uptake were significantly related to each other at the field, regional and continental scales (Table 1) indicating no major difference in interpretation of data by either approach (Fig.S7-S9).

Discussion

4.1. Substrate C use efficiency

Our soils displayed a wide range of CUE values for the 16 different C substrates tested here (Fig. 1). This variability in CUE reflects the use of substrate-C within a diverse array of metabolic pathways present within the microbial community, some of which preferentially feed key anabolic processes (e.g. cell wall production, protein synthesis) while others are predominantly used for energy production. The differences may also partially reflect differences in microbial community composition (e.g. fungal-to-bacterial or copiotroph-to-oligotroph ratios), or the degree of competition/stress being experienced by the community and therefore the relative abundance of specific metabolic pathways operating in the soil (Rath et al., 2016; Maynard et al., 2017). The results do, however, clearly indicate the adaptability of the community to split the C derived from 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*

Overall, our data showed a reasonably consistent pattern of CUE across the diverse range of samples for the different C substrates. Despite this, however, significant variation in CUE existed for some individual organic and amino acids (Table S6). This suggests that a universal set of constants cannot be applied to CLPP mineralization data for individual substrates to routinely convert them back to actual C uptake values (e.g. as only mineralization is measured in typical non-isotopically labelled CLPP studies). Despite this caveat, and the obvious underestimation of rates of substrate use within conventional CLPP assays, statistical analysis revealed that accounting for CUE did not greatly alter the separation pattern between samples. The group of compounds largely responsible for driving the statistical separation between the isotopic-based (uptake) and conventional (mineralization) CLPP approaches were slightly different (Table S5, Fig. S7). Consequently, care is needed when inferring dominant processes occurring in soil from conventional CLPP profiles.

To ensure comparability across the samples, we standardized the climatic conditions for the study, however, mean annual temperature was known to vary both at the local and continental scale. Based on previous studies, where CUE was relatively insensitive to temperature, we expect this to have had a minor outcome on the CLPP profiles (Roberts and Jones, 2012; Oquist et al., 2017), however, further work is required to confirm this. Following convention, our CLPP study was performed in the laboratory in the absence of plants and associated mycorrhizas and consequently this may have affected our CLPP profiles (Blagodatskaya et al., 2014). Although CLPP has largely been confined to the laboratory, the approach has also been adapted for use in the field (Glanville et al., 2012; Lehman et al., 2013). The benefits of *in situ* field measurements

include the lack of perturbation of microbial activity caused by soil preparation (e.g. sieving) and the inclusion of intact rhizosphere communities. While these effects can be minimized in the laboratory (Swallow and Quideau, 2015), it has been demonstrated that substrate C partitioning does vary slightly between laboratory and field samples (Oburger and Jones, 2009). In spite of this Glanville et al. (2012), suggested that substrate use profiles were more likely to vary inter-annually in the field relative to differences between the laboratory and the field. In conclusion, our results suggest that conventional CLPP profiling represents a good way of distinguishing between communities with different abilities to assimilate labile C, however, some caution is required in ascribing these differences to processes occurring in the field.

Acknowledgements

DLJ and PWH were supported by funding provided by the UK Natural Environment Research Council (NERC reference NE/I011722/1). MF, NCB and DVM received funding from the Australian Grains Research and Development Corporation. DVM was the recipient of an Australian Research Council Future Fellowship (FTFT110100246). TG received funding from the Royal Society.

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Figure legends

Fig. 1. Box plots showing microbial carbon substrate 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 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. 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.

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

Figure

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.

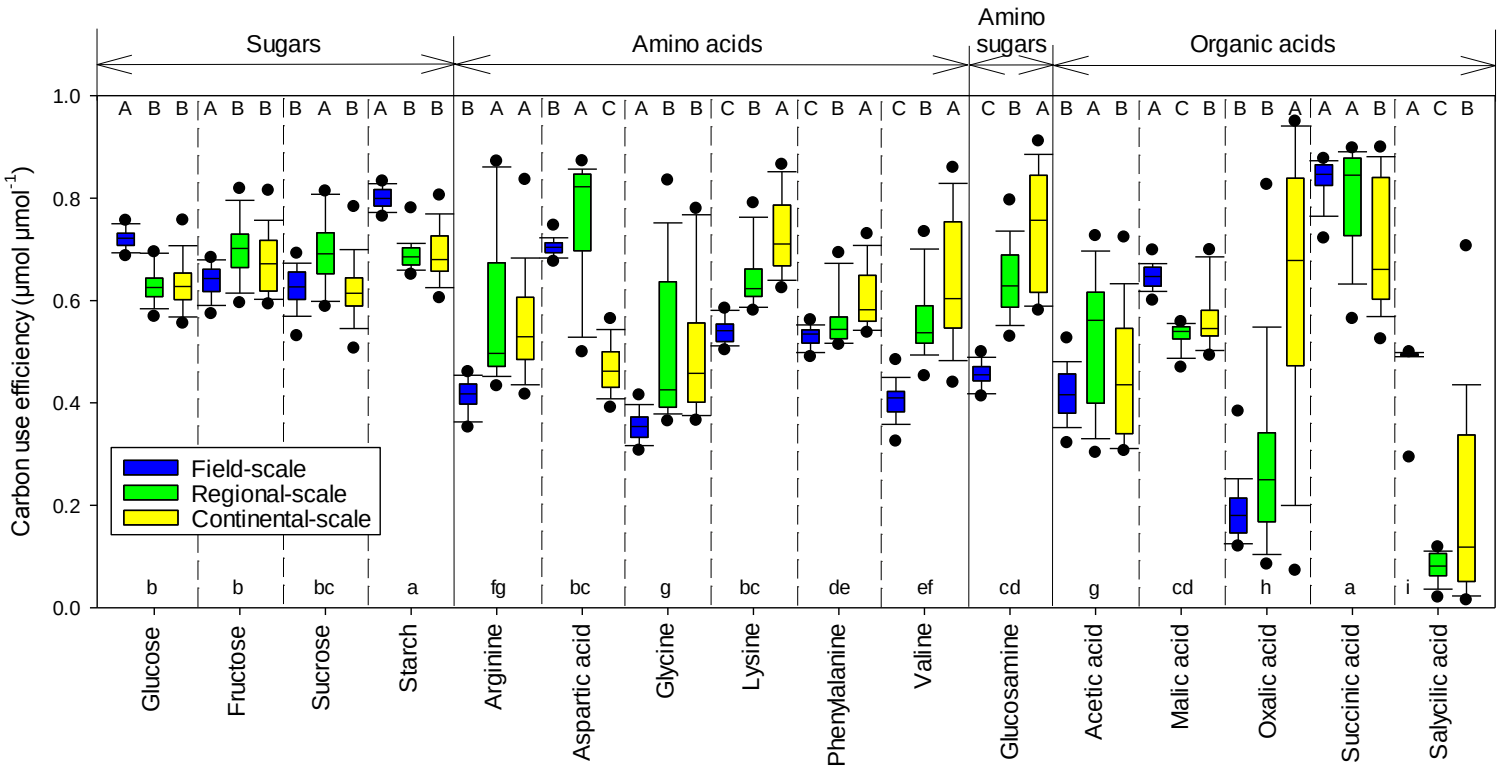
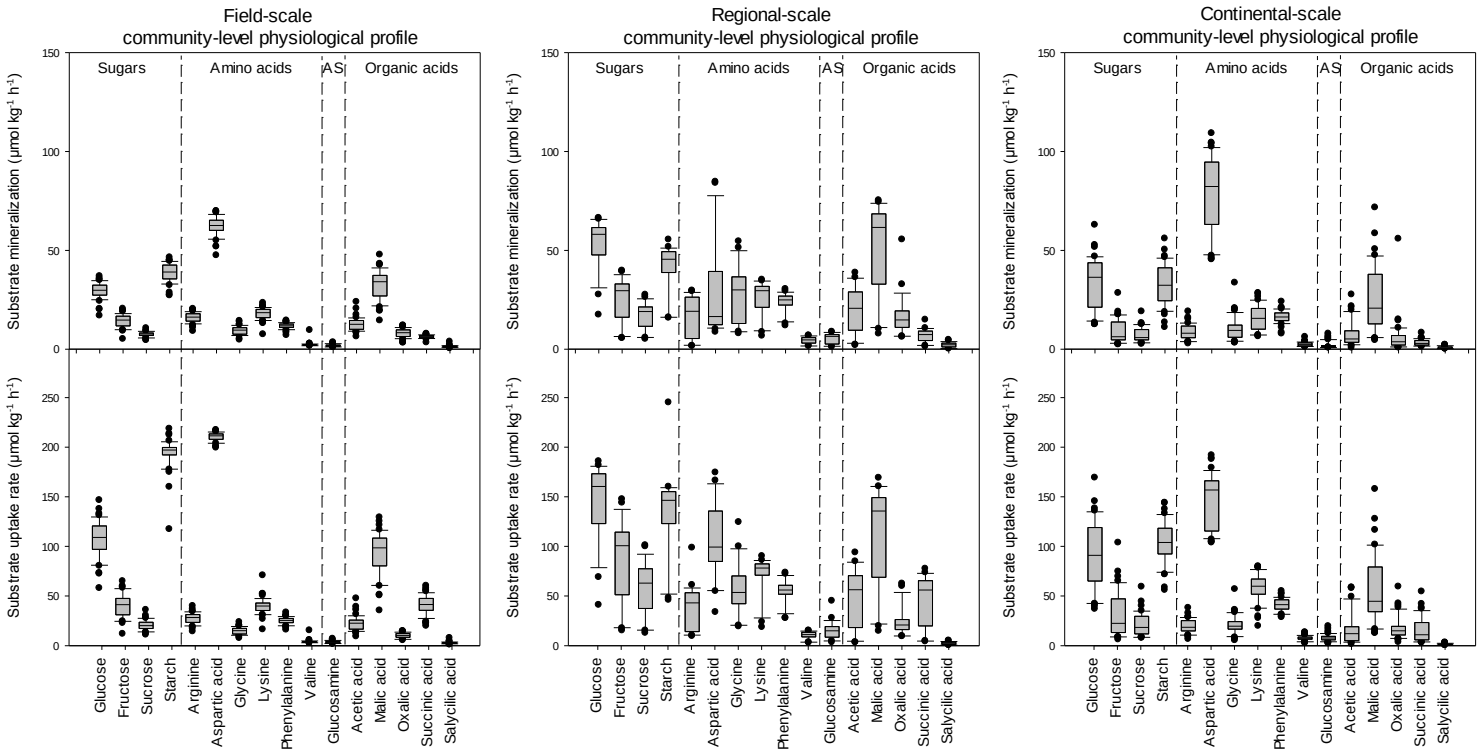


Fig. 2. Box plots showing substrate uptake rate (lower panels) and mineralization rate (upper panels) for a range of different carbon substrates in soils collected at three contrasting spatial scales (field scale (n = 48), regional scale (n = 24) or continental scale (n = 42)). The boundary of the box 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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