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Using metabolic tracer techniques to assess the impact of tillage and straw management on microbial carbon use efficiency in soil

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ABSTRACT

Tillage practices and straw management can affect soil microbial activities with consequences for soil organic carbon (C) dynamics. Microorganisms metabolize soil organic C and in doing so gain energy and building blocks for biosynthesis, and release CO_2 to the atmosphere. Insight into the response of microbial metabolic processes and C use efficiency (CUE; microbial C produced per substrate C utilized) to management practices may therefore help to predict long term changes in soil C stocks. In this study, we assessed the effects of reduced (RT) and conventional tillage (CT) on the microbial central C metabolic network, using soil samples from a 12-year-old field experiment in an Irish winter wheat cropping system. Straw was removed from half of the RT and CT plots after harvest or incorporated into the soil in the other half, resulting in four treatment combinations. We added 1-^{13}C and 2,3-^{13}C pyruvate and 1-^{13}C and U-^{13}C glucose as metabolic tracer isotopomers to composite soil samples taken at two depths (0–15 cm and 15–30 cm) from each of the treatments and used the rate of position-specific respired $^{13}CO_2$ to parameterize a metabolic model. Model outcomes were then used to calculate CUE of the microbial community. Whereas the composite samples differed in CUE, the changes were small, with values ranging between 0.757 and 0.783 across treatments and soil depth. Increases in CUE were associated with a reduced tricarboxylic acid cycle and reductive pentose phosphate pathway activity and increased consumption of metabolic intermediates for biosynthesis. Our results suggest that RT and straw incorporation do not substantially affect CUE.

1. Introduction

Concerns about climate change have stimulated research into management practices to reduce greenhouse gas emissions from cropland (Linquist et al., 2012; Smith and Martino, 2007). Considerable emissions results from tillage, which accelerates the breakdown of soil organic matter and releases it as CO_2 to the atmosphere (Smith, 2004). Reduced tillage (RT) and straw incorporation have been suggested to promote soil carbon (C) storage in cropping systems, reduce erosion and greenhouse gas emissions, improve soil health, and lower costs and energy use (Smith, 2004).

Soil C stocks are ultimately determined by the difference between the rate of organic matter input and decomposition. Hence, predictions about long-term changes in soil C stocks in agricultural systems need to consider the impact of management practices on soil microbial decomposition processes. Heterotrophic soil microbes use organic substrates for the production of energy-rich compounds such as ATP, NADP, NADPH (collectively referred to as ATPeq in this paper), and for the production of biosynthetic building blocks. When a larger fraction of C is released as CO_2, less can be incorporated into new microbial biomolecules. For this reason, physiological controls over the partitioning of substrate-C to biosynthesis (Carbon Use Efficiency, CUE) have consequences for soil C storage (Allison et al., 2010; Anderson and Domsch, 2010; Chapman and Gray, 1986; Frey

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et al., 2013; Manzoni et al., 2012; Schimel et al., 2007; Tucker et al., 2013).

Carbon use efficiency can be affected by various biotic and abiotic factors (Frey et al., 2001; Manzoni and Porporato, 2009), including substrate availability (Bremer and Kuikman, 1994). Low substrate availability results in a low CUE, presumably because most substrate is used for ATPeq production to support maintenance processes (Anderson and Domsch, 2010; Rühl et al., 2010). At intermediate substrate availability, while other resources are not limiting, CUE is increased and production of new microbial cells stimulated. Carbon use efficiency may decline again at high C availability, especially when the availability of other nutrients is insufficient (Gombert et al., 2001; Manzoni and Porporato, 2009; Manzoni et al., 2012).

In many measurements of CUE, relatively large amounts of ^{13}C-labeled substrates (100s–1000s μg substrate C g^{-1} soil) are added to soil, after which CUE is calculated by measuring substrate consumption, $^{13}CO_2$ production, and/or incorporation into microbial biomass (Frey et al., 2001; Thiet et al., 2006). Some approaches use ^{14}C-labeled substrates instead (e.g. Bremer and Kuikman, 1994; Sugai and Schimel, 1993), allowing for CUE estimates with only small amounts of added substrate, thereby minimizing the disturbance of the soil microbial community. However, all approaches described above involve relatively long-term incubations lasting 12 h to several days (see Frey et al., 2001). As such, they may underestimate CUE due to turnover of biosynthetic compounds and cells initially produced from the added substrate (Tucker et al., 2013).

Dijkstra et al. (2011a,b) proposed a "metabolic tracer probing" approach in which $^{13}CO_2$ production from pairs of position-specific ^{13}C-labeled metabolic tracers was measured over 1 h or less, minimizing problems arising from biosynthesis and label turnover. This approach uses relatively small amounts of substrate compared to some other studies (Dijkstra et al., 2011a,b; Frey et al., 2001). Rates of $^{13}CO_2$ production from different C-atoms within the tracers were used to inform a metabolic model, which calculated CUE and metabolic process rates through the various steps in glycolysis, the tricarboxylic acid (TCA) cycle and the pentose phosphate pathway (Dijkstra et al., 2011b).

Tillage alters the distribution of C throughout the soil profile, disrupts soil aggregates, and increases aeration (Doran, 1980; Hendrix et al., 1986), all of which affect soil microbial processes. Reduced tillage practices have been found to increase soil C contents (Ogle et al., 2005; van Groenigen et al., 2011), whereas straw incorporation can both increase soil C contents and substrate availability (Lemke et al., 2010; Jensen et al., 1997; Xu et al., 2011). Here, we explore whether the microbial central C metabolic process rates and CUE are affected by tillage and straw incorporation management using a long-term experiment in and Irish winter wheat field. We hypothesized that, at this well-fertilized site, RT and straw incorporation reduce CUE.

2. Methods

2.1. Study site

In the autumn of 2000, sixteen 27 × 30 m plots were established in a winter wheat (*Triticum aestivum* L.) field at Teagasc Crops Research Centre near Carlow, Ireland. The soil at this site is a haplic luvisol with a sandy loam texture (72% sand, 23% silt, 5% clay) and a pH of 6. Mean annual precipitation and temperature are 824 mm and 9.4 °C. Fertilizer N was supplied in the form of calcium ammonium nitrate at a rate of 200 kg N ha^{-1} y^{-1} in three applications over the growing season (van Groenigen et al., 2010).

Half of the plots were conventionally tilled (CT). The CT plots were ploughed to a depth of 20–25 cm, usually in late September. Ploughing was followed by secondary cultivation using a rotary power harrow (Lely Roterra), to a depth of approximately 10 cm, prior to sowing. The other eight plots were subjected to a reduced tillage (RT) treatment, consisting of shallow non-inversion tillage with a single pass of a tined stubble cultivator (Horsch Terrano FX) to a depth of 7–10 cm, carried out in August soon after harvest. Starting in 2001, straw was chopped and incorporated into the soil of half of the RT plots and half of the CT plots by the ploughing and stubble cultivation operations described above (RT+, CT+), while it was baled and removed from the other plots (RT−, CT−). Straw incorporation represented a soil C input of approximately 2.8 Mg C ha^{-1} y^{-1} (van Groenigen et al., 2011). In 2007, each plot was divided into 20 subplots (2.5 × 15 m) with different N fertilizer treatments. The subplots sampled in this study received the original N fertilizer regime of 200 kg N ha^{-1} y^{-1}. Further details about site history and management can be found in van Groenigen et al. (2010, 2011).

In April 2012, five soil cores (diameter 2 cm) were collected per plot at two depths (0–15 cm and 15–30 cm). The soil was stored at 4 °C in the dark for a month, and then sieved through a 2 mm mesh screen in Flagstaff, Arizona. A 10 g subsample of soil from each plot was oven-dried, after which soil C and N concentrations were determined using an NC 2100 Elemental Analyzer interfaced with a Finnigan Delta Plus XL isotope ratio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory (http://www.isotope.nau.edu/). Prior to the start of the metabolic tracer experiment, soil from the four replicate plots per treatment combination was pooled for both soil depths, resulting in 8 composite samples (i.e., CT+, CT−, RT+ and RT−, each sampled at 2 depths).

2.2. Metabolic tracer probing

The method for metabolic tracer probing is described in Dijkstra et al. (2011a,b). We repeated the tracer application procedure described below four times over a 10-day period, resulting in four replicate estimates of metabolic flux rates and CUE for each tillage × straw × depth combination.

In each tracer application run, four 20 g aliquots per composite sample were weighed into specimen cups and pre-incubated overnight in airtight Mason jars (473 ml volume) at 20 °C in the dark. After eighteen hours of pre-incubation, the jars were opened and the headspace was replaced. After closing, 10 ml of pure CO_2 ($\delta^{13}C = -6.8‰$) was added to the headspace. This initial injection with pure CO_2 was done to collect enough CO_2 in a 10 ml sample for a 10 min measurement on the Picarro G1101-*i* CO_2 cavity ring-down isotope spectrometer (Picarro Inc., Sunnyvale, CA, USA) at a CO_2 concentration between 300 and 2000 ppm. Two ml of a 3.6 mmol l^{-1} 1-^{13}C or 2,3-^{13}C-labeled sodium pyruvate solution or 2 ml of a 1.8 mmol l^{-1} 1-^{13}C or U-^{13}C glucose solution was injected through a septum onto the surface of the soil. All jars received 1.08 μmol tracer-C per g soil. Pyruvate and glucose isotopologues were 99 atom% ^{13}C-enriched at the indicated C positions (Cambridge Isotope Laboratories, Andover, MA, USA).

A 10 ml headspace sample was taken immediately before and 20, 40 and 60 min after tracer addition. Jars were not opened between samples. The headspace sample was injected into a Tedlar air-sample bag (Zefon International, Ocala, FL, USA) and diluted with CO_2-free air to generate enough volume to enable a 10 min analysis. The isotope signature of respired CO_2 at 20, 40 and 60 min was corrected for the signature of CO_2 measured before tracer addition. We then calculated the ratios of position-specific

$^{13}CO_2$ production from the two isotopologues for each metabolic tracer as

$$C_U/C_1 = {}^{13}CO_2 \text{ from U-}{}^{13}C \text{ glucose}/{}^{13}CO_2 \text{ from 1-}{}^{13}C \text{ glucose} \quad (1)$$

and,

$$C_1/C_{2,3} = {}^{13}CO_2 \text{ from 1-}{}^{13}C \text{ pyruvate}/{}^{13}CO_2 \text{ from 2,3-}{}^{13}C \text{ pyruvate} \quad (2)$$

2.3. Modeling microbial metabolic processes and CUE

For a quantitative interpretation of the ratios of position dependent $^{13}CO_2$ production, we used the model described by Dijkstra et al. (2011b). Briefly, the model consists of 21 reactions ($v1$–$v21$; Fig. 1) describing the steady-state partitioning of glucose-C across the reactions of glycolysis, pentose phosphate pathway, and TCA cycle, assuming that glucose is the main substrate. Pyruvate carboxylation ($v9$) was considered the main anaplerotic reaction, balancing the consumption of biosynthesis precursors from TCA cycle pools. Each node in Fig. 1 generates one equation assuming input equals output. This creates a set of equations with 10 unknowns. By expressing all rates in moles per time unit relative to $v1$ (i.e., glucose uptake rate, set at 100%), the number of unknowns is reduced to 9. The rates of $v15$–$v21$ were estimated as a fixed function of $v14$ (G6P consumption) using previously reported relative precursor demand for fungi and bacteria (Dijkstra et al., 2011b), reducing the model to two unknowns. For our model we assumed a community composition of 50% bacteria and 50% fungi (% of total activity). The remaining two rates ($v10$ and $v14$) were estimated using the experimentally determined C_U/C_1 ratio of glucose and the $C_1/C_{2,3}$ ratio of pyruvate (Eqs. 1 and 2). The model equations were solved using 'Solver', a linear programming tool in Excel, by matching the $C_1/C_{2,3}$ and C_U/C_1 ratios calculated by the model to the experimentally observed ratios.

Model solutions are sensitive to the composition of the microbial community (Dijkstra et al., 2011b). To assess the impact of the microbial community composition on model outcomes, we also solved the model for hypothetical communities consisting of 100% bacteria or 100% fungi.

Carbon use efficiency of the microbial community was calculated as

$$CUE = \left(6 \times v1 - \sum CO_2\right) \Big/ (6 \times v1) \quad (3)$$

where $v1$ indicates the glucose uptake rate and ΣCO_2 is the modeled CO_2 released during pentose phosphate pathway, glycolysis and TCA cycle activity. To further characterize metabolic flux patterns, we calculated partitioning ratios at important branch points in the metabolic network. These branch points are responsible for much of the flexibility of the central C metabolic network in response to environmental factors. For example, the partitioning of C over pentose phosphate pathway and glycolysis is affected by temperature (Dijkstra et al., 2011c). Flux partitioning between glycolysis and pentose phosphate pathway was calculated as

$$\phi 1 = v2/v10 \quad (4)$$

Flux partitioning between pentose biosynthesis and the reductive pentose phosphate pathway was calculated as

$$\phi 2 = v21/v11 \quad (5)$$

Finally, flux partitioning between pyruvate carboxylase and pyruvate dehydrogenase was calculated as

$$\phi 3 = v9/v5 \quad (6)$$

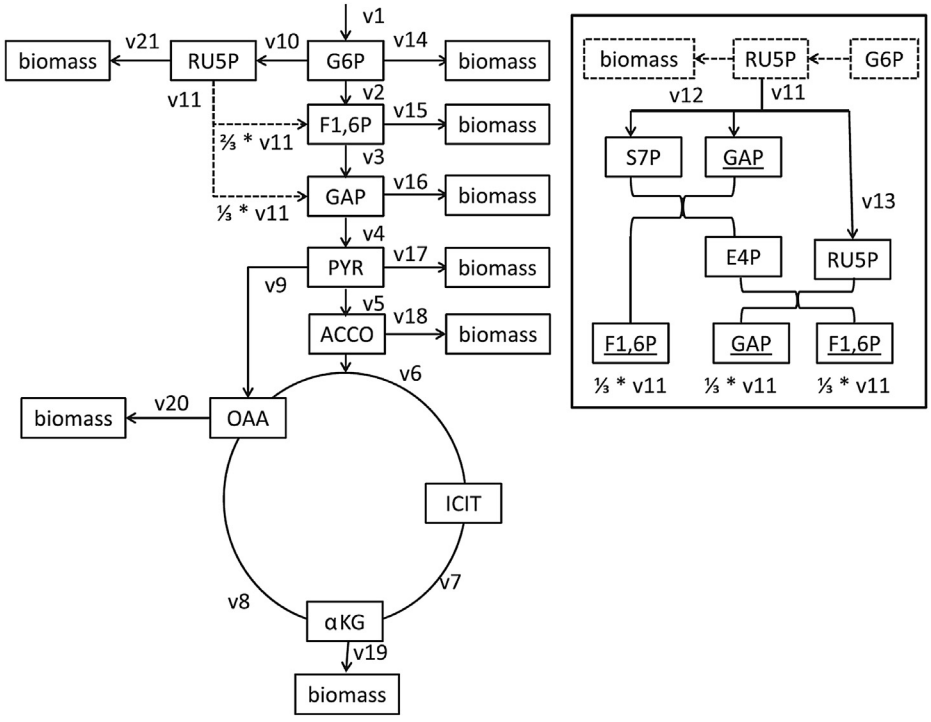

Fig. 1. A simplified model for metabolic processes in soil microbial communities. Reaction rates ($v2$–$v21$) are normalized relative to glucose uptake rate ($v1$, set at 100) on a molar basis. Insert depicts details of the pentose phosphate pathway. Abbreviations: G6P = glucose-6P; F1,6P = fructose-1,6P2; GAP = glyceraldehyde-P; PYR = pyruvate; ACCO = acetyl-CoA; ICIT = isocitrate; αKG = α-ketoglutarate; OAA = oxaloacetate; RU5P = ribulose-5P; S7P = sedoheptulose-7P; E4P = erythrose-4P (from Dijkstra et al., 2011b).

2.4. Statistics

An ANOVA was conducted on metabolic tracer ratios and modeling results using SPSS (version 20). Tillage, straw management and soil depth were included as fixed factors. Whenever ANOVA indicated significant main effects and/or interactions, differences between multiple means were tested using Fisher's LSD. Pseudo-replication resulting from compositing field replicates into one soil sample per treatment and soil depth means that statistical significance should be interpreted as to apply to differences between composite samples, rather than differences between treatment combinations in the field. However, for each treatment combination, each of the four field replicate plots contributed equally to the composite sample. As such, means (but not standard errors) for metabolic tracer ratios and modeling results can be considered representative for treatment means at the field level. Soil C concentrations and C/N ratios were determined from field replicates that had not been composited. Because the field experiment had a complete randomized block design, an ANOVA was conducted for these data, with tillage and straw management as fixed factors, and blocks as a random factor. In all statistical analyses, significance was determined at a level of $P < 0.05$.

3. Results

The C_U/C_1 glucose ratio (Eq. (1)) for the composite samples was not significantly affected by tillage, straw incorporation or soil depth (Fig. 2a). A significant depth by tillage by straw interaction ($P < 0.001$) was observed for the $C_1/C_{2,3}$ pyruvate ratio (Eq. (2)). This ratio was significantly lower for the composite CT+ samples than for CT− samples from both soil depths. In contrast, the composite RT+ sample from the 0–15 cm soil layer showed a significantly higher $C_1/C_{2,3}$ ratio than the RT− sample (Fig. 2b).

We used the isotopomer ratios of glucose and pyruvate to model the central C metabolic network consisting of pentose phosphate pathway, glycolysis, and TCA cycle (Dijkstra et al., 2011b). As an example, we show the pattern of reaction rates for the 0–15 cm soil layer in the CT− treatment (Fig. 3). The pattern found for this composite sample, as well as for all other samples, resembled closely what was observed for other soils (Dijkstra et al., 2011b,c). Compared to the CT− composite samples, the CT+ samples from both soil depths showed significantly lower flux rates for anaplerotic and biomass reactions and higher TCA cycle activity. The RT+ sample from the 0–15 cm soil layer showed increased glycolysis, anaplerotic, and biomass reactions and decreased flux through the TCA cycle and pentose phosphate pathway reactions relative to the RT− sample (Fig. 4; Table 1).

The differences in flux patterns through the central C metabolic network between composite samples were reflected in the partitioning coefficients ($\phi 1$, $\phi 2$, and $\phi 3$, Table 1). The C flux through pyruvate carboxylase ($v9$) relative to pyruvate dehydrogenase ($v5$; $\phi 3$) was significantly lower for CT+ samples than for CT− samples from both soil depths. Compared to the RT− sample from the 0–15 cm layer, the RT+ sample showed significantly increased glycolysis relative to pentose phosphate pathway activity ($\phi 1$), pentose biosynthesis relative to reductive pentose phosphate pathway activity ($\phi 2$), and pyruvate carboxylase relative to pyruvate dehydrogenase activity ($\phi 3$).

The CT+ composite samples from both soil layers showed significantly lower CUE values than the corresponding CT− samples. Compared to the RT− sample from the 0–15 cm soil layer, the RT+ sample showed increased CUE. However, we found no significant difference between the CUE of the RT− and RT+ composite samples taken from the 15–30 cm soil layer (Fig. 5).

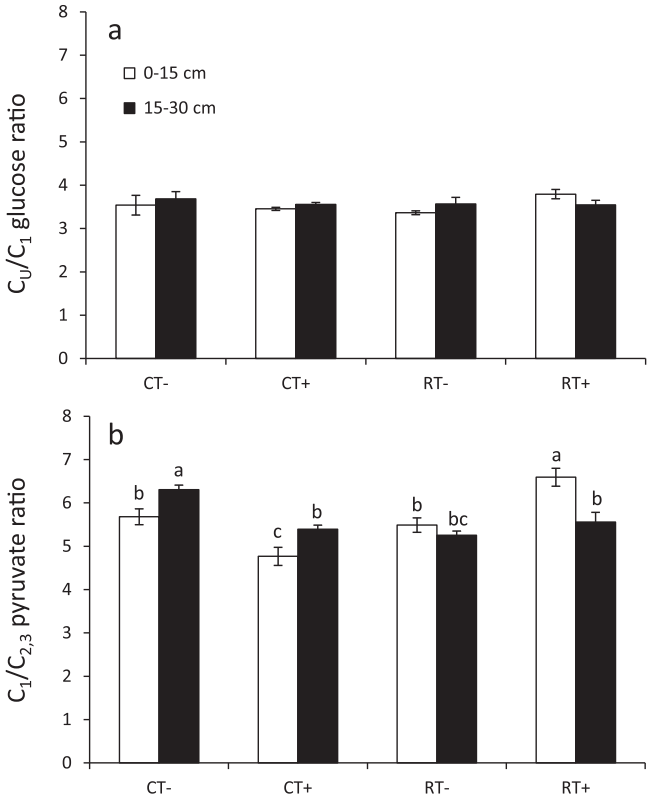

Fig. 2. C_U/C_1 ratio of glucose (a) and $C_1/C_{2,3}$ ratio of pyruvate (b) as affected by 12 years of soil tillage and 11 years of straw incorporation. Results are shown for replicate measurements on composite soil samples from all treatment combinations: CT with straw removal (CT−), CT with straw incorporation (CT+), RT with straw removal (RT−) and RT with straw incorporation (RT+). Error bars indicate SE values ($n = 4$), letters indicate significant differences between means.

The model solutions for CUE and metabolic process rates were affected by the assumed composition of the microbial community; estimates for CUE were slightly but significantly higher when the microbial community was assumed to consist of fungi only. Averaged over all management practices and soil depths, CUE equaled 0.767 when the community was assumed to consist of 100% bacteria, 0.770 when the community consisted of 50% bacteria and 50% fungi, and 0.785 when 100% of the microbial community consisted of fungi. Differences in CUE between tillage and straw incorporation treatments were not affected by assumption about community composition (results not shown). Thus, even if these management practices have dramatic effects on soil microbial community

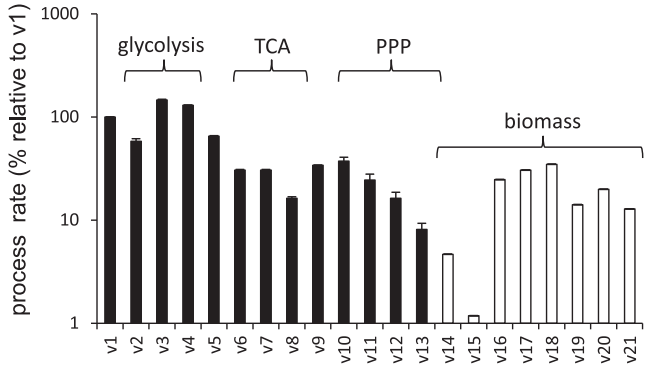

Fig. 3. Reaction rates $v1$–$v21$ (logarithmic scale) for the composite sample of the 0–15 cm soil layer of the CT− treatment. Black bars indicate reactions related to biomass formation. Error bars indicate SE values ($n = 4$).

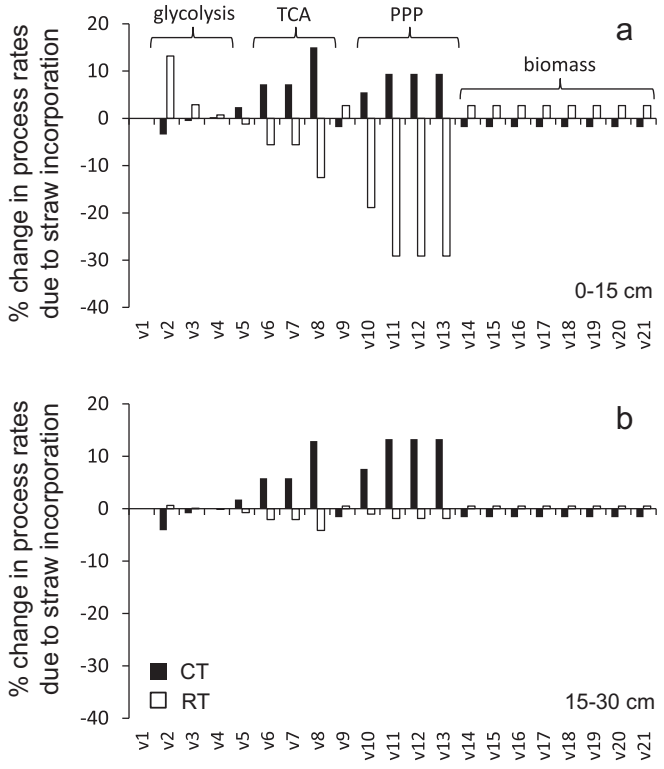

Fig. 4. The effect of straw incorporation for CT and RT treatments on reaction rates $v1$–$v21$ of the central C metabolic network (% change relative to the respective straw removal treatment) for the 0–15 cm (a) and 15–30 cm (b) soil layers. Effect sizes were derived from differences between measurements on composite samples from each treatment combination. The significance of differences between means is reported in Table 1.

composition, the above estimates of their CUE will change only slightly.

Reduced tillage and straw incorporation both significantly increased soil C concentrations in the 0–15 cm layer, with the highest soil C concentrations occurring in RT plots with straw incorporation (Table 2). In the 15–30 cm layer, RT decreased soil C concentrations. Straw incorporation did not significantly affect soil C/N ratios in either of the soil layers, but RT decreased C/N ratios in the 15–30 cm layer. The average soil C concentration for the 8 treatment × depth combinations showed no significant correlation with the CUE of their corresponding composite sample ($r^2 = 0.19$, $P = 0.29$).

Table 1
Effect of straw incorporation on metabolic reaction rates and flux partitioning ratios under reduced (RT) and conventional (CT) tillage, as measured in composite samples taken from two soil depths. Effect sizes are reported in Fig. 4.

	Soil depth			
	0–15 cm		15–30 cm	
Metabolic reaction	CT	RT	CT	RT
Glycolysis ($v2$–$v4$)	ns	↑	ns	ns
Pyruvate dehydrogenase ($v5$)	ns	ns	↑	ns
TCA cycle ($v6$–$v8$)	↑	↓	↑	ns
Pentose phosphate pathway ($v10$–$v13$)	ns	↓	ns	ns
Anaplerotic and biomass reactions ($v9$, $v14$–$v21$)	↓	↑	↓	ns
Flux partitioning ratios				
$\phi 1$	ns	↑	ns	ns
$\phi 2$	ns	↑	ns	ns
$\phi 3$	↓	↑	↓	ns

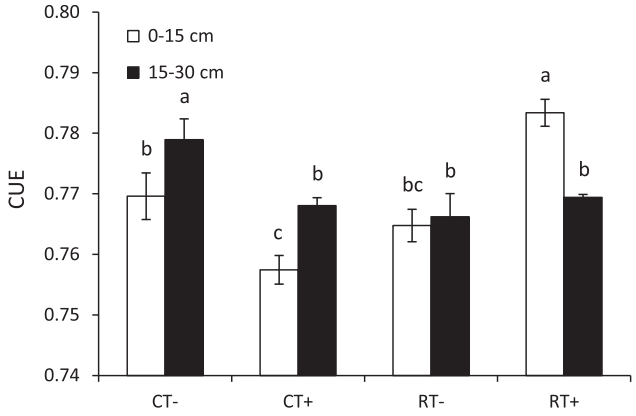

Fig. 5. Effect of straw incorporation on C use efficiency (CUE) calculated from the metabolic model as affected by tillage and straw incorporation. Error bars indicate SE values for replicate measurements on composite samples from each treatment combination ($n = 4$). Letters indicate significant differences between means. See Fig. 2 for abbreviations.

4. Discussion

4.1. Carbon use efficiency

The values for CUE observed in this study are similar to those observed in other soils (Dijkstra et al., 2011b,c; Frey et al., 2013; Tucker et al., 2013), and are at the high end of CUE measurements made with other methods (Frey et al., 2001; Manzoni et al., 2012). One explanation for our relatively high values is that these measurements are conducted only for one hour, while other methods typically involve longer incubations periods, thereby increasing the likelihood of substrate-C recycling. Indeed, a recent study estimates the mean residence time of C in the soil microbial biomass to be around 29–30 days (Blagodatskaya et al., 2011), suggesting that CUE determined from incubations lasting several days may be underestimated.

An alternative explanation for the relatively high CUE values is that our model does not differentiate between C built into microbial biomass and C that is actively secreted or lost. In many studies where incorporation of C into microbial biomass is measured, secretion of microbial products is not considered microbial biomass (Frey et al., 2001). Our model does include these microbial products as biomass, because they are available for long-term C sequestration and their production requires substrate, ATPeqs and biosynthetic precursors.

The hypothesis that increased soil C input decreases CUE was supported by the results for the composite samples from the CT treatments: the CT+ samples from both soil depths showed a slightly, but significantly lower CUE than the CT− samples.

Table 2
Soil C concentrations and C/N ratios in an Irish winter wheat field at two sampling depths, as affected by 12 years of soil tillage and 11 years of straw incorporation ($n = 4$).

Straw	Tillage	C (%)		C/N	
		0–15	15–30	0–15	15–30
Incorporated	RT	2.19 ± 0.03	1.50 ± 0.03	11.4 ± 0.3	10.3 ± 0.3
	CT	1.69 ± 0.03	1.61 ± 0.05	10.6 ± 0.1	10.7 ± 0.3
Removed	RT	1.91 ± 0.11	1.43 ± 0.12	11.0 ± 0.5	9.7 ± 0.3
	CT	1.65 ± 0.06	1.59 ± 0.09	10.4 ± 0.1	10.5 ± 0.3
ANOVA					
Straw		$P < 0.05$	NS	NS	NS
Tillage		$P < 0.01$	$P < 0.01$	NS	$P < 0.01$
Straw × tillage		NS	NS	NS	NS

mean ± SE.

However, the RT+ sample from the surface soil showed a higher CUE than the RT− sample, while no significant difference was observed between the RT+ and RT− samples from the 15–30 cm soil layer. Moreover, the average soil C concentration for the 8 treatment × depth combinations showed no relation with the CUE. Together, these findings are at odds with our hypothesis that CUE decreases with increased straw incorporation. This may be because straw incorporation did not increase substrate C availability at the time of sampling, or because the relationship between CUE and substrate availability is not as strong as previously hypothesized. Either way, these results suggest that at our site, CUE is regulated by more factors than straw incorporation alone.

Microbial community composition may be one such factor. Theoretically, when the community shifts dramatically from a bacterial to a fungal dominated community, our estimates of CUE would increase. However, at this experimental site, the effect of tillage on the relative importance of fungal and bacterial decomposition pathways appears limited (van Groenigen et al., 2010). Alternatively, soil food web structure may have been altered. For example, Frey et al. (2001) report that the presence of protozoa reduces CUE. Treatment effects on substrate quality may have played a role as well (Gommers et al., 1988). For instance, RT may have affected the chemical composition of crop residue (e.g. Franzluebbers et al., 1995), so that straw incorporation affected substrate availability in RT treatments differently than in CT treatments.

The effect of substrate availability on CUE depends on the availability of other nutrients (Manzoni et al., 2012, and straw incorporation is known to affect soil N dynamics (e.g. Ocio et al., 1991; Nieder and Richter, 1986). Moreover, straw addition has been shown to affect soil N availability differently over time, with N immobilization shortly after straw addition, and re-mineralization later on (e.g. Nieder and Richter, 1986). Since straw incorporation did not significantly affect soil C/N ratios, and because our plots were well fertilized, we expect that straw addition did not cause N limitation of microbial growth at our site at the time we sampled. Similarly, plant activity and therefore C and nutrient availability vary throughout the season, which might affect microbial processing. As such, the CUE values reported here may not be representative for the microbial activity during the rest of the growing season. To fully evaluate the differences in CUE between treatments, repeated sampling over time is needed.

4.2. CUE and underlying metabolic processes

Increases in CUE were associated with a shift of activity from pentose phosphate pathway (*v*10) to glycolysis (*v*2 and *v*3), and decreased TCA activity (*v*6–8). Rühl et al. (2010) studied the impact of C availability on metabolic flux patterns for riboflavin-overproducing *Bacillus subtilis*. They observed a transition from rapid microbial growth during C excess to ATPeqs production (maintenance-dominated energy metabolism) under C limitation, associated with a transition from high to low CUE. In accordance with our results, Rühl et al. (2010) observed lower pentose phosphate pathway and TCA cycle activities at higher CUE. In response to a short-term temperature increase, we observed a small but significant increase in CUE at higher temp (from 0.73 at 4 °C to 0.75 at 20 °C), which was also associated with a shift in activity from pentose phosphate pathway to glycolysis (Dijkstra et al., 2011c). In contrast to our current study, the higher CUE at higher temperature was associated with greater C flux through the TCA cycle.

4.3. CUE and soil C storage

The largest differences in CUE observed were between composite samples of the 0–15 cm soil layer of the CT+ (0.757) and the RT+ treatment (0.783); a difference of 3.4%. Field measurements from 2009 showed a 25% difference in soil C stocks between the CT+ and RT+ treatments in the 0–15 cm soil layer (27.7 vs. 34.6 Mg C ha^{-1}; van Groenigen et al., 2011). Can these differences in soil C stocks be attributed to changes in CUE? According to most C cycling models, including the Century model (Parton et al., 1987), microbes respire fixed proportions of labile C they take up, while the remainder is transferred to the next more-stable C pool. A higher CUE means a greater production of microbial cells, which directly leads to a greater C transfer to the long-term C storage pools. The equilibrium increase in C stored in long-term pools is roughly proportional to the change in CUE, assuming all other factors remain the same (Manzoni, personal communication). According to these model assumptions, the increase in CUE of the RT+ sample relative to the CT+ sample would increase soil C stocks by approximately 3.4%. This would suggest that higher CUE in the RT+ treatment was responsible for at most 14% of the increase in soil C stock after 9 years in the 0–15 cm soil layer.

On the other hand, not all models predict that increased CUE results in net soil C sequestration. In a new class of models (Schimel and Weintraub, 2003; Fontaine and Barot, 2005), which emphasize microbial controls via the production of soil extracellular enzymes, an increase in CUE led to more microbial cells and therefore more enzyme production, which caused an increase in substrate consumption, ultimately leading to reduced soil C content. Allison et al. (2010) calculated that in the long term, a decrease in CUE of 8% (due to warming) could offset a 30% decline in soil C content. This suggests that a 3.4% increase in CUE as observed in this study could decrease the soil C content by approximately 12.8% (Allison, personal communication). In other words, this new class of models suggests that the observed increase in CUE could form a feedback mechanism limiting the C accumulation rate in the RT+ treatment.

When we averaged CUE values across the 0–30 cm soil profile, differences between composite samples became even smaller; the largest difference in CUE values was observed between CT+ (0.763) and RT+ (0.776); a difference of only 1.8%. This suggests that whereas treatment effects on CUE likely affected soil C dynamics at our site, their net effect on total soil C stocks was probably of minor importance. van Groenigen et al. (2010, 2011) suggested that differences in soil C stocks between treatments at our site could largely be explained by differences in soil C input rates due to straw incorporation and by physical protection against decomposition due to RT. Both mechanisms are widely recognized as driving factors for soil C storage in cropping systems (Kong et al., 2005; Six et al., 2000). We conclude that these mechanisms likely overshadowed treatment effects on CUE in determining soil C storage at our site.

Although the above mentioned models suggest very contrasting relationships between CUE and soil C dynamics, they open up fascinating questions regarding manipulation of CUE to stimulate soil C storage. For example, Ladha et al. (2011) showed that fertilizer N additions reduced the rate at which soil organic C declined in cultivated soils. However, it is unclear whether this is solely due to increased soil C input, or whether microbial feedbacks also play a role. Manzoni et al. (2012) observed that N addition increased CUE, suggesting the latter. Metabolic tracer probing and modeling provide insights into microbial metabolism that will enhance our ability to understand, and possibly manipulate the processes underlying soil C sequestration.

In soil C models such as RothC and Century, the microbial processing of organic matter results in three products; i.e. microbial biomass, CO_2 and humified organic matter (Parton et al., 1987; Coleman and Jenkinson, 1999). Biochemically, microbes don't produce soil organic matter, they turn into it. This means that the impact of microbes on soil C stocks is determined by both CUE and turnover of the microbial biomass. In theory, tillage and residue

treatments could affect turnover of the microbial biomass (e.g. Heinze et al., 2010), which would alter long term soil C stocks even when CUE remains the same. To better predict the effect of microbial activity on long term soil C stocks, future studies should therefore simultaneously measure treatment effects on both CUE and the turnover rate of microbial biomass.

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