ACCESS TO RESEARCH INFRASTRUCTURES

Carbon stable isotopes measurement of the transitory carbon pool as an early indicator of land use impact on soil carbon sequestration

IsoTrans

Gabriel Moinet, John Hunt, Andrew Midwood, Cornelia Rumpel, Peter Millard, Abad Chabbi

1. Introduction and motivation

Continued emissions of greenhouse gases from anthropogenic activities are very likely to cause severe and irreversible impacts globally, including climate warming and threats to food security. Limiting or reversing these impacts can be achieved partly by changing the way we manage net GHG emissions from the terrestrial biosphere (Smith et al., 2013; Paustian et al. 2016). For instance the 4 per 1000 initiative, launched by France in 2015, has the potential to deliver co-benefits in the form of reduced GHG emissions and increased carbon sequestration (Chabbi et al. 2017). As a result, land productivity and other ecosystem services offered by agro-ecosystems will increase (Lal, 2009). This indeed requires full consideration of SOC stocks; it also calls for the development of SOC management strategies that will allow these stocks to be maintained or increased. However important uncertainties are associated with estimates of SOC stocks and their historical and future changes. This has implications for how investments to increase SOC stocks are planned and monitored. Uncertainty will decrease over time as experience increases. Therefore, sound knowledge management and rigorous tracking and monitoring are required.

Recently a method based on the measurement of the ^{13}C isotopic signature of CO_2 respired from soils after roots are removed ($\delta^{13}CR_H$) has been introduced as an indicator to assess SOC stock dynamics (Millard et al., 2010; Zakharova et al., 2015). Once soils are disturbed, $\delta^{13}CR_H$ can change rapidly. Typically $\delta^{13}CR_H$ is relatively enriched a few minutes after soil disruption ($\delta^{13}CR_{INITIAL}$) and becomes progressively more depleted with time, to reach an equilibrium 60 to 100 minutes after the disturbance has been imposed ($\delta^{13}CR_{DISRUPTED}$).
In previous experiments, we showed that sieving the soil resulted in the release of a pool of carbon depleted in ^{13}C (i.e. the transitory carbon pool) from its physical protection within the soil matrix, making it available for microbial mineralisation (Zakharova et al., 2015). We also observed that $\delta^{13}CR_{DISRUPTED}$ was more enriched than the isotopic signature of CO_2 respired from the roots ($\delta^{13}CR_{ROOT}$). We hypothesized that $\delta^{13}CR_{DISRUPTED}$ represents the mix of two sources of carbon: the transitory carbon pool (the signature of which can be identified as $\delta^{13}CR_{ROOT}$) and the active component of the stable SOC pool ($\delta^{13}CR_{INITIAL}$). From this we can calculate the proportion of carbon coming from the transitory carbon pool (p_t), using a simple mass balance mixing model as follow:
$p_t = (\delta^{13}CR_{DISRUPTED} - \delta^{13}CR_{INITIAL}) / (\delta^{13}CR_{ROOT} - \delta^{13}CR_{INITIAL})$.

Changes in p_t in response to changes in management practices would indicate changes in the balance between the gains and losses of carbon from stable SOC. Therefore, a simple measure

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of p_t before and after a change in land management practices may be used as an indicator to project future changes in SOC stocks.

1. Multidisciplinary approach

The approach combined expert knowledge in agronomy, soil biochemistry and physics, gas exchange at the interface soil-atmosphere and the use of stable isotopes of carbon.

2. Scientific objectives

This project aimed at verifying that measurements of p_t can provide reliable assessment of the impact of different management practices on future changes in soil organic carbon stocks.

3. Methodology and experimental set-up

To test for the validity of p_t as a reliable indicator, a series of measurements were conducted at the SOERE platform of INRA in Lusignan, France. The platform affords a series of soil which have been under different farming management practices for 14 years and where their impacts on SOC stocks have been determined. At these sites, we proposed to evaluate our indicator p_t and test our findings against the observations from the long-term SOC measurements.

The experiment took place in June 2017, during a significant heat wave that affected the region. Weather conditions were extremely dry at the time of measurements. The drought meant that sampling soil cores for rapid measurements of respired $^{13}CO_2$ was technically problematic, and measurements were sometimes impossible due to low respiration rates. Therefore, we modified our experimental set up and investigated the effects of irrigation on respired $^{13}CO_2$. This irrigation effect was evaluated at different times after water addition.
In permanent pastures under either cut and carry management or grazing by dairy cows, water was added to an equivalent of 125 mm of rain over a few hours on 4 by 1 m plots randomly distributed across the paddock. We monitored the indicator p_t by measuring the $^{13}CO_2$ respired from roots ($\delta^{13}C\mathit{R}_{ROOT}$) and from soils over time after roots were removed by sieving ($\delta^{13}C\mathit{R}_H$) in irrigated plots and in non-irrigated plots (only for the grazed pasture). As well, $\delta^{13}C\mathit{R}_{ROOT}$, $\delta^{13}C\mathit{R}_{INITIAL}$, $\delta^{13}C\mathit{R}_S$, the $^{13}CO_2$ respired from the undisturbed soils (including respiration from both the roots and from heterotrophic organisms decomposing SOC) as well as total soil respiration (R_S) were measured through time before irrigation and over 8 days after irrigation and the grazing and cut and carry management were compared.

4. Preliminary results and conclusions

Effect of irrigation on $\delta^{13}C\mathit{R}_H$ and p_t

No significant difference was found for $\delta^{13}C\mathit{R}_{ROOT}$ between irrigated and non-irrigated plots (Table 1). Contrastingly, both $\delta^{13}C\mathit{R}_{INITIAL}$ and $\delta^{13}C\mathit{R}_{DISRUPTED}$ appeared significantly more enriched for the irrigated plots compared to non-irrigated plots (Fig. 1 and Table 1). This did not result in differences in the indicator p_t (Table 1). This may be explained by the fact that the difference in the $\delta^{13}C\mathit{R}_{INITIAL}$ is reflecting microbial decomposers getting access to a more enriched pool of SOC in the bulk soil under well-watered conditions. This pool is not accessible for decomposition under dry conditions. With nearly identical values of p_t, we argue that the contributions of the decomposition of the transitory carbon pool and of the

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stable SOC pool are identical for irrigated and non-irrigated plots. The more enriched value for $\delta^{13}CR_{DISRUPTED}$ in the irrigated plots reflects access to a more enriched pool of the stable SOC.

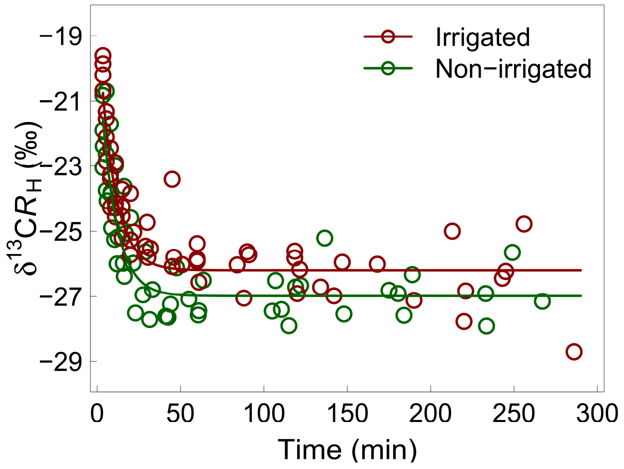

Figure 1 *Evolution of $^{13}CO_2$ signature respired from soils ($\delta^{13}CR_H$) with time after disturbance for irrigated (in red) and non-irrigated (in green) plots.*

	Irrigated	Non-irrigated	*p* value
$\delta^{13}CR_{ROOT}$	-28.8 ± 0.3	-29.4 ± 0.3	0.2
$\delta^{13}CR_{INITIAL}$	-20.1 ± 0.2	-22.0 ± 0.5	0.02
$\delta^{13}CR_{DISRUPTED}$	-26.2 ± 0.3	-27.0 ± 0.3	0.01
p_t	0.72 ± 0.02	0.68 ± 0.05	0.5

Table 1 *Mean values ± standard errors (n = 4) of $\delta^{13}CR_{INITIAL}$, $\delta^{13}CR_{DISRUPTED}$, $\delta^{13}CR_{ROOT}$ and p_t. The p-values represent significance from mean comparison Student t-tests.*

Time sequence after irrigation

Because of hot and dry conditions, there was only a limited effect of the irrigation treatment on the soil water content (W_S), with W_S decreasing rapidly and reaching smaller values than those measured before irrigation (Fig. 2).

On this range of soil water content, $\delta^{13}CR_{INITIAL}$ appeared weakly positively correlated to W_S for the grazed management ($p = 0.02$, $R^2 = 0.14$), confirming the result from the incubation of $\delta^{13}CR_H$ over time after root removal. The $^{13}CO_2$ respired from undisturbed soils ($\delta^{13}CR_S$) presented a strong inverse correlation with W_S (Fig. 3, $p < 0.0001$, $R^2 = 0.53$). $\delta^{13}CR_{ROOT}$, on the other hand, did not correlate with W_S ($p = 0.8$). For the cut and carry management, no correlations were found between W_S and $\delta^{13}CR_{INITIAL}$ $\delta^{13}CR_S$ (Fig. 3) or $\delta^{13}CR_{ROOT}$ and ($p > 0.2$).

In the grazed pasture, increasing values of $\delta^{13}CR_{INITIAL}$ with increasing water content, as suggested above, may reflect increasing accessibility to a more enriched pool of the stable SOC. This, however, did not seem to influence $\delta^{13}CR_S$ with values following an opposite trend. Increasing respiration rates from the roots and associated rhizosphere microorganisms decomposing recently released root exudates have been observed as a consequence of irrigation (Fei et al., 2015; Moinet et al., 2016b).

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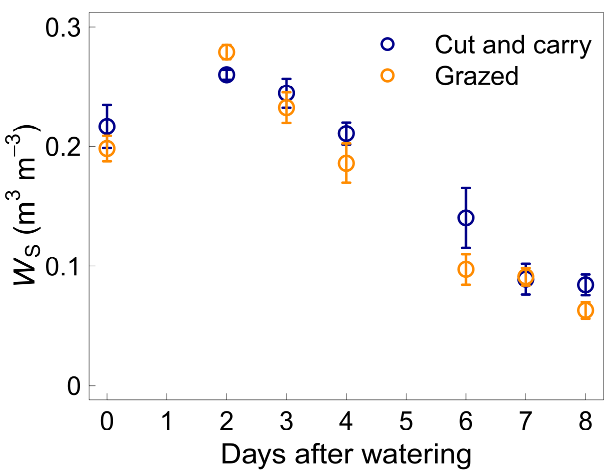

Figure 2 *Soil volumetric water content (W_S) over time after irrigation for pastures under the cut and carry (blue dots) and grazed management (orange dots). Irrigation was applied on day 1 and measurements started 24h afterwards. Vertical bars represent one standard error of the mean (n = 4).*

We attribute the decreasing values of $\delta^{13}CR_S$ with increasing water content to increasing contribution of root and rhizosphere respiration to the total undisturbed soil CO_2 efflux. An increase in total soil respiration was also observed after irrigation, with R_S and W_S being positively correlated (p = 0.01, R^2 = 0.19) and corroborates the explanation of an increasing rate of respiration from the roots and rhizosphere. Furthermore, the response of $\delta^{13}CR_S$ to changes in soil water content was very rapid and the pattern of changes in $\delta^{13}CR_S$ after irrigation was very similar to that of W_S (Fig. 4). Carbone et al. (2011) observed that root respiration responded more slowly than microbial respiration after watering events, and it is generally admitted that microbial respiration is strongly influenced by soil water content (Moyano et al., 2013). Our approach does not allow concluding whether root respiration itself, respiration from rhizosphere microorganisms, or both, increased with W_S. Nonetheless, the rapid response of $\delta^{13}CR_S$ to irrigation suggests that the microbial respiration component contributed significantly. Thus, as for the enrichment of $\delta^{13}CR_H$, we attribute the depletion of $\delta^{13}CR_S$ to an increased accessibility to ^{13}C depleted SOC in the rhizosphere. We hypothesize that this represents an increase in the turnover of the transitory carbon pool. With the p_t indicator remaining constant after irrigation, we also propose that increased root exudates and carbon inputs to the rhizosphere match the increased losses from the transitory carbon pool to maintain a constant carbon stock in this transitory pool. However, the validity of the indicator p_t remains to be tested to validate this hypothesis.

In the pasture under cut and carry management, we did not observe any significant changes in the isotopic signatures of respired CO_2 after irrigation. We argue that accessibility to the enriched pool of SOC in the bulk soil and to the transitory carbon pool in the rhizosphere were not increased after irrigation in these conditions. The lack of a relationship between R_S and W_S (p = 0.3) seems to confirm this observation.

More analyses are underway to test the hypothesis that changes in soil water content modify microbial accessibility to soil carbon pools in the grazed pasture only, giving access to more enriched SOC in the bulk soil and more depleted SOC in the rhizosphere.
Measurements of microbial and fungal community structures and their specific ^{13}C isotopic signatures are being undertaken and will provide valuable information to confirm or refute

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this hypothesis. If differences appear before and after irrigation for the grazed management only, it would partly confirm our hypotheses.

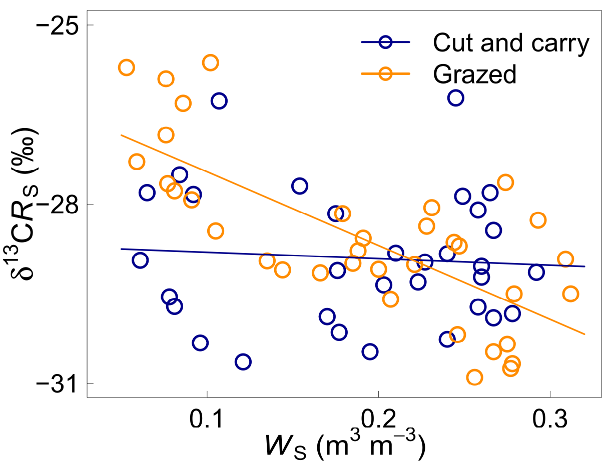

Figure 3 *$^{13}CO_2$ respired from undisturbed soils ($\delta^{13}CR_S$) for pastures under the cut and carry (in blue) and the grazed (in orange) management as functions of soil volumetric water content (W_S). The lines represent the fit of linear relationships for each management.*

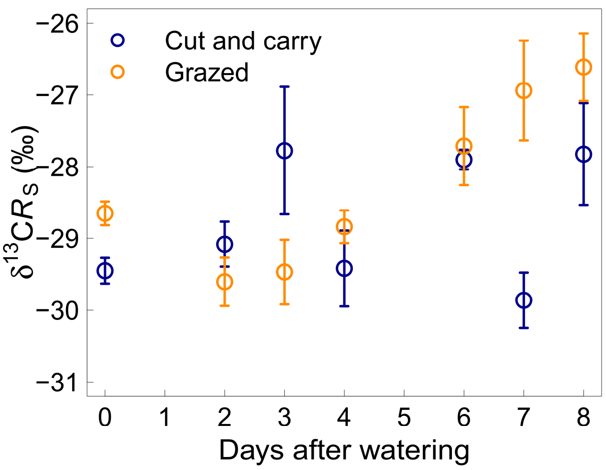

Figure 4 *$^{13}CO_2$ respired from the undisturbed soil ($\delta^{13}CR_S$) over time after irrigation for pastures under the cut and carry (in blue) and the grazed (in orange) management. Irrigation was applied on day 1 and measurements started 24h afterwards.*

5. Outcome and future studies

Exceptionally dry conditions at the time of the experiment prevented the direct validation of p_t as an indicator of future changes in carbon stocks and this should be the subject of future research. In well-watered conditions, the indicator p_t should be measured for a range of agricultural management practices known to be resulting in contrasting directions and rates of SOC stock changes. The SOERE platform of INRA in Lusignan present a large range of practices including croplands, most of them resulting in continued SOC losses, and grasslands mostly resulting in SOC stock increases. Therefore, the site is suitable for validating the indicator p_t, but this may require including croplands as well as grasslands amongst the experimental treatments.

As shown in the present experiment, the dynamics of $^{13}CO_2$ respired from different components of the soil are affected by soil water content. It is likely that other environmental variables may affect these dynamics and although it was not the case for soil water content, it

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is not known whether other variables may result in changes in the indicator p_t. Particularly, under an experimental set up including croplands, the effect of soil disturbance through ploughing should also be assessed.

6. References

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