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Carbon dynamics in topsoil and in subsoil may be controlled by different regulatory mechanisms

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Abstract

It is estimated that in excess of 50% of the soil carbon stock is found in the subsoil (below 20–30 cm). Despite this very few studies have paid attention to the subsoil. Although surface and subsurface horizons differ in pedological, environmental and physicochemical features, which are all likely to affect the mechanisms and biological actors involved, models of carbon dynamics tend to assume that the underlying processes are identical in all horizons, but with lower gross fluxes in the subsurface. The aim of this study was to test this assumption by analysing factors governing organic matter decomposition in topsoil (from depths of 5–10 cm) and subsoil (from depths of 80–100 cm). To this end, we established incubations that lasted 51 days, in which factors that were thought to control organic matter mineralization were altered: oxygen concentration, soil structure and the energetic and nutritional status. At the end of the incubation period, the microbial biomass was measured and the community level physiological profiles established. The mineralization per unit organic carbon proved to be as important in the subsoil as it was in surface samples, in spite of lower carbon contents and different catabolic profiles. Differences in the treatment effects indicated that the controls on C dynamics were different in topsoil and subsoil: disrupting the structure of the subsoil caused a 75% increase in mineralization while the surface samples remained unaffected. On the other hand, a significant priming affect was found in the topsoil but not in the subsoil samples. Spatial heterogeneity in carbon content, respiration and microbial communities was greater in subsoil than in topsoil at the field scale. These data suggest greater attention should be paid to the subsoil if global C dynamics is to be fully understood.

Keywords: ^{13}C, C dynamics, microbial community structure, stable isotopes, subsoil

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Introduction

The effect of global warming on carbon turnover in subsoil may have important consequences for the organic carbon stock held in soils as >50% of the total soil organic carbon is stored in the subsoil (Batjes, 1996). Carbon turnover models that explicitly describe the subsoil tend to assume that the underlying mechanisms that control C dynamics are the same in both topsoil and subsoil (e.g. Lomander *et al.*, 1998b; Jenkinson & Coleman, 2008). In these models the equations describing carbon mineralization are the same for topsoil and subsoil but are either parameterised differently or a factor affecting the rate of decomposition is introduced for subsoil layers (Jenkinson & Coleman, 2008). In essence, subsoil is treated merely as a 'less concentrated' topsoil. The corollary of this assumption is that both compartments will respond in the same way to changes in conditions or external perturbations, the only difference being the intensity of the response. Although based on many observations of decreasing microbial biomass and activity with depth (e.g. Taylor

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et al., 2002), this description of C dynamics in subsoil may not be accurate.

The few comparative studies of C dynamics in topsoil and subsoil suggest that there may very well be significant differences between the two. Fierer *et al*. (2003) showed that a subsurface soil was more susceptible to nutrient inputs and temperature increases but less so to moderate drought than its surface counterpart. The temperature effect they detected corroborated that found by Lomander *et al*. (1998a). On the other hand, several studies have found that subsurface soil was less responsive to temperature increases than that of the upper horizons (Winkler *et al*., 1996; MacDonald *et al*., 1999). A range of different explanations have been given for the different behaviours of topsoil and subsoil, the most frequently cited of which is that substrate quality decreases with depth, meaning that the organic matter at depth is less biodegradable than the organic matter in topsoil. Other explanations put forward are that there are differences in microbial community structure or that the nature of organic C interactions with the physical matrix varies with depth. A recent study has provided some corroboratory evidence for the first explanation. It showed that a supply of fresh plant-derived carbon to the subsoil stimulated the microbial degradation of ancient buried organic matter, estimated to be as much as 2500 years old, suggesting that subsoil organic matter was in fact recalcitrant (Fontaine *et al*., 2007). The stimulation of soil organic matter by addition of fresh organic matter is a phenomenon known as the 'priming effect' (Bingemam *et al*., 1953).

A number of studies have remarked upon the fact that microbial biomass or activity in subsurface soil is considerably more variable than in surface soil (Lomander *et al*., 1998a; Tessier *et al*., 1998; Nunan *et al*., 2002; LaMontagne *et al*., 2003). These observations suggest that microbial 'hot-spots' contribute more to the overall functioning of subsurface soil than of surface soil and that the spatial relationship between organic matter and microbial communities may be an important factor in subsoil C dynamics.

There is sufficient evidence to suggest that the factors controlling C dynamics in topsoil and subsoil may be different and yet, with the notable exception of the study by Fierer *et al*. (2003), this possibility has not really been considered or investigated.

The aim of the work presented here was to test the hypothesis that the factors controlling C dynamics in topsoil and subsoil are different. This was achieved by comparing the effects of a range of treatments on the mineralization of soil organic matter in topsoil and in subsoil. The treatments represented many of the mechanisms believed to regulate C dynamics in soil, namely, O_2 availability, substrate accessibility and nutritional and energetic limitations. Drought was not tested as this is not a usual occurrence at the study site.

Materials and methods

Soil, sampling and sample preparation

Samples were collected from the 'Closeaux' field experiment in the INRA research centre in Versailles, France. The field experiment is a wheat–maize (C3–C4) chronosequence that was established in 1992. Thirty-two research plots of 15 m × 6.4 m each were put in place on a wheat field that had been cropped to C3 plants for a period of at least 50 years previously. Each year until 2001, three (four in 1993) randomly-selected plots were sown to maize (*Zea mays* L.), the others remaining under winter wheat (*Triticum aestivum* L.). Four control plots remained under wheat throughout the experiment. The soil is classified as a Eutric Cambisol. There is some lateral variability however, and more clay illuviation has been observed in places, although not at our sampling site. When this occurs the soil can be described as a Luvisol. The topsoil and subsoil characteristics are presented in Table 1. During the sample preparation slight differences in the colour of a number of the subsoil samples were noted. The subsoil samples were principally brown but a number were reddish. The red samples were randomly distributed among treatments. However, the colour was recorded (as either brown or red) and the colour code served as a factor in subsequent statistical analyses. After the incubation a textural analysis was carried out on the two colour groups which indicated the two groups could also be distinguished by the coarse sand content. Their characteristics are also presented in Table 1. Sampling was carried out in March 2007 by collecting 30 undisturbed cores (diameter: 4.5 cm) to a depth of 1 m from three plots that had been sown to maize for 10 years. Samples were stored at 5 °C until use within a fortnight.

Thirty undisturbed subsamples, 5 cm in height, were excised from the 1 m cores, one subsample per core, at a depth equivalent of between 80 and 100 cm. These were the subsoil samples. Twenty-one topsoil samples were obtained in the same manner from the 5–10 cm depth equivalent of the soil cores. These depths were selected because previous analyses indicated that the presence of calcium carbonate was negligible (Rasse *et al*., 2006). Before commencing the incubations subsamples were tested for the presence of calcium carbonate by addition of 1 M HCl to soil that was directly adjacent to the excised subsamples. No effervescence was detected. Three subsamples of each soil type were used to deter-

Table 1 Characteristics of topsoil (depth: 5–10 cm) and subsoil (depth: 80–100 cm) samples

		Subsoil		
Variable	Topsoil	All	Red	Brown
C (mg g^{-1} soil)	12.56 (0.8)	3.39 (1.09)	1.92 (0.34)	4.01 (0.5)
N (mg g^{-1} soil)	1.23	0.43 (0.12)	0.26 (0.03)	0.50 (0.06)
C/N	10.82	7.94 (0.59)	7.76 (1.04)	8.02 (0.29)
$\delta^{13}C$	−24.5	−25.6 (0.6)	−24.9 (0.3)	−25.8 (0.5)
Biomass (μg g^{-1} soil)	121.3 (37.9)	28.8 (12.3)	24.1 (8.4)	30.7 (13.3)
Clay (g kg^{-1} soil)	174 (8)	232 (38)	229 (19)	234 (57)
Fine silt (g kg^{-1} soil)	208 (7)	222 (39)	212 (39)	232 (44)
Coarse silt (g kg^{-1} soil)	322 (25)	245 (67)	213 (87)	277 (23)
Fine sand (g kg^{-1} soil)	242 (32)	146 (76)	96 (74)	196 (35)
Coarse sand (g kg^{-1} soil)	52 (2)	156 (108)	250 (6)	61 (49)
pH	6.8 (0.2)	7.4 (0.3)	7.6 (0.3)	7.3 (0.2)
CEC	12 (0.3)	11.4 (2.0)	10.3 (1.1)	12.5 (2.3)

Subsoil samples were further divided into two colour-coded classes. Values in brackets are standard deviation.

mine the water content at pF 2.7 which was the desired pF for the incubations.

Incubations

Three potential controls of C dynamics were tested in subsoil samples by measuring their effect on CO_2 production kinetics during a 51-day incubation in a $2 \times 2 \times 2$ factorial design (Fig. S1). The potential controls tested were the physical separation of substrate and micro-organisms, low O_2 concentrations and organic matter recalcitrance. Topsoil samples were subjected to the same treatments, with the difference that the low O_2 treatment was only applied to structured, unamended samples (Fig. S1). The other combinations of treatments that included low O_2 levels were omitted for the topsoil samples because it was felt that O_2 deprivation was unlikely to be as important a factor in topsoil C dynamics.

The spatial segregation of substrate and micro-organisms was tested by comparing sieved (<1 mm) and undisturbed samples. In the low oxygen treatment the atmosphere was flushed with reconstituted CO_2-free air composed of N_2 (95%) and O_2 (5%). Other samples were flushed with reconstituted air with 19% O_2 and 81% N_2. Oxygen levels of 5% were based on a previous report that stated that these were the prevalent O_2 levels in a similar, though slightly more hydromorphic, soil (Sierra & Renault, 1998). Recalcitrant organic matter was considered to be organic matter that did not contain sufficient energy for microbial decomposition to proceed (Fontaine *et al*., 2007), i.e. that the energetic return on energy invested by micro-organisms to produce the enzymes necessary for degradation was not sufficient to justify the investment. In order to test the hypothesis that the recalcitrance of organic C increases with depth a treatment in which labile substrate in the form of ^{13}C-labelled fructose ($\delta^{13}C = 4355$; 13.3 μg fructose C mg^{-1} soil C) was added and the amount of soil organic C mineralized during the incubation determined (Hamer & Marschner, 2005). Soil organic C mineralization was calculated using the following equation:

$$C_{SOC} = C_{measured} \times \left(\frac{\delta^{13}C_{measured} - \delta^{13}C_{fructose}}{\delta^{13}C_{SOC} - \delta^{13}C_{fructose}} \right), \quad (1)$$

where $\delta^{13}C_{measured}$ is the isotopic signature of the respired C, $C_{measured}$ is the amount of total C respired, $\delta^{13}C_{SOC}$ is the isotopic signature of the soil organic C, C_{SOC} is the amount of soil C respired, $\delta^{13}C_{fructose}$ is the isotopic signature of the fructose C added to the samples. An underlying assumption of this equation is that the isotopic signatures of the fructose and soil organic C are homogeneous. While this was true for the fructose solution, it was not so for the soil organic carbon. The heterogeneous isotopic signature arose because samples were taken from a wheat–maize chronosequence. As a result, the soil organic C was made up of wheat C (C3; $\delta^{13}C = -27.6$) and of maize C (C4; $\delta^{13}C = -12.5$). This means that the isotopic signature of the soil organic C may not have reflected the isotopic signature of the C that was mineralized. Therefore, the average δ^{13} CO_2-$C_{measured}$ of the control samples was used as a proxy for $\delta^{13}C_{SOC}$ as this was considered to be closer to the $\delta^{13}C$ of the organic C mineralized in the fructose amended samples.

An additional treatment in which undisturbed samples were incubated at ambient O_2 levels, without fructose but with a mineral nutrient amendment, consisting of a solution of N (100 μg g^{-1} soil as NH_4NO_3) and of P (6 μg g^{-1} soil as K_2HPO_4), was also imposed

(Fig. S1). All treatments were carried out in triplicate. Organic C mineralization was expressed as a percentage of soil organic C.

The incubations were carried out at 20 °C, in the dark and at a water content of $0.19\,g\,g^{-1}$ soil (dry weight equivalent) which corresponded to a pF of approximately 2.7. Before the incubation, the samples were allowed to dry to the desired water content (they were close to saturation at the time of sampling). The samples that were amended with fructose or mineral nutrients were allowed to dry slightly more and were brought to the desired water content by addition of approximately 1 mL of the appropriate solution.

Samples were placed on sample holders in 1 L air-tight jars with spring-lock catches. Jars were sealed with rubber gaskets covered with a thin film of silicon gel and a glass lid fitted with a septum for headspace sampling. The wide opening of the jars allowed us to incubate undisturbed cores. Water (20 mL) was placed at the bottom of the jars to ensure that the atmosphere was moist and that the soil did not dry during the incubation.

At the end of the incubation the microbial biomass and community level physiological profiles (CLPP) of each sample were determined.

Analytical

Soil respiration. The jars' headspace was sampled on days 1, 3, 7, 15, 30 and 51 of the incubation. The CO_2 content was determined by gas chromatography (3000 Micro GC, Agilent, Massy, France) and the $\delta^{13}C$ of the headspace CO_2 by gas chromatography coupled to an Isochrome III isotope ratio mass spectrometer (Micromass-GVI Optima, Isoprime Ltd, Manchester, UK).

Microbial measurements. Microbial biomass was determined by fumigation-extraction (Vance *et al.*, 1987). CLPP were constructed using Biolog® Ecoplates (Biolog Inc., Hayward, CA, USA). Soil dilutions (150 μL) were inoculated into each of the microplate wells. The microplates were incubated at 20 °C for 7 days and colour development (carbon utilization) was measured as absorbance at 590 nm (A_{590}) every 24 h from day 3 onwards using a microplate reader (Xenius, Safas SA, Monaco). The 'single-point reading' approach was adopted because the inoculum density was not standardized before microplate inoculation (Garland *et al.*, 2001).

Statistical analysis

Treatment effects on C mineralization and biomass C and their interactions were determined by residual error maximum likelihood (REML) in order to account for the effect of the different textural classes found in the subsoil. An additional factor identifying coarse sand and fine sand samples was used as a fixed effect in the analysis in order to remove the variability due to the differences in texture. REML was used for this rather than ANOVA because the different textural classes resulted in an unbalanced design. The dimensionality of the CLPP data was reduced by principal components analysis and REML was also used to test the significance of differences among principal component (PA) score means. Significant differences in isotopic signature of CO_2-C on successive sampling dates were determined by repeated measurements analysis of variance. Statistical analyses were carried out using GENSTAT 7th Edition (VSN International Ltd, Hemel Hempstead, UK).

Results

Organic carbon mineralization

The total C content of the topsoil was approximately four times that of the subsoil samples (Table 1). Topsoil N content was three times the N content of subsoil, meaning that the topsoil C/N ratio was higher than that of the subsoil (Table 1). The subsoil samples could be divided into two groups based on the sample colour (brown or red) and on the coarse and fine sand contents (Table 1). When the subsoil samples were grouped accordingly, significant differences ($P<0.01$) in the C and N contents were identified, with the brown samples containing more than twice the C and N of the red samples. The amounts of C mineralized per gram of soil were significantly higher in topsoil than in subsoil in all treatments (data not shown) but, when calculated as a proportion of total organic C, there were no significant differences (Fig. 1). The range of values observed in the subsoil (0.6–6.2% soil organic C) was greater than that found for the topsoil samples (1.6–5.1% organic C). When subsoil samples were grouped into colour classes, significant differences were apparent: C mineralization was significantly higher ($P<0.01$) in the subsoil samples of the red group than in the topsoil or the other subsoil samples, between which there was no significant difference.

The treatment effects were somewhat different, depending on the sample origin. Sieving significantly ($P<0.001$) increased subsoil C mineralization by 75% but had no effect on topsoil samples (Figs 2 and 3). Fructose amendments caused a significant priming effect ($P<0.01$) in both undisturbed and disturbed topsoil samples but had no effect on the respiration of soil organic C in subsoil samples (Figs 2 and 3). The low O_2 treatments did not affect CO_2 emissions from either surface samples (data not shown) or subsurface samples (Fig. 3). The nutrient treatment did not affect C

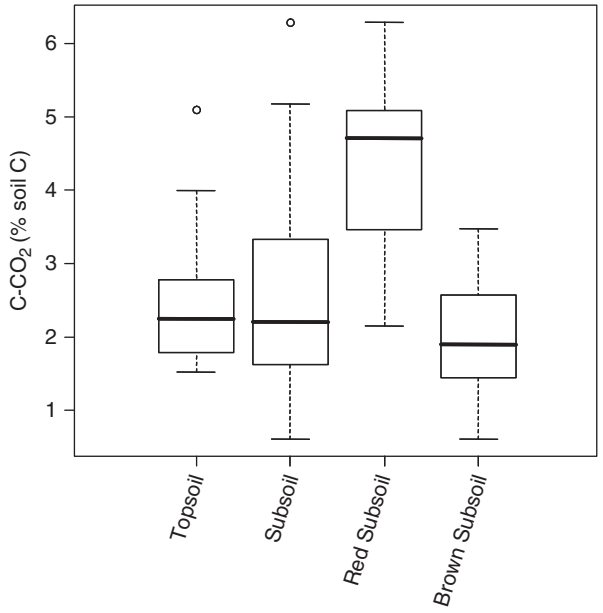

Fig. 1 Box plots showing the amount of soil C respired as a percentage of total soil C for topsoil ($n = 18$) and subsoil ($n = 27$) samples and for red and brown subsoil samples, regardless of treatment. Treatments are not distinguished in order to provide a clear overview of differences among soil types. Treatment effects are shown in subsequent figures. The bow represents the median, upper and lower quartiles while the whiskers show the smallest and largest observations. The points above the whiskers may be considered as outliers, but were not in this case.

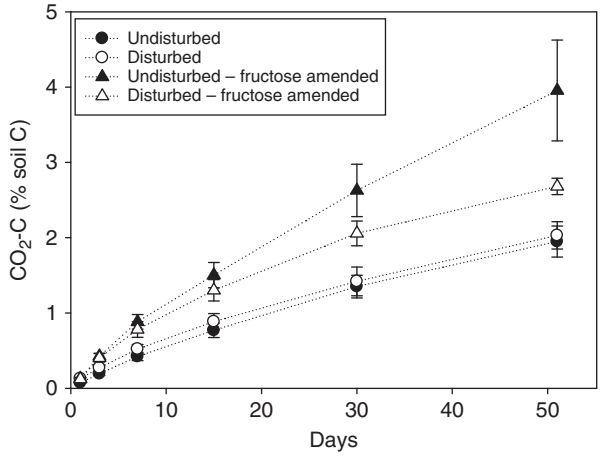

Fig. 2 Cumulative respiration of topsoil organic C. The difference in soil C respired in control (circles) and fructose amended (triangles) samples is the priming effect. There were three replicates per treatment. Bars are standard errors.

mineralization in topsoil or subsoil either (data not shown). It is pertinent to note at this point that the mean values presented in Fig. 3 may be slightly misleading if it is not born in mind that the red subsoil samples mineralized significantly more than the brown subsoil samples, regardless of treatment, and that the red samples were randomly distributed among the treatments. Certain treatments contained several samples from the red group while others did not contain any resulting in apparent effects due to the significantly higher mineralization rates of the samples from the red group. An example of this is the apparent priming effect seen in Fig. 3c, where two of the fructose-amended samples were from the red group, while all of the unamended samples were from the brown group. Therefore, the analysis of the treatment effects had to account for this important unexpected effect. If the three panels of Fig. 3 are viewed together it can been seen that, regardless of the treatment combinations, sieved samples always mineralized more than their undisturbed counterparts (Fig. 3). This was not the case for any of the other treatments.

Significantly more ($P < 0.001$) fructose, as a percentage of the fructose added, was mineralized in the topsoil than in the subsoil samples (Fig. 4). The percentage of fructose mineralized ranged from 57% to 60% of the fructose added in the topsoil samples. The variability was much greater in the subsoil where between 14% and 73% of the added fructose was mineralized. The greater variability in the subsoil was due to the fact that the samples from the red group mineralized significantly more ($P < 0.01$) than the other samples and that the samples in the undisturbed, anaerobic treatment mineralized less ($P < 0.01$) than those in the other treatments. On the other hand, there was no treatment effect on fructose mineralization in the topsoil; both treatments mineralized 59% of the fructose added. There were also slight differences in mineralization kinetics of surface and subsurface samples. The topsoil samples mineralized the fructose more rapidly, reaching maximal, or near maximal, values after 1 day. The mineralization rate declined rapidly thereafter. In excess of 80% of the fructose mineralization occurred during the first 3 days. Fructose mineralization in subsoil samples increased dramatically to reach a peak on day 3 and declined more slowly thereafter. Between 50% and 70% fructose mineralization occurred during the first 3 days (Fig. 4).

It should be noted that the variability among replicates in the undisturbed, fructose-amended topsoil samples was much greater than that of the disturbed topsoil samples (Fig. 2). Variability among replicates of undisturbed treatments was always greater than that of disturbed treatments. This was not the case for subsurface samples. Here, the variability among replicates was generally more important than that of topsoil treatments and was primarily due to the presence of samples from the red colour group which mineralized significantly more than the other subsoil samples (Fig. 1).

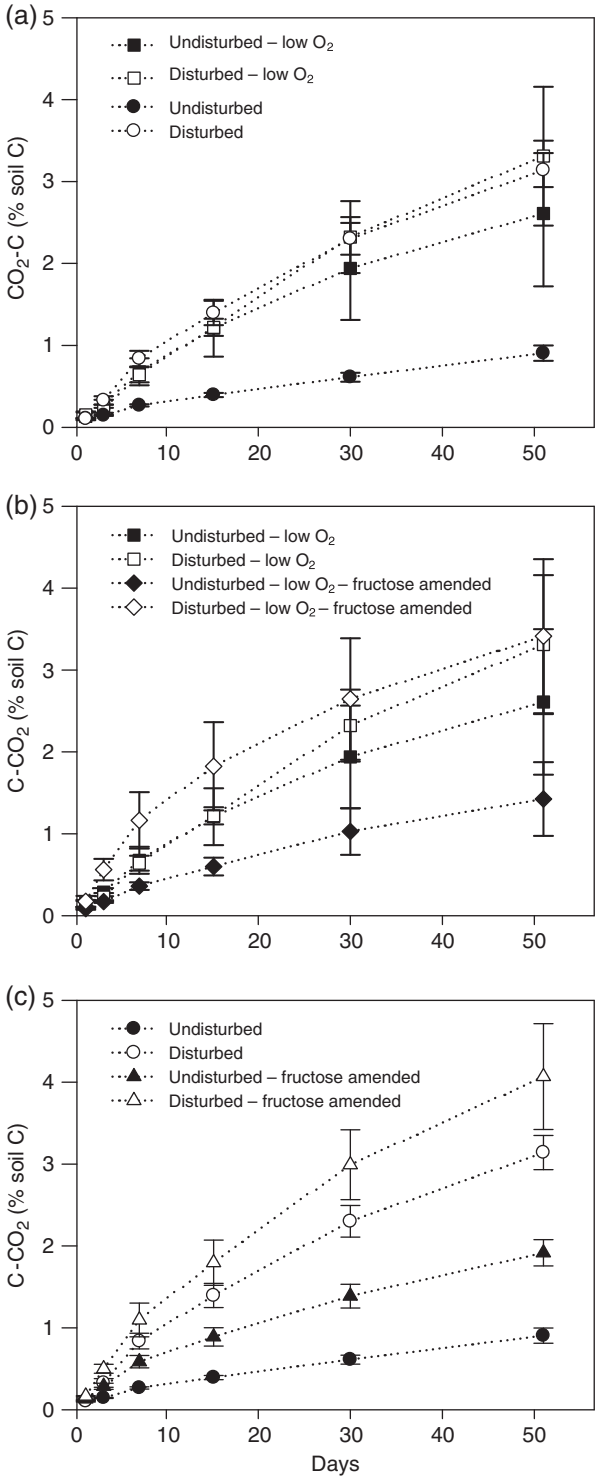

Fig. 3 Cumulative respiration of soil organic C in the subsoil. Disturbed samples (open symbols) respired more than their undisturbed counterparts (solid symbols) in all cases. There were three replicates per treatment. Bars are standard errors. Large standard errors are due to the presence of red samples. The apparent priming effect seen in (c) is due to the presence of a red sample amongst the fructose-amended soils.

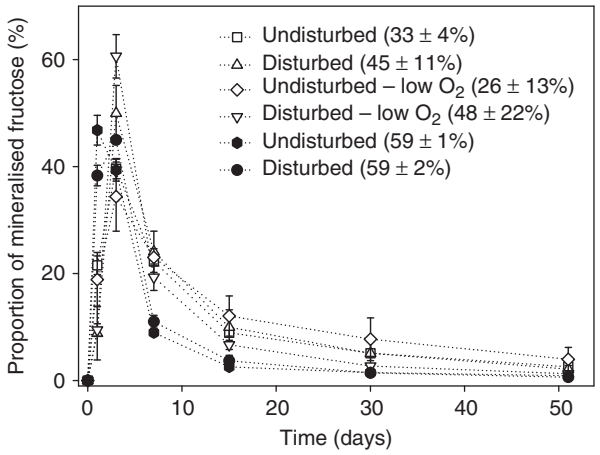

Fig. 4 Daily fructose mineralization, as a percentage of total fructose mineralised, in topsoil (black symbols) and subsoil (white symbols). There were three replicates per treatment. Bars are standard errors of the means. The percentage of added fructose mineralized in each treatment is indicated in brackets.

Isotopic signatures

There were also differences in the isotopic signature of the CO_2-C evolved from topsoil and subsoil samples not amended with fructose during the incubation (Fig. 5). The isotopic signature of the topsoil CO_2-C decreased slightly during the initial stages of the incubation and increased gradually thereafter. The decrease in isotopic signature was significant ($P<0.05$) in the disturbed samples, while there was a significant increase ($P<0.01$) for both undisturbed treatments (Fig. 5a). The isotopic signature of the CO_2-C respired from the disturbed samples were significantly higher ($P<0.05$). In the case of the subsoil, the isotopic signal of the undisturbed samples incubated at ambient O_2 levels was significantly different from the disturbed samples at ambient O_2 levels and the undisturbed low O_2 samples (Fig. 5b). Furthermore, with the exception of the undisturbed low-O_2 treatment, the isotopic signature decreased significantly ($P<0.05$) from approximately −12.5 (the isotopic signature of maize C) to about −24.5 during the course of the incubation (Fig. 5b). The undisturbed low-O_2 treatment followed the same trend but the differences were not significant due to the high variability of measurements made on one of the sampling dates (Fig. 5b). Finally, the greater the respiration of the subsoil samples, the faster the isotopic signature evolved towards the signature of the soil organic matter.

Despite the tests indicating that there was no calcium carbonate at the site, the fact that the isotopic signature of the CO_2 released at the beginning of the incubation of one of the treatments was significantly higher than that of maize C (δ of −8‰ vs. −12.5‰) casts doubt on the

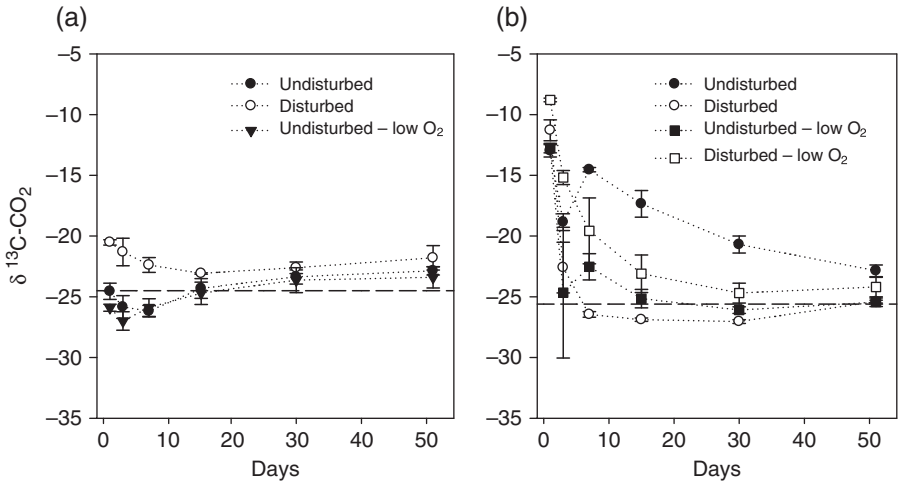

Fig. 5 $^{13}\delta$C of CO_2 respired from topsoil (a) and subsoil (b). The isotopic signature of the soil organic matter is indicated by a dashed line (24.5 and 25.5 for topsoil and subsoil, respectively).

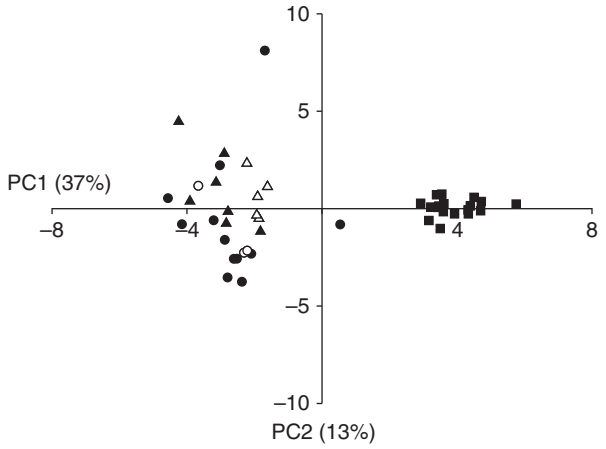

Fig. 6 Principal components scores for topsoil (■), undisturbed subsoil (●) and disturbed subsoil (▲) samples in the first two PCs of CLPP data. Subsoil samples were grouped into brown (solid symbols) and red samples (open symbols). Percentage of total variance accounted for by each PC is indicated in brackets. CLPP, community level physiological profiles; PC, principal component.

tests. The release of CO_2 from carbonates can result in an overestimation of the mineralization of soil organic matter (Bertrand *et al.*, 2007). In order to estimate the likely impact of the presence of CO_2 of carbonate origin on the treatment effects observed, it was assumed that the differences in isotopic signature between the CO_2 released from the soil and that of the soil organic matter were solely due to the contribution of carbonate-C. The isotopic signature of the carbonate-C was estimated at $\delta = -1 \pm 0.3$‰ by acidifying samples taken from layers known to contain calcium carbonate (Rasse *et al.*, 2006) in sealed glass jars that had been flushed with CO_2-free air and measuring the signature of the CO_2 released. Differences among treatments, other than those amended with fructose, remained unaffected, i.e. the hierarchy and significance of differences were unchanged. In the case of the fructose amended samples, the isotopic signature of the CO_2 that originated from soil calcium carbonate or from the mineralization of native soil organic matter was masked by the presence of highly labelled fructose C in the CO_2 respired. Therefore, a number of different scenarios were considered. First of all, it was assumed that there were no carbonates in the fructose-amended samples. While this increased the apparent difference in soil organic C mineralization between amended and unamended samples, it did not lead to a significant priming effect. Subsequently, the range of isotopic signature profiles observed in the unamended samples was used to estimate the amount and kinetics of carbonate CO_2 released in fructose amended samples: either 10 or 25 μg carbonate-C released in 3, 15 or 51 days. The following equation was then used to estimate the amount of soil organic C mineralized:

$$C_{SOC} = \frac{\left(\delta^{13}C_{measured} - \delta^{13}C_{fructose}\right) \times C_{measured} - \left(\delta^{13}C_{carb} - \delta^{13}C_{fructose}\right) \times C_{carb}}{\delta^{13}C_{SOC} - \delta^{13}C_{fructose}}. \quad (2)$$

The scenarios in which the mineral C was released rapidly (in 3 or 15 days), as might be expected with the burst of microbial activity following the addition of fructose, resulted in periods of net immobilization of

atmospheric CO_2, which is not particularly plausible (Fig. S2). These calculations suggest that the presence of soil carbonates in the samples had a negligible effect on the results obtained and can therefore be ignored.

Microbial communities

There were significant effects of soil type ($P<0.001$) and colour group ($P<0.01$) on the first PC of the CLPP (Fig. 6). No treatment effects were seen in the topsoil samples. The CLPP for subsoil samples that had been disturbed were grouped together but their PC scores on the first and second PCs were not significantly different from those of other subsoil samples. Inspection of the loadings on the first PC revealed that topsoil microbial communities had a greater relative usage of polymers (tween 40, tween 80 and cyclodextrin), disaccharides (D-cellobiose and α-D-Lactose), L-phenylalanine and itaconic acid while microbial communities in the subsoil tended to use relatively more compounds commonly found in root exudates (L-serine, L-asparagine, 4-hydroxy benzoic acid), other organic acids (γ-hydroxybutyric acid, pyruvic acid methyl ester) and putrescine. The overall average well colour development (overall substrate usage of the communities) however, was an order of magnitude greater in the topsoil than in the subsoil (data not shown).

It is known that microorganisms that grow in Biolog microplates are not necessarily representative of the original community used to inoculate the microplates and that fast growing bacteria adapted to high substrate concentrations are responsible for much of the colour development (Smalla *et al*., 1998). Nevertheless, the approach can reveal some aspects of communities' catabolic capacities (e.g. Nunan *et al*., 2006) and has been used to distinguish different soils (e.g. Widmer *et al*., 2001).

The microbial biomass was significantly higher ($P<0.001$) in the topsoil than in the subsoil (Table 1). The fertilized subsoil samples had significantly ($P<0.05$) less biomass than the other subsoil samples and the disturbed topsoil samples contained significantly ($P<0.001$) more biomass than the undisturbed samples; though the difference was solely due to a large difference in the amended samples, the unamended treatments having virtually identical microbial biomass contents (data not shown). No other treatment effects were apparent, nor were there significant differences between red and brown subsoil samples (Table 1).

There were no differences in the amount of CO_2 mineralized per unit biomass C in the topsoil (3.4 μg CO_2-C μg^{-1} biomass C) and in the subsoil (3.1 μg CO_2-C μg^{-1} biomass C) during the incubation period. However, in the fructose amended samples, the amount of fructose C mineralized per unit microbial biomass was significantly higher ($P<0.001$) in the undisturbed topsoil samples (1.61 μg C μg^{-1} C biomass) than in the undisturbed subsoil (0.65 μg C μg^{-1} C biomass), disturbed subsoil (0.42 μg C μg^{-1} C biomass) or the disturbed topsoil (0.6 μg C μg^{-1} C biomass) samples.

Discussion

Factors controlling C dynamics in subsoil

Variations of respiration normalized for C content have been interpreted in the past as indicating variations of the decomposability of SOM, the two being positively related (Lomander *et al*., 1998a; Mikan *et al*., 2002; Fierer *et al*., 2003). In this study the proportion of total C mineralized was either significantly higher in subsurface than in surface soil (in the case of samples from the red group) or there was no significant difference between the two, suggesting that the decomposability of the organic matter available to the resident communities did not decrease with depth but rather remained constant or increased, contrary to what has been found elsewhere (Lomander *et al*., 1998a; Fierer *et al*., 2003). CLPP indicated that a portion of the subsoil communities were adapted to preferentially use simple molecules of the type commonly found in root exudates such as amino and organic acids rather than more complex molecules. The isotopic signature of the CO_2-C released at the beginning of the incubation from subsoil samples was close to that of maize C, suggesting that recently added (via root exudation or root death) C was the primary source of substrate for microbial activity, providing further corroboratory evidence that relatively labile organic matter was mineralized. The stimulation of organic C mineralization in the subsoil after sieving was quite dramatic (Fig. 3), suggesting that the physical accessibility of the organic C to micro-organisms is a major control on C dynamics in this subsoil. Xiang *et al*. (2008), in a study of the respiratory responses of microbial communities to multiple wet/dry cycles, also concluded that physical processes, such as diffusion, were the primary limitations on organic matter decomposition in subsoil.

The lack of effect of nutrient amendment may be due to the fact that the site studied is a well-managed site that receives regular fertiliser treatment, meaning that microbial communities were not N or P limited. Low oxygen levels did not appear to significantly affect C mineralization either. This suggests that the level of oxygen (5%) was sufficiently high not to become limiting, at least for the duration of the incubation.

The picture that emerges, then, is one in which subsoil organic C is in readily biodegradable forms,

but physically removed from degraders, rather than intrinsically recalcitrant, as suggested elsewhere (Fontaine *et al*., 2007). The latter have argued that an additional energy source is necessary to prime microbial decomposition of recalcitrant subsoil C. The differences between the results obtained here and those of Fontaine *et al*. (2007) may be due to the rooting depth of the vegetation cover at the two sites, resulting in differences in the chemical form of organic matter inputs at depth. The greater rooting depth of the wheat–maize succession, compared with that of the grassland studied by Fontaine *et al*. (2007), might have resulted in greater inputs of labile root exudates into the subsoil at this site. However, Xiang *et al*. (2008) also conducted their study at a grassland site and concluded that subsoil organic matter was relatively labile. An alternative explanation therefore, might reside in the use of the substrate to prime decomposition. Fontaine *et al*. (2007) used cellulose whereas fructose was used here. Fungi are known to be important cellulolytic micro-organisms (Edwards *et al*., 2008) and, through hyphal spread (Otten *et al*., 2001), have a greater ability to explore the soil environment than bacteria. It may be that the addition of cellulose stimulated the growth of fungal hyphae through the soil, resulting in an increase in the rate of encounter between decomposers (fungi) and the soil organic matter. In this study fructose was used, which may not have stimulated fungal activity and growth to the same extent (Hobbie *et al*., 2003), reducing their ability to bridge the distance between organic matter patches.

Topsoil vs. subsoil

The most striking finding of the study and the one with, potentially, the most far reaching consequences was that there were clear differences in the regulation of C cycling in the topsoil and subsoil at this particular site. The data suggest that contact between degraders and substrate or, at the very least, exoenzyme access to substrate was the main regulatory mechanism of C mineralization in the subsoil. The significant priming effect observed in the topsoil indicated that the supply of fresh organic matter was the most important limiting factor on C dynamics in the surface horizon. The consequences of such different regulatory mechanisms could be dramatic. For example, it is believed that one of the effects of elevated atmospheric CO_2 will be an increase in root exudation and therefore an increase in the supply of fresh organic matter (e.g. Philips *et al*., 2006). Were this the case then an increase in topsoil organic matter turnover and decomposition might be expected, as has been found elsewhere (Carney *et al*., 2007; Peralta & Wander, 2008). In the subsurface soil on the other hand, there is no reason to believe that this would be the case, as no priming effect was observed. In fact, an increase in soil organic matter would be a likely scenario. Root exudates tend to be relatively small soluble molecules that have relatively high diffusion rates and so could diffuse away from the zones of microbial activity and not be degraded.

Microbial communities

CLPP indicated that the subsoil 'fast-growing' communities preferentially used simple molecules of the type usually found in root exudates such as amino and organic acids. The topsoil 'fast-growing' communities on the other hand, were adapted to using slightly more complex molecules such as polymers or disaccharides. Soil organic matter is probably composed of, among others, molecules that are more complex than root exudates. These data do not mean that subsoil microbial communities were better able to utilise simple molecules than topsoil communities, but rather that simple substrates accounted for a greater proportion of the average well colour development of subsoil samples than of topsoil samples. However, the average well colour development was an order of magnitude greater in the topsoil than in the subsoil, indicating that the overall activity was far higher in the topsoil. The microbial biomass in the topsoil was four times that in the subsoil and although mineralization per unit biomass was the same, overall, substrate usage was greater in the topsoil. The fructose mineralization kinetics confirms the ability of the topsoil communities to use simple substrates more rapidly than communities in the subsoil.

Natural isotopic signatures

The isotopic signature of the CO_2-C released at the beginning of the incubation from subsoil samples was close to that of maize C, suggesting that recently added C (via root exudation or root death) was a readily available source of substrate for microbial activity. As the incubation proceeded, the isotopic signature tended towards the signature of the soil organic matter, indicating that the maize derived substrate was exhausted and the microbial communities increased their use of the soil organic matter. The maize derived substrate was consumed more rapidly in the samples that respired more. The isotopic evidence is consistent with the scenario whereby the organic C available to the microbial communities in the subsoil was readily decomposable. The relative stability of the isotopic signature in the topsoil, at a value close to that of the soil organic matter, indicates that the contribution of fresh maize C to the mineralized C was lower than in the subsoil,

probably because much of the maize inputs would have been mineralized in the period between harvest (in October 2006) and the sampling date (March 2007). It should be noted that, at the beginning of the incubation, the isotopic ratio of the disturbed samples was significantly higher than those of the other treatments, indicating that this treatment caused the released of small quantities of maize C. However, the quantities were too small to significantly affect the total amount of C mineralized.

In conclusion, we suggest that modellers of soil C dynamics should be aware of the fact that the controls on dynamics in the topsoil and subsoil may be different, as this study suggests, and so may respond differently to future perturbations and changes. Contrary to the recent work on the stability of subsurface C (Fontaine *et al*., 2007), this work does not suggest that C mineralization in subsurface soils is energy limited, but rather is limited by the physical separation of substrate and decomposer, as also suggested by Xiang *et al*. (2008). The spatial separation of decomposer and substrate appears to play a more important role in the subsoil. Were this to prove widespread, then agricultural practices that promote exudation of low molecular weight, soluble compounds might lead to an increase in C stocks in the subsoil through increases in diffusion of C away from decomposition hot spots. Practices that increase the ratio of exudates C to root C would then be of particular interest (Swinnen *et al*., 1995) as a greater proportion of the plants below ground C allocation would not be mineralized.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Graphical representation of the experimental design. A detailed description of the design is provide in the Materials and Methods.

Figure S2. Cumulative estimated mineralisation of soil organic C assuming $25\,\mu g\,g^{-1}$ soil (black solid symbols) of carbonate-C or $10\,\mu g\,g^{-1}$ soil (open symbols) released over a period of 3 (◆ or ◇), 15 (■ or □) or 51 (● or ○) days for undisturbed samples incubated with ambient O_2. Assuming $25\,\mu g\,g^{-1}$ soil of carbonate-C is released rapidly (3 days), C immobilisation lasted 7 days. If released over 15 days, C was mineralised, but then immobilised between days 7 and 15. If $10\,\mu g\,g^{-1}$ soil were released rapidly (3 days) there was very little mineralisation during the first three days of the incubation. The rather unusual mineralisation curves suggest that either carbonate-C was not released or was released in rather small quantities. The mineralisation curves of the other treatments showed similar temporal changes. Estimated error bars are not presented because it is the form of the mineralisation curves that is of interest.

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