Hobley, E., Baldock, J., Hua, Q., & Wilson, B. (2016). Land-use contrasts reveal instability of subsoil organic carbon. *Global Change Biology*, *23*(2), 955-965. http://dx.doi.org/10.1111/gcb.13379

Received Date : 19-Apr-2016
Accepted Date : 24-May-2016
Article type : Primary Research Articles

Land-use contrasts reveal instability of subsoil organic carbon

Authors: Eleanor Hobley[1]*†, Jeff Baldock[2], Quan Hua[3], Brian Wilson[1,4]

Affiliations:

[1]University of New England, Armidale, New South Wales, Australia

[2]CSIRO Agriculture, Urrbrae, South Australia, Australia

[3]Australian Nuclear Science and Technology Organisation, Lucas Heights, New South Wales, Australia

[4]Office of Environment and Heritage, Armidale, New South Wales, Australia

*Correspondence to: nellie.hobley@wzw.tum.de

+49 8161 71 4186

†Current address: Technische Universität München, Weihenstephan, Germany

Running head: Deep soil carbon stability

Keywords: Land-use change, radiocarbon, fractions, agriculture, climate, deep soil carbon

Original research article

Abstract

Subsoils contain large amounts of organic carbon which is generally believed to be highly stable when compared with surface soils. We investigated subsurface organic carbon storage and dynamics by analyzing organic carbon concentrations, fractions and isotopic values in 78 samples from 12 sites under different land-uses and climates in eastern Australia. Despite radiocarbon ages of several millennia in subsoils, contrasting native systems with agriculturally managed systems revealed that subsurface organic carbon is reactive on decadal timeframes to land-use change, which leads to large losses of young carbon down the entire soil profile. Our results indicate that organic carbon storage in soils is input driven down the whole profile, challenging the concept of subsoils as a repository of stable organic carbon.

Introduction

Soil organic carbon (SOC) constitutes a major component of the global carbon cycle (Batjes, 1996, Jobbagy & Jackson, 2000). Agriculture has a profound influence on soil carbon storage and the global carbon cycle: cropped soils generally contain less carbon than native soils, whereas grazed pasture soils generally contain similar amounts of carbon to native soils (Batjes, 1996, Guo & Gifford, 2002, Wang *et al.*, 1999). However, the effects of agriculture on SOC storage may be secondary to other influences such as climate and soil fertility, which in turn determine agricultural practices (Rabbi *et al.*, 2015).

The effects of agriculture on SOC cycling have been widely investigated but studies have frequently focused on carbon dynamics in topsoils (Poeplau *et al.*, 2011, Rabbi *et al.*, 2014). Globally, over 70 % of the SOC stored in 0-3 m depths was estimated to be located in subsurface depths (> 20 cm) (Jobbagy & Jackson, 2000) and the drivers of SOC storage vary with depth (Hobley *et al.*, 2015, Hobley & Wilson, 2016, Jobbagy & Jackson, 2000, Salome *et al.*, 2010). Investigation of SOC dynamics based solely on surface soils may therefore result in incorrect conclusions as to what drives profile SOC storage. Furthermore subsurface SOC is frequently reported to be much older than surface SOC (Hobley *et al.*, 2013, Rumpel *et al.*, 2002), and subsoils (>20 cm) have been suggested to be a repository of slow-cycling organic carbon. The reasons for this apparent SOC stability remain unclear (Rumpel & Kögel-Knabner, 2011, Schmidt, 2011). Recently, substrate limitation was proposed as a stabilisation mechanism in subsoils (Don *et al.*, 2013), but it has also been suggested that subsoil SOC stability is largely a function of soil type (Mathieu *et al.*, 2015). However, evidence of actively cycling subsoil C has been associated with deep root activity (Trumbore *et al.*, 1995), and incubation experiments of subsoils have showed C turnover attributed to priming effects (Fontaine *et al.*, 2007) or wetting-drying cycles (Schimel *et al.*, 2011) , so that a better understanding of subsoil C dynamics is still required.

We investigated profile SOC cycling under native vegetation and agriculture (cropping and grazed pastures) at 12 sites selected across a climate gradient in eastern Australia (Fig. S1 and Table S1) from the dataset reported in (Hobley *et al.*, 2015, Hobley *et al.*, 2016, Hobley & Wilson, 2016). To investigate the drivers of profile C cycling as related to land-use, environmental and site factors we analysed 78 samples for total SOC concentrations, fractions (particulate, humus and resistant OC fractions – POC, HOC and ROC respectively) and C isotopic values ($\delta^{13}C$, ^{14}C). Using a combination of ANOVA, partial regression and stepwise linear regression modelling, we identified drivers of SOC dynamics across the whole data set, and then contrasted specific sites within the data set to model the SOC reacting to land-use change down the soil profile to depths of up to 1 m.

Materials and Methods

Sites and soils

Twelve sites were selected from the dataset described in Hobley & Wilson (2016) to contrast the effects of land-use and climate (rainfall, temperature, seasonality) on SOC profile (0-80 or 0-100 cm) dynamics, whilst minimising variance due to other site characteristics (e.g. soil type, Table S.1). The sites originated from two separate projects with different sampling depths so that for some sites, specific depths were combined according to bulk density ratios in order to create comparable sampling depths (e.g. 0-5 cm sample and 5-10 cm samples were combined to 0-10 cm samples).

Organic carbon analysis

Sample analysis follows Hobley *et al*. (2015). In brief, sieved (<2 mm), dried (40 °C) and ground (<100 µm) samples were analysed for carbon (C) content ($\%_{mass}$) using LECO dry combustion. C content was adjusted for water content (105 °C dry samples). The presence of carbonates in samples was tested using HCl and samples containing carbonates were analysed after acidification and heating with 6 % H_2SO_3 at 100 °C (repeated until no effervescence remained in sample) to remove carbonates, so that all results represent the organic carbon in the soils.

Radiocarbon analysis

Preparation of the radiocarbon samples (n=78) follows Hobley *et al*. (2013). In brief, aliquots of samples prepared for SOC analysis were heated in 2M HCl at 40 °C on a hotplate for at least 24 hr until dry. The dry samples were converted to CO_2 at 900 °C, which was then graphitized in excess H_2 over an Fe catalyst to produce a graphite target for accelerator mass spectrometry (AMS) analysis. A small portion of the graphite target was analysed for $\delta^{13}C$ (reported relative to Vienna Pee Dee Belemnite) using an elemental analyser - isotope ratio mass spectrometer (vario microcube EA, Elementar, Germany, and IsoPrime Isotope Ratio Mass Spectrometer (IRMS), GV Instruments, UK). AMS ^{14}C analysis was carried out using the STAR accelerator at ANSTO. Radiocarbon content of SOC samples, after correction for machine background, procedural blank and isotopic fractionation using measured $\delta^{13}C$, are reported as percent Modern Carbon (pMC) and conventional radiocarbon ages (in years Before Present, yr BP, where 0 yr BP is AD 1950).

Soil organic carbon fraction analyses

Three SOC fractions (particulate, humus and resistant organic carbon - POC, HOC and ROC respectively) were estimated using partial least square calibrations developed using truncated (1030-6000 cm^{-1}), baseline-offset and mean-centred mid-infrared (MIR) spectra according to the methodology reported in Baldock *et al*. (2013a). Two different calibrations were used to predict the fraction estimates, based upon different spectral processing algorithms: square-root transformation and Savitzky-Golay smoothing. Further, these different estimates were averaged, resulting in three different estimates for each of the fractions (i.e. square-root estimate, Savitzky-Golay estimate and average estimate). To overcome issues of collinearity between the content of the fractions and SOC (and depth), the proportional contribution of each fraction to SOC was calculated by dividing the fraction concentration by the sum of the three fractions, a proxy for SOC content (Hobley *et al.*, 2016). MIR estimates were validated using full physico-chemical fractionation estimates obtained for 6 sites (N1, G1, C1, N3, G3, C3) according to the methodology described in Baldock *et al.* (2013b). Full fractionations were performed on unground 40 °C dried samples to enable the physical separation of fractions according to particle-size.

Data analyses and models

Effects of land-use on SOC concentrations, $\delta^{13}C$ values and SOC fractions were assessed using analysis of variance (ANOVA) and partial regressions, which enabled investigation of land-use effects whilst controlling for other highly collinear covariates (e.g. depth). Significance was assessed at probability of error $p<0.05$. Throughout this manuscript, the term 'significant' refers to p-values ≤ 0.05; 'highly significant' refers to p-values ≤ 0.01.

In order to identify the drivers of radiocarbon content down the soil profile, stepwise multiple linear regressions were bootstrapped (n=100) with variable selection based upon the Akaike Information Criterion improvement in individual models. Four different model algorithms were applied: forward selection, backward selection, bidirectional starting with the null model (bidirectional forward), and bidirectional starting with the full models (bidirectional backward). The potential predictor variables described climate, land-use, topography and site variables (Table S.2) and were selected based upon their importance to SOC dynamics (Hobley *et al.*, 2015, Hobley *et al.*, 2016, Rabbi *et al.*, 2015).

The predictors selected across all of the four different stepwise regression model algorithms were used to create base multiple regression models, which were then updated by progressively adding the other predictors selected in only some models. The individual multiple regression models were then compared using ANOVA to identify the best model across the whole dataset for each of the three OC fractions estimates (square-root transformation, Savitkzy-Golay smoothing and average of the two). The final multiple linear regression models were cross-validated (using ten-fold cross-validation) to estimate performance accuracy (cross-validated R^2). Explained variance of these final models was apportioned to the predictors using the proportional marginal variance decomposition in the relaimpo package in R (Grömping, 2006). Partial regressions were then used to assess the influence of the selected predictors on radiocarbon content of the soils whilst controlling for other important predictors.

The depth distribution of soil organic radiocarbon was investigated preliminarily using linear and non-linear (exponential) functions. Simple linear models were selected as they provided a good fit to the distribution of the radiocarbon content as a function of soil depth:

$$SO^{14}C(z) = SO^{14}C_0 + k \cdot z \tag{1}$$

where $SO^{14}C(z)$ is the radiocarbon content (pMC) of SOC at the depth z (cm), $SO^{14}C_0$ is the radiocarbon content at the soil surface and k is the depth depletion constant of radiocarbon in the soil. The effects of land-use and environmental factors on the model parameters were then investigated using regression analysis, ANOVA and partial correlations.

In addition to the analysis of the entire data-set, specific sites from within climate-soil type combinations were contrasted to investigate the effects of land-use on SOC. The difference in SOC concentration and its radiocarbon content between two sites were compared at each depth and modelled to identify the 'dynamic' reaction of SOC to land-use change:

$$^{14}C_{\Delta SOC} \cdot \Delta SOC = {}^{14}C_{Site1} \cdot SOC_{Site1} - {}^{14}C_{Site2} \cdot SOC_{Site2} \tag{2}$$

Where $^{14}C_{\Delta SOC}$ is the radiocarbon content (pMC) of the SOC lost (or gained) and ΔSOC is the change in SOC concentration (%) as a result of land-use change from the land-use at Site 1 (with radiocarbon content $^{14}C_{Site1}$ and SOC concentration SOC_{Site1}) to the land-use at Site 2 (with radiocarbon content ${}^{14}C_{Site2}$ and SOC concentration SOC_{Site2}). Assuming that the SOC concentration and radiocarbon content of Site 2 was the same (or similar) as that of Site 1 prior to land-use change, we obtain:

$$ {}^{14}C_{\Delta SOC} = \frac{{}^{14}C_{Site1} \cdot SOC_{Site1} - {}^{14}C_{Site2} \cdot SOC_{Site2}}{SOC_{Site1} - SOC_{Site2}} \quad (3) $$

Results

Land-use effects on SOC, its isotopic signatures and component fractions

SOC concentration was highest at the surface and decreased highly significantly (and exponentially) with increasing depth (Fig. 1). Similarly, soil organic radiocarbon (SO^{14}C) content was greatest at the surface and declined highly significantly with increasing depth (Fig. 1), yielding greater apparent ages of SOC at depth. The δ^{13}C increased highly significantly with increasing depth and decreasing SOC concentration (Fig. 1).

Cropped sites had significantly lower SOC concentrations than native sites. The difference was most pronounced at the surface (0-10 cm) with SOC concentration under cropping averaging less than half that measured in native soils (Fig. 2). In contrast, although SOC concentration was on average higher under native vegetation than under grazing, the difference was not significant, nor were the differences between grazed and cropped sites significant. The specific contrasts of land-use within climate-soil systems indicated reduced surface SOC concentrations under both cropping and grazing than under native vegetation (Table 1), although with increasing depth, the differences between native and agricultural systems were diminished.

The effect of land-use on SOC was mirrored in $SO^{14}C$, which was reduced at all sites under agriculture compared with native systems (Fig. 1). The highest $SO^{14}C$ was under native vegetation, which has significantly higher $SO^{14}C$ than cropped sites. On average the radiocarbon content at the surface (0-10 cm) was 11 pMC higher under native vegetation than under cropping (Fig. 2). The reduction in radiocarbon was not limited to the surface but also observed in the subsoil. Despite the overall lower radiocarbon content in the grazed than the native soils, the differences were not significant. Although there was a trend to surface $\delta^{13}C$ enrichment under agriculture than native vegetation (Fig. 2), the effects were neither significant at the surface nor at depth (Fig. 1, Table 1).

These results are reflected in the site specific contrasts (Table 1), where the native sites had higher $SO^{14}C$ at almost all depths - both at the surface and in the subsoil - than the comparable agricultural sites. This resulted in apparent age differences in the order of decades to several millennia in the subsoils between native and agricultural sites. The models of the radiocarbon content in the 'dynamic' SOC (i.e. the SOC change as a result of land-use conversion) indicate that this dynamic SOC is fresh carbon at all sites to a depth of at least 30 cm, with evidence of young carbon losses in greater depths at all sites. At some (cropped) sites, the change in SOC is potentially due to fresh carbon losses down the entire soil profile. Overall, the reactive SOC in the subsoils was younger than, or of a similar apparent age to, the bulk SOC under the native site, however due to the small changes in SOC concentration, uncertainties were at times very large.

The proportions of POC and HOC were highly significantly correlated with depth and SOC, but the proportion of ROC was not (Fig. 3). Whereas the proportion of POC decreased with decreasing SOC concentration and increasing depth, the proportion of HOC increased with decreasing SOC concentration and increasing depth. Across all depths, the proportion of POC was significantly reduced under cropping compared with native systems and this relationship remained highly significant after controlling for depth via partial correlation. However, grazing did not significantly alter the proportion of POC and the proportions of the other fractions were not significantly affected by land-use. Furthermore, the specific site contrasts (Table 1) show that at the soil surface, all three SOC fractions were depleted at most – though not all - agricultural sites and with increasing depth, the POC fraction was less affected by agriculture than the HOC and ROC fractions.

Modelling radiocarbon depth distribution and drivers

The multiple linear regression models used to investigate the drivers of profile $SO^{14}C$ were able to account for 86.8±0.1% of variation within $SO^{14}C$ and selected five predictor variables: depth, seasonal rainfall, land-use class, the proportion of POC, and the surface clay content of the soils. Radiocarbon content was negatively associated with soil depth, which was by far the most important variable to the models, accounting for 73.5±0.8 % of model explained variance (MEV). Seasonal rainfall was positively associated with $SO^{14}C$, contributing 13.7±0.1% to MEV, land-use contributed a further 5.8±0.2% to MEV. The proportion of POC was positively associated with $SO^{14}C$ (5.0±0.9% to MEV) and clay content at the soil surface was negatively associated with $SO^{14}C$, (2.0±0.1% MEV).

In contrast to the exponential decline in the SOC concentrations (Hobley & Wilson, 2016), simple linear models of radiocarbon to depth fit very well, with explained variances averaging 94 % (Range 87-99%). Whereas land-use significantly affected surface $SO^{14}C$, accounting for around 41% of variance (Fig. 2), land-use was not significantly associated with the depth depletion parameter of the fit, *k*. *S*imilarly, k was unaffected by soil type, the proportion of any fraction, textural gradient from surface to subsoil, mean annual temperature or mean annual precipitation. However, k was highly significantly associated with the seasonality of rainfall, which accounted for around 67% of variance in the depth depletion of radiocarbon (Fig. 4).

Discussion

Land-use effects on SOC, its isotopic signatures and component fractions

The SOC concentrations at the sites are consistent with global observations of SOC depth distribution (Batjes, 1996, Jobbagy & Jackson, 2000) as well as within the study region (Hobley *et al.*, 2015, Hobley & Wilson, 2016), and support the current understanding of the effects of agriculture on SOC storage (Guo & Gifford, 2002, Hobley *et al.*, 2015), namely a large reduction in SOC due to cropping and a less significant effect due to grazing.

These effects of land-use on SOC proportional fractions indicate that at the surface POC was the most sensitive indicator of land-use change (Chan *et al.*, 2002). However, the specific site contrasts indicate that land-use affected all three fractions and in the subsoil POC was less sensitive to change than the other fractions. This is consistent with different depth distributions of the three fractions, resultant from different inputs at different depths (Hobley *et al.*, 2016), so that the reaction to change (i.e. reduction in input after conversion from native to agriculturally managed systems) differs between fractions and depths. Specifically, in the subsoil the dominant fraction was HOC (which originates from turnover and translocation of POC), so that in the subsoil this fraction was the most affected by land-use change. Although the lack of clear response of ROC to land-use change could reflect a greater stability of this fraction, it could also be attributable to a higher variance of this fraction across all sites and depths (Tables S3 and S4), resulting in difficulties in detecting differences.

The reduction in radiocarbon down the entire soil profile under agricultural management compared with native systems corroborates findings that land-use affects sub-surface SOC storage in the region (Hobley & Wilson, 2016), which had previously been difficult to detect (Wilson *et al.*, 2011), and highlights the power of radiocarbon dating to identify small changes in ecosystem carbon cycling.

Drivers of SOC profile dynamics

The depth distribution of SOC has been associated with climate, site factors, and vegetation (Hobley *et al.*, 2015, Jobbagy & Jackson, 2000) and in the study region, land-use was found to be the most influential driver of the depth distribution of SOC (Hobley & Wilson, 2016), but studies into the drivers of $SO^{14}C$ vertical distribution are less common.

Decreasing radiocarbon content with increasing soil depth such as we observed is often reported (Hobley *et al.*, 2013, Rumpel *et al.*, 2002). Depth was by far the most influential variable to the models of radiocarbon content, so that it would therefore appear that depth is intimately linked with soil radiocarbon content. However, depth should not be viewed as a direct driver of radiocarbon content but is instead a proxy for other processes (such as input, translocation, turnover). The other factors important to the models reflect our understanding as to the drivers of SOC dynamics, namely climate, human influence and site factors, such as clay content (Jenny, 1941). However, similar to the findings of Rabbi et al. (2015) investigating land-use effects on SOC storage, the effect of land-use on $SO^{14}C$ indicated by the models appears to be relatively minor.

Furthermore, the unimportance of land-use to the radiocarbon depth depletion parameter implies that land-use affects $SO^{14}C$ content down the whole soil profile: the loss in radiocarbon after disturbance is not confined to the topsoil but is reflected at depth. This implies that the drivers of profile SOC concentration – which is affected by land-use (Hobley & Wilson, 2016) - differ to those of profile $SO^{14}C$. We explain this via the fact that land-use effects on SOC concentration are greatest near the surface where concentrations are highest (Table 1, Hobley *et al.*, 2015). Land-use also impacts the soil physical environment, with cropped soils typically having higher bulk densities than native soils (Hobley *et al.*, 2015). This will directly affect the pore-size distribution and pore continuity and indirectly affect SOC redistribution and input (e.g. via effects on illuviation and percolation, faunal activity and therefore bioturbation, or rooting depth), thereby altering the depth distribution of SOC (Hobley & Wilson, 2016). However, $SO^{14}C$ represents a *content* - not an absolute *concentration* - within SOC, and land-use does not alter the depth distribution of $SO^{14}C$ because, as indicated by our modelling (Table 1), the SOC responsive to land-use change is of a similar (young) age down much of the profile.

Although only three soil types were represented in our data set, they represent large contrasts in clay content and activity. It is therefore interesting that soil type did not influence SOC profile dynamics, as soil type was recently suggested to be a highly important driver of profile SOC dynamics (Mathieu *et al.*, 2015). Instead, our results indicate that the season in which rainfall predominately occurs was the most important factor to the depth distribution of radiocarbon in these profiles. Amount and seasonality of rainfall were found to be highly influential to surface SOC concentration, but was not influential to SOC depth distribution in the study region (Hobley & Wilson, 2016). This was attributed to the influence of water availability on SOC production which predominates near the surface. It is therefore interesting that seasonality of rainfall appears to be important to radiocarbon content of the entire dataset in this study. We hypothesize that the season of rainfall is a key driver of radiocarbon content because it can translocate fresh, young organic carbon (e.g. as dissolved organic carbon, DOC) from the surface to the subsoil. This may lead to a large shift in radiocarbon content, particularly at depth where radiocarbon is highly depleted, without having a sizeable effect on total SOC concentration.

Specifically, the significantly smaller depth depletion parameter of radiocarbon indicates a greater input of younger carbon at depth in summer dominant rainfall regions compared with regions experiencing uniform rainfall (Fig. 4). This could be attributable to a downward flush of fresh DOC during summer rains, occurring as non-equilibrium (preferential) flow due to rainfall intensity and soil wetting and drying (Jarvis, 2007). Subsequent degradation of the fresh DOC may also be limited, as the profile dries out due to decreased rainfall, resulting in retention of younger carbon at depth. In contrast, in soils under uniformly distributed rainfall, although a translocation of DOC will occur with rainfall events, the translocation is more likely to occur as equilibrium (matrix) flow, allowing temporal immobilization, microbial processing and re-release of DOC into the soil matrix (Kaiser & Kalbitz, 2012), thereby limiting overall DOC translocation rates. Other factors such as rooting depth may also be influenced by seasonality of rainfall (although this is less likely in annually cropped systems), and we recommend future investigations into the influence of climate factors on SOC, DOC and radiocarbon depth distribution in soils.

Further evidence of translocation driving subsurface SOC dynamics is provided by the $\delta^{13}C$ analyses as well as the fraction data. The observed enrichment of ^{13}C with depth is a well-documented phenomenon (Balesdent *et al.*, 1993, Wynn *et al.*, 2004), which is hypothesized to reflect a shift in the composition of SOC from plant towards microbial residues (Ehrleringer *et al.*, 2000) due to systematic microbial fractionation of SOC (Wynn *et al.*, 2004). Thus our observed enrichment of $\delta^{13}C$ with depth implies that subsurface SOC has undergone more microbial processing than surface SOC (Ehrleringer *et al.*, 2000).

These results are consistent with the overall decrease in the proportion of POC and increase in the proportion HOC observed with enhanced sampling depth, as POC is (primarily) produced by plants, whereas HOC is produced by microbial degradation processes. As such, it would appear that subsurface SOC is comprised more of microbial than plant products. This is consistent with input of plant materials predominately at the surface which are then progressively transformed and cycle downwards (Kaiser & Kalbitz, 2012). The presence of ROC at depth in subsoils also indicates that polyaromatic material consistent with pyrogenic origins is distributed down the entire soil profile, well below its near-surface production depth (Chafer, 2008, Doerr *et al.*, 2006), indicating vertical movement of pyrogenic material (Hobley *et al.*, 2014). Although this is potentially due to bioturbation, the mismatch between organisms contributing to bioturbation and the depth distribution of SOC (Ekschmitt *et al.*, 2008) suggest that this explanation is insufficient. Furthermore, the depth distribution of ROC has been associated with interactions of precipitation and texture, suggesting that vertical mobility of this particulate fraction results from illuvation (Hobley *et al.*, 2016). Together with the $SO^{14}C$, these results strongly suggest that vertical translocation is a key driver of subsurface SOC dynamics in these soils and that this vertical translocation is climate driven.

SOC stability down the profile

The observed increases in apparent radiocarbon age down the profile are consistent with the current concept of enhanced stability of subsurface SOC, which has been proposed to have turnover times in the order of centuries to millennia (Wang *et al.*, 1999). However, the generally lower SOC and radiocarbon contents down the profiles under agriculture than native vegetation strongly suggest that subsurface SOC is not as stable as previously hypothesized. Indeed, evidence of dynamic subsurface SOC responses to land-use change suggest that ‘deep’ soil carbon may be actively cycling (Don *et al.*, 2011).

A potential driver of subsurface SOC cycling is the supply of fresh carbon to subsoil horizons, which enhances microbial mineralisation of old carbon, suggesting that stability of deep soil carbon is a function of substrate availability (Fontaine *et al.*, 2007). However, our study indicates that change from native vegetation to agriculture results in an overall *loss* of SOC and radiocarbon down the entire soil profile. The modelled radiocarbon content of the reactive SOC was generally higher than that of the native SOC, indicating that disturbance of native systems leads to a young carbon deficit in soils, with no evidence of a priming effect (Keiluweit *et al.*, 2015, Sorensen, 1963) at any depth.

A shift to lower radiocarbon contents after disturbance of native sites has been observed within topsoils and attributed to preferential loss of SOC from young C pools (Wang *et al.*, 1999). Preferential turnover of young SOC could also explain our lower $SO^{14}C$ content under agriculture, supported by the modelled radiocarbon concentrations of the SOC reacting to land-use change, which indicate that, in general, fresh carbon is lost due to disturbance of the native system. This is most noticeable under cropping, where the sites indicate a potential lack of fresh carbon down the entire soil profile (Table 1).

Losses of DOC via translocation with rainfall (see above) could also lead to a reduction in $SO^{14}C$ in the profile, if the DOC is washed down below the sampling depth of the soil. This is supported by the fact that subsoil SOC changes appear to be predominately in the HOC and ROC fractions, not POC, implying that translocation of fresh POC is limited. Regardless of which mechanism (turnover, translocation or both) is responsible for the observed reduction in $SO^{14}C$, it must be stressed that the driver of change is the lack of supply of young, fresh carbon to the soil as the biomass produced is harvested from the system: soil carbon storage in these soils is input driven.

With increasing depth, there was some evidence of dynamic ‘old’ carbon (i.e. apparent radiocarbon ages in the order of centuries to millennia) at depth after land-use change, especially under grazed pastures. These results were not associated with a loss but with a slight gain of SOC in the subsoil after land-use change, potentially suggesting destabilisation and subsequent translocation of old carbon, specifically within the sandy soils (N1, C1, G1) profiles. This gain was detected in both HOC and ROC fractions, suggesting translocation of old carbon to subsurface soils in these fractions, e.g. as old DOC which is incorporated into the HOC fraction, or as stable charcoal (Hobley *et al.*, 2014). However, given the small changes in SOC and large uncertainties in the modelled results, these results should be interpreted with caution.

The individual site contrasts indicate that agricultural use leads to a dynamic response in all three fractions at the surface, but mainly affects HOC (and to a lesser degree ROC) below a depth of around 30 cm. These two fractions – HOC and ROC – are theoretically more stable than POC, as ROC is chemically resistant and HOC has been microbially processed and can be bound to mineral surfaces in the soil. However, the concepts of enhanced SOC stability in different fractions have largely been developed on investigations of surface soils (Baldock & Skjemstad, 2000, Skjemstad *et al.*, 1996). Isolating functional SOC pools of different stability is challenging (von Lützow *et al.*, 2007) and our analysed fractions are not necessarily discrete, but are probably (potentially overlapping) components of a SOC continuum. Nevertheless, our results suggest that when viewing the soil system across the whole profile, these supposedly more stable SOC fractions (i.e. HOC and ROC) are not more stable than the ‘labile’ POC, and that all three fractions respond dynamically to change (Hobley *et al.*, 2016). Future research into the dynamics and ^{14}C content of SOC fractions down the entire profile under different land-uses will help to decipher the complex interrelationships between SOC fractions and stability.

Outlook

Our results indicate that agriculture affects SOC concentration, fractions and radiocarbon content down the whole soil profiles studied. There was no evidence for enhanced subsoil stability and evidence for older carbon fractions was limited to specific subsoil depths in sandy soils, where translocation of pyrogenic and humus carbon may represent older carbon pools. However, given that these fractions reacted to land-use change, the idea that they are stable is questionable.

So where does this leave soil carbon stability? We believe that the apparent radiocarbon ages down the soil profile do not indicate enhanced subsurface stability. We propose a conceptual model whereby the apparent radiocarbon age of subsurface SOC predominately reflects the input of SOC via translocation processes as well as the microbial pool of SOC. Root inputs may also contribute to the young carbon pool in subsoils, but our results suggest that their importance is limited. We hypothesize that the microorganisms in the subsoil preferentially use the supply of fresh, translocated SOC for catabolism, rather than anabolism, retaining a pool of old carbon in their biomass. The old carbon is not stable, but is not actively cycling due to its storage in microorganisms, which can remain alive but inactive for thousands of years. When this input is reduced, the younger carbon is preferentially removed from the system, both via microbial activity and washing out of the system. As the carbon in microbial biomass is relatively old, a small increase or decrease in the amount of young carbon results in a drastic shift in radiocarbon content, highlighted by the increase in radiocarbon age in the orders of millennia in agricultural subsoils mere decades after land-use change. Similarly, all fractions are dynamic upon change. Although there may be different ages in specific fractions at given depths, this does not reflect an overall enhanced stability when the entire soil profile is viewed as a system. Our results strongly suggest that SOC storage and dynamics are determined by inputs. Given the reactive nature of all fractions at all depths, we cannot expect singular applications of carbon (e.g. as ‘stable’ biochar or ‘labile’ manures) to result in long-term carbon enrichment in soils.

Acknowledgments

This research was funded by the Australian Government Department of Agriculture. Thank you to NSW OEH staff for laboratory work and A Wilkie for the map. Thank you to Martin Wiesmeier for comments and feedback. We thank the anonymous reviewers who helped to improve this manuscript. The authors declare no conflicts of interest.

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Supporting Information

Fig. S1 Site Locations

Table S1 Site attributes

Table S2 Model predictors

Table S3 Organic carbon concentration, fractions and isotopic composition

Table S4 Physico-chemically isolated carbon fractions

Table 1. Change in organic carbon (totals, fractions and $\delta^{13}C$) and modelled change in radiocarbon content due in specific land-use contrasts.

	Mass proportion (or change therein Δ) of SOC§, fraction* ($\%_{mass}$) or $\delta^{13}C$** (‰)						Radiocarbon content (pMC) (age in brackets, yBP)***		
Depth (cm)	Comparison 1a: N1-G1								
	SOC_{Native}	ΔSOC	ΔPOC	ΔHOC	ΔROC	$\Delta\delta^{13}C$	pMC_{Native}	ΔpMC	pMC in ΔSOC
0-10	1.29 ± 0.14	-0.30±0.01	-0.09 ± 0.00	-0.13 ± 0.01	-0.05 ± 0.00	2.7	107.0 (Modern)	-7 (NA)	129±2 (Modern)
0-20	0.96 ± 0.20	-0.31±0.14	-0.07 ± 0.01	-0.15 ± 0.07	-0.10 ± 0.04	-0.1	95.8 (350)	-11 (970)	119±7 (Modern)
20-30	0.76 ± 0.33	-0.02±0.22	0.01 ± 0.01	0.05 ± 0.12	-0.01 ± 0.03	0.8	89.7 (870)	-12 (1160)	123±62 (Modern; 3880-Modern)
30-50	0.46 ± 0.15	0.09±0.08	0.00 ± 0.01	0.10 ± 0.01	0.01 ± 0.00	-0.5	81.6 (1630)	-6 (620)	83±58 (1460; 10950-Modern)
50-70	0.24 ± 0.12	0.12±0.06	0.01± 0.00	0.09 ± 0.00	0.04 ± 0.01	-1.4	71.1 (2740)	-2 (250)	65±2 (3510; 3780-3260)
70-80	0.18 ± 0.11	0.13±0.04	0.00 ± 0.00	0.10 ± 0.00	0.05 ± 0.01	-0.7	62.1 (3830)	-12 (1680)	34±5 (8580; 9880-7460)
70-100	0.34 ± 0.06	0.01±0.07	0.00 ± 0.00	0.02 ± 0.02	0.02 ± 0.00	-0.5	54.5 (4890)	-7 (1180)	42±57 (7040; Ancient-130)
	Comparison 1b: N1-C1								
0-10	1.29 ± 0.14	-0.04±0.06	-0.07 ± 0.00	-0.02 ± 0.04	0.03 ± 0.02	2.0	107.0 (Modern)	-5 (NA)	219±54 (Modern)
0-20	0.96 ± 0.20	-0.26±0.02	-0.06 ± 0.01	-0.15 ± 0.01	-0.06 ± 0.01	0.4	95.8 (350)	-6 (550)	113±5 (Modern)
20-30	0.76 ± 0.33	-0.25±0.11	-0.02 ± 0.00	-0.09 ± 0.04	-0.09 ± 0.04	1.2	89.7 (870)	-10 (990)	111±4 (Modern)
30-50	0.46 ± 0.15	-0.10±0.05	-0.01 ± 0.00	-0.02 ± 0.02	-0.04 ± 0.01	-0.5	81.6 (1630)	-4 (360)	98±6 (160; 650-Modern)
50-70	0.24 ± 0.12	-0.06±0.03	-0.01 ± 0.00	-0.03 ± 0.01	-0.01 ± 0.01	-0.8	71.1 (2740)	-10 (1220)	99±4 (120, 470-Modern)
70-80	0.18 ± 0.11	0.06±0.05	-0.01 ± 0.01	0.06 ± 0.01	0.03 ± 0.01	-0.8	62.1 (3830)	-12 (1690)	25±10 (11250; 15360-8550)
70-100	0.34 ± 0.06	-0.07±0.03	0.00 ± 0.00	-0.03 ± 0.01	-0.03 ± 0.00	-0.5	54.5 (4890)	-15 (2590)	134±55 (Modern; 1900-Modern)
	Comparison 2: N2-C2								
0-10	1.35 ± 0.07	-0.61±0.08	-0.16 ± 0.01	-0.23 ± 0.03	-0.14 ± 0.04	1.4	110.9 (Modern)	-10 (NA)	123±2 (Modern)
0-20	0.69 ± 0.06	-0.15±0.06	-0.04 ± 0.01	-0.05 ± 0.05	-0.01 ± 0.01	0	99.9 (Modern)	-7 (560)	127±15 (Modern)
20-30	0.55 ± 0.05	-0.11±0.03	-0.02 ± 0.00	-0.04 ± 0.03	-0.01 ± 0.01	-1.2	93.8 (520)	-8 (690)	130±15 (Modern)
30-50	0.62 ± 0.04	-0.17±0.04	-0.02 ± 0.01	-0.03 ± 0.04	-0.08 ± 0.03	0.1	97.0 (250)	-10 (840)	124±11 (Modern)
50-70	0.48 ± 0.08	-0.09±0.06	-0.01 ± 0.00	0.01 ± 0.02	-0.07 ± 0.03	-0.5	85.5 (1260)	-4 (340)	111±29 (Modern; 1600-Modern)
70-80	0.49 ± 0.06	-0.23±0.07	-0.02 ± 0.01	-0.10 ± 0.05	-0.10 ± 0.02	0.3	81.1 (1680)	-4 (360)	85±1 (1270; 1400-1130)

	Comparison 3: N3-C3								
0-10	3.04 ± 0.25	-2.40±0.32	-0.85 ± 0.02	-1.09 ± 0.10	-0.47 ± 0.04	3.3	114.2 (Modern)	-13 (NA)	118±1 (Modern)
0-20	1.56 ± 0.17	-0.97±0.19	-0.24 ± 0.03	-0.50 ± 0.03	-0.19 ± 0.04	4.0	103.3 (Modern)	-15 (1050)	113±3 (Modern)
20-30	0.92 ± 0.17	-0.44±0.09	-0.02 ± 0.00	-0.11 ± 0.07	-0.12 ± 0.04	-0.1	90.8 (780)	-10 (940)	102±6 (Modern; 340-Modern)
30-50	0.68 ± 0.23	-0.32±0.06	-0.02 ± 0.02	-0.21 ± 0.05	-0.19 ± 0.08	-1.2	80.5 (1750)	-13 (1460)	94±6 (490; 1020-Modern)
50-70	0.35 ± 0.20	-0.11±0.16	0.00 ± 0.01	0.00 ± 0.02	0.00 ± 0.05	-1.0	65.8 (3360)	-20 (2940)	161±95 (Modern; 3360-Modern)
70-80	0.23 ± 0.12	-0.10±0.08	0.00 ± 0.00	-0.06 ± 0.05	-0.01 ± 0.02	-0.2	53.6 (5010)	-20 (3730)	131±109 (Modern; 12120-Modern)
	Comparison 4: G2-C4[+]								
0-10	1.16 ± 0.16	-0.49±0.30	-0.06 ± 0.02	-0.16 ± 0.06	-0.20 ± 0.05	-0.7	98.4 (140)	-7 (620)	113±9 (Modern)
0-20	0.97 ± 0.29	-0.36±0.25	-0.03 ± 0.00	-0.15 ± 0.06	-0.14 ± 0.05	-0.8	93.8 (510)	-9 (770)	122±26 (Modern; 390-Modern)
20-30	0.85 ± 0.12	-0.25±0.23	-0.01± 0.01	-0.09 ± 0.04	-0.19 ± 0.01	0.1	89.9 (860)	-9 (840)	242±259 (Modern; Ancient-Modern)
30-50	0.82 ± 0.19	-0.23±0.11	-0.01 ± 0.01	-0.14 ± 0.01	-0.14 ± 0.05	0.9	84.8 (1330)	-8 (840)	112±12 (Modern)
50-70	0.79 ± 0.25	-0.16±0.16	-0.01 ± 0.01	-0.11 ± 0.05	-0.03 ± 0.01	1.4	79.8 (1810)	-2 (180)	71±31 (2780; 7400-Modern)
70+[$]	0.49 ± 0.13	-0.01±0.04	0.00 ± 0.00[$]	-0.02 ± 0.03[$]	-0.02 ± 0.00[$]	1.1[$]	65.2 (3440) [$]	0 (-60) [$]	78±14 (2040; 3650-700)
	Comparison 5: N4-G4								
0-10	2.19 ± 0.62	-0.92±0.74	0.11 ± 0.03	-0.20 ± 0.03	-0.31 ± 0.09	1.0	107.0 (Modern)	1 (NA)	104±2 (Modern)
0-20	0.88 ± 0.24	-0.36±0.23	-0.04 ± 0.01	-0.13 ± 0.01	-0.01± 0.02	5.0	109.3 (Modern)	-2 (NA)	113±2 (Modern)
20-30	0.56 ± 0.17	-0.30±0.13	-0.04± 0.04	-0.09 ± 0.01	-0.04 ± 0.04	5.2	103.4 (Modern)	-5 (120)	109±2 (Modern)
30-50	0.40 ± 0.13	-0.22±0.07	-0.03 ± 0.02	-0.10 ± 0.02	-0.02 ± 0.03	4.9	95.1 (400)	-1 (80)	96±1 (330, 280-370)
50-70	0.42 ± 0.16	-0.17±0.06	0.01 ± 0.00	-0.19 ± 0.04	0.04± 0.01	0.7	86.0 (1210)	-6 (630)	99±8 (120, 810-Modern)
70-100	0.37 ± 0.26	-0.22±0.08	0.00 ± 0.00	-0.29 ± 0.11	0.02 ± 0.03	2.1	75.8 (2230)	1 (-130)	75±1 (2320, 2370-2260)

[§] The SOC shown is the mean ± standard deviation of LECO and MIR estimates. * Positive values indicate a gain of C after land-use conversion, negative values indicate a loss of C after land-use conversion. Values show mean and range of two MIR estimates (SR, SG). ** Positive values indicate enrichment of ^{13}C after land-use conversion, negative values indicate depletion of ^{13}C after land-use conversion. *** Radiocarbon ages are reported as years Before

Present (1950) and are not calibrated. Ages are mean values and range calculated from upper and lower values of ΔSOC. [+]The grazed site is the baseline for this comparison. [$]The lower depth was 70-80 cm for the grazed site and 70-100 cm for the cropped site so that this depth comparison is approximate.

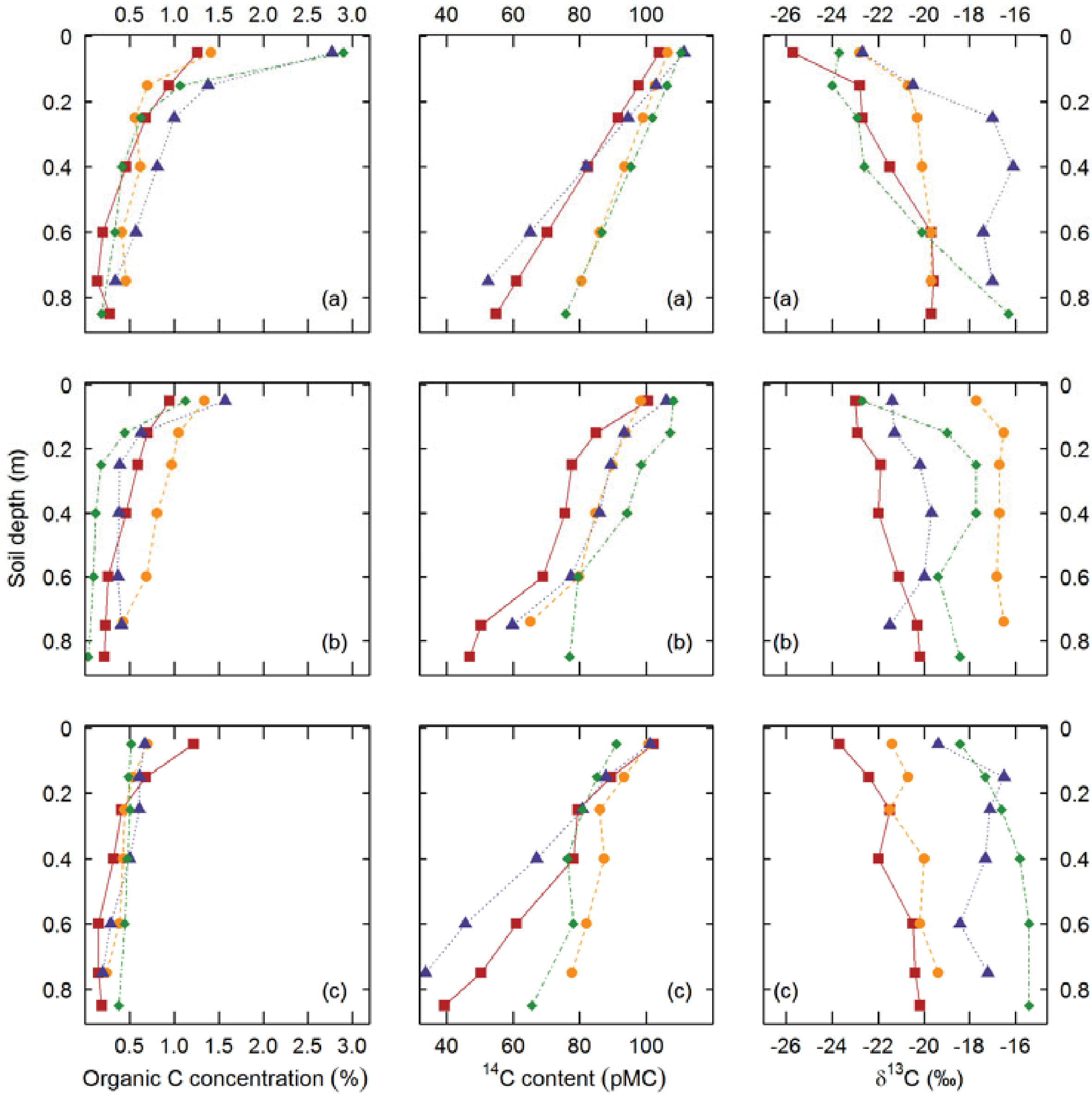
List of Figures

Fig. 1. LECO organic carbon content (left), radiocarbon content (centre) and $\delta^{13}C$ ratios (right) as a function of soil depth under native vegetation (a), grazed pastures (b) and cropping (c). Colours relating to sites (see Table 1): Red – site 1; orange – site 2; blue – site 3; green – site 4.

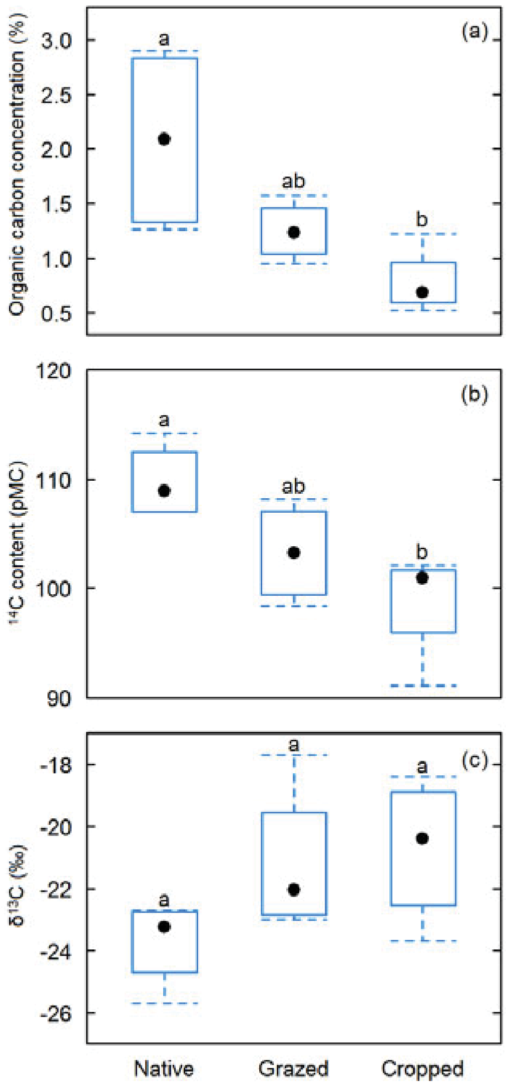
Fig. 2. Surface (0-10 cm) LECO organic carbon concentration (a), radiocarbon content (b) and $\delta^{13}C$ (c) under different land-use. Dots represent means, boxes indicate 1st and 3rd quartiles, whiskers represent 5 and 95% confidence intervals. Different letters indicate significant differences ($p<0.05$) between groups ($n=4$ per group).

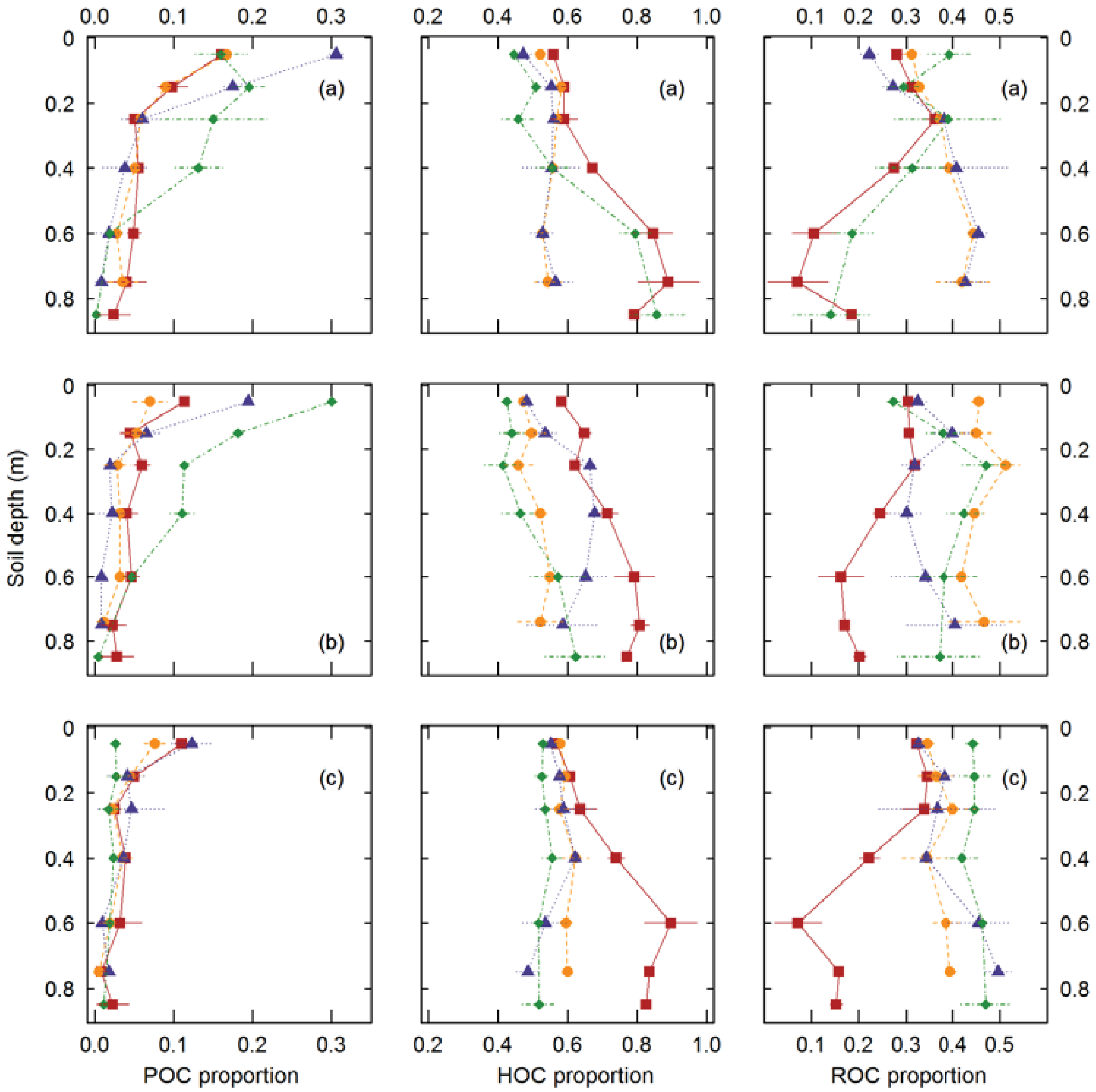
Fig. 3. Relative proportion of organic carbon fractions (particulate organic carbon – POC, humus organic carbon – HOC, resistant organic carbon – ROC) as a function of soil under native vegetation (a), grazed pastures (b) and cropping (c). Colours relating to sites (see Table 1): Red – site 1; orange – site 2; blue – site 3; green – site 4.

Fig. 4. Radiocarbon depth depletion constant, k, within rainfall seasonality zones. Dots represent means, boxes indicate 1st and 3rd quartiles, whiskers represent 5 and 95% confidence levels. Different letters indicate highly significant differences between groups ($n=6$ per group).

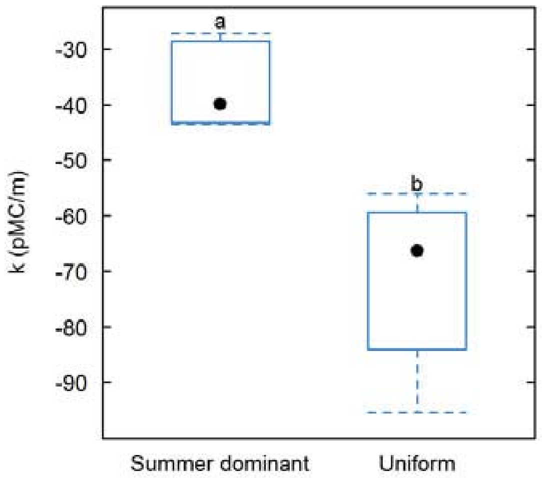


Soil depth (m)
(a)
(b)
(c)
Organic C concentration (%)
^{14}C content (pMC)
δ^{13}C (‰)


Organic carbon concentration (%)
3.0
2.5
2.0
1.5
1.0
0.5
(a)
a
ab
b
120
110
100
90
^{14}C content (pMC)
(b)
a
ab
b
-18
-20
-22
-24
-26
δ^{13}C (‰)
(c)
a
a
a
Native
Grazed
Cropped



0.0
0.1
0.2
0.3
0.2
0.4
0.6
0.8
1.0
0.1
0.2
0.3
0.4
0.5
0
0.2
0.4
0.6
0.8
(a)
(b)
(c)
Soil depth (m)
POC proportion
HOC proportion
ROC proportion


a
b
-30
-40
-50
-60
-70
-80
-90
k (pMC/m)
Summer dominant
Uniform