Vegetation and microbial determinants of soil carbon isotopic composition.

Jacques A. Nel

NLXJAC018

Thesis presented for the degree of Master of Science
In the department of Biological Sciences
University of Cape Town
November 2017

Supervisor
Prof MD Cramer

Declaration

I, Jacques A. Nel, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signed by candidate

Signature: Signature removed

Date: ……02/11/2017……..

Abstract

Typically, soil $\delta^{13}C$ and $\delta^{15}N$ values tend to increase with depth across a wide range of ecosystems. Changes in $\delta^{13}C$ with depth have been attributed to vegetation changes (i.e. C_3/C_4 shifts), but the similarity in $\delta^{13}C$ and $\delta^{15}N$ profiles suggest that microbial decomposition may play an important role. The determinants of soil $\delta^{13}C$ and $\delta^{15}N$, however, are complex and microbial decomposition and vegetation shifts are not the only mechanisms that drive the fractionation of the isotopes with depth. We explored the utility of using $\delta^{13}C$ as a proxy for vegetation change by considering alternative mechanisms for the changes in soil $\delta^{13}C$ with depth. These alternate mechanisms may weaken the interpretation of soil $\delta^{13}C$ as an indicator of vegetation change if the measured $\delta^{13}C$ changes are small. We hypothesized that: (1) if soil-related processes such as mineralization and dark CO_2-fixation by microbes and roots contribute significantly to the $\delta^{13}C$ signature of bulk soil at depth, one cannot simply determine whether the $\delta^{13}C$ value of the soil at depth is indicative of a past vegetation assemblies (i.e. C_3/C_4 transitions); (2) changes in soil $\delta^{13}C$ and $\delta^{15}N$ values are linked through common microbially mediated decomposition-related processes; (3) anaplerotic CO_2 fixation by microbes and roots may contribute significantly to soil $\delta^{13}C$ values, while N_2-fixation may contribute to soil $\delta^{15}N$ values with depth. Microbial processing of soil organic matter (SOM) during decomposition leads to ^{13}C-enrichement of SOM with depth and has been modelled using a Rayleigh distillation process. Anaplerotic fixation of soil CO_2 is, however, known to occur in microbes and roots and we suggest that this has a role in determining soil SOM $\delta^{13}C$ values through cumulative incorporation of bulk atmosphere CO_2 into SOM. These processes vary greatly between soils and environments.

The correspondence between soil $\delta^{13}C$ and $\delta^{15}N$ was assessed by compiling data from soil depth profiles from widely distributed sites and conducting an analysis of global $\delta^{13}C$ and $\delta^{15}N$ variations in surface soils in order to determine relationships between

soil isotopes and with climate and soil properties. Strong positive correlations between $\delta^{13}C$ and $\delta^{15}N$ values through soil profiles were found at a number of sites and were found to be independent of vegetation type. Globally, soil $\delta^{13}C$ and $\delta^{15}N$ values were also found to be significantly positively correlated across a wide range of climates and biomes. The global correspondences between $\delta^{13}C$ and $\delta^{15}N$ values may suggest a mechanistic link between $\delta^{13}C$ and $\delta^{15}N$ through the process of SOM decomposition and microbial processing.

Anaplerotic CO_2 fixation by soil microbes and roots was assessed using soils from 10 sites across South Africa differing in soil properties and incubated in the dark for 3 d under continuous exposure to $^{13}CO_2$- and $^{15}N_2$-enriched atmospheres with varying soil moisture (10, 50 and 100% of field capacity) and temperature (4, 25, 40°C). There was no evidence of significant N_2 fixation in any treatment. Significant soil anaplerotic CO_2 fixation, however, occurred in all soils. Highest rates of anaplerotic CO_2 fixation occurred in soils at 50% field capacity and 25°C, suggesting a link with microbial biotic activity. Soils with low C and N concentrations and low C:N ratios exhibited the highest rates of CO_2 fixation in soils, indicating a link between anaplerotic CO_2 fixation rates and soil nutrient status. The higher rates of CO_2 fixation in soils with low nutrients may indicate that soil microbes rely increasingly on anaplerotic fixation as SOM-N declines, forcing greater reliance on de novo amino acid synthesis, and thus anaplerotic CO_2 fixation. The ubiquitous occurrence of anaplerotic $^{13}CO_2$ fixation in these soils indicates that anaplerotic fixation is likely important in contributing to determining soil $\delta^{13}C$ values. Diffusion of low $\delta^{13}C$ bulk atmospheric CO_2 (ca. -10‰) into the soil atmosphere (<< -10‰) will drive soil CO_2 $\delta^{13}C$ towards ca. -10‰, and constant anaplerotic CO_2 fixation will result in SOM $\delta^{13}C$ also tending towards -10‰ in more highly processed SOM deeper in the soil.

The consequences of decomposition and the linked anaplerotic activity for soil $\delta^{13}C$ values may be erroneously interpreted as evidence for C_4 vegetation being invaded by C_3 vegetation, potentially leading to incorrect conservation decisions. We argue that

$\delta^{13}C$ should only be used as a proxy for vegetation change where decomposition rates and anaplerotic CO_2 fixation are low and/or their effect on soil $\delta^{13}C$ values can be accounted for.

Acknowledgments

Firstly, I would like to thank my supervisor Prof Mike Cramer for his ongoing support and the encouragement he brought to the project, even when things seemed dire. Thank you for always being willing to debate and explore concepts and ideas and for sharing your wisdom and experience with me, I have learnt a lot.

For assistance with data collection I would like to thank Edmund February and Emma Grey for providing me soil data that proved to be extremely useful in my thesis.

I would like to thank the technical staff in the H.W. Pearson building, Des Barnes, Dawood Hattas and Edward Chirwa for their assistance in protocols and experimental guidance and for providing assistance with technical analyses. I would also like to thank Ian Newton from Archeometry for the isotope analysis and for the ongoing assistance with the project.

This project would not have been possible without the financial support from the National Research Foundation (NRF).

Many thanks to my peers for all their interest and discussion with regards to my thesis and for providing encouragement when it was needed. Thank you to the 112 crew Joel, Ed and Scoen for your ongoing support and willingness to discuss and understand my project as it progressed.

Finally, thank you to my family, Ilze, Dries, Roelf, Charne and my girlfriend Grace for all your ongoing support both emotionally and financially over the years. It has been a long journey to get here and all of you made it possible.

Table of Contents

Chapter 1

General Introduction

The pitfalls of using soil $\delta^{13}C$ as an indicator for C_3–C_4 vegetation alternate states.

Understanding the past is often key to understanding the future. By reconstructing historical vegetation assemblages we are able to illustrate past plant diversity and the stability, or lack thereof, of ecosystems (Van Der Hammen & Hooghiemstra 2000; West et al. 2000). Furthermore, due to the strong association between vegetation and climate (temperature, precipitation), the documentation of past vegetation changes allows us to recreate climate variability in the past (Davis & Shaw 2001; Prentice 1986). This is essential for understanding the response of these ecosystems to changing climates, CO_2 and land usages as well as informing conservation strategies and decisions (Thuiller et al. 2005; Engler et al. 2011; Whitlock et al. 2003). In particular, increasing global CO_2 concentrations are thought to increase competitive abilities of C_3 woody vegetation over C_4 grasses (Bond & Midgley 2000), and thus atmospheric CO_2 has been interpreted as being responsible for a shift of savanna ecosystems to a more forested state in which woody cover increases over time (Archer et al. 2001). This has prompted the suggestion that savannas should be conserved by removal of woody encroachment (Gillson 2015a).

There are a number of techniques used to determine historical vegetation changes including historical land-use maps (Flinn & Vellend 2005), repeat photography (Zier & Baker 2006), pollen analysis (Haberle & Maslin 1999; Coombes et al. 2009) and dendroecological analysis (Cowell 1998; Splechtna et al. 2005). These methods provide different time-scales of analysis; repeat photography and dendroecology are powerful tools in determining tree density and community composition in the short

term (± 50 years, Fensham & Fairfax 2002; Splechtna et al. 2005), while pollen analysis can provide reconstruction of paleo-vegetation at the taxonomical level over much greater time periods (Haberle & Maslin 1999; Coombes et al. 2009). The use of palynology requires sampling of lake sediments which integrate pollen from over a variable, but usually extensive area thus representing community composition (Prentice 1986). The identification of pollen in sediment cores is laborious and requires expertise, commonly resulting in few replicate samples, especially in more arid ecosystems (Birks & Birks 2000; Zech et al. 2011). Other measures or proxies for vegetation change are thus desperately required.

Soil profile $\delta^{13}C$ analysis is now being used more commonly to provide a proxy for changes between C_3 and C_4 vegetation due to their ease of use and flexibility (Krull & Bray 2005; Pessenda et al. 1996). This technique has the advantage that the vegetation signal is extremely localized and that the accompanying radiocarbon soil age provides a time axis for changes in $\delta^{13}C$ associated with vegetation shifts. Natural abundance isotope analysis has been used to document transitions between C_3 forests and C_4 grasslands or increases in woody cover, at an intermediate temporal resolution of hundreds to thousands of years (e.g. Sanaiotti et al. 2002; Gillson et al. 2004). This is because C_3 and C_4 plants fractionate carbon isotopes differently during photosynthesis, and this imprints the plants with a carbon isotopic composition that is characteristic of the photosynthetic pathway. C_3 plants have $\delta^{13}C$ values between -22‰ and -32‰ (Troughton 1979) while C_4 plants have values between -9.2‰ and -19.3‰ (Hattersley 1982), with variation in $\delta^{13}C$ within the C_3 and C_4 groupings being caused by climatic and geographical factors (Damesin et al. 1997). The use of $\delta^{13}C$ as a proxy for vegetation change relies on the process through which the imprinted characteristics of the photosynthetic pathway (i.e. the $\delta^{13}C$ values) are transferred from the plant into the SOM through litter fall, rhizodeposition and vegetation turn-over. This imprints the soil C with the characteristic vegetation type of the area, and as soil formation processes continue, the original signal is preserved at depth.

This has driven a number of studies in which $\delta^{13}C$ values at depth or in ancient soils have been associated with historical plant communities existing during soil formation and in order to determine whether C_3 or C_4 vegetation types were common in the area historically. For example, West et al. (2000) investigated $\delta^{13}C$ values of SOM in the forests of the Hluhluwe-Umfolozi Game Reserve in South Africa and found that $\delta^{13}C$ values increased with depth (from 24.1–22.7‰ to 19.1–14.8‰) under the current forest, and concluded that the area now covered by tall mature C_3 trees was once a C_4 grassland. Similarly, Gillson (2015a) completed a more detailed study of the same area and found, without exception, that the current forest, thicket and densely wooded savanna were all previously C_4 grasslands, although there was no availability of other proxies (e.g. pollen analysis) as an independent check. Other studies in Brazil have included buried charcoal fragments for ^{14}C dating and pollen analysis of lake sediments in order to provide better resolution of the timing of ancient savanna-forest transitions, and found agreement in timing and species compositions relating to $\delta^{13}C$ values at different depths (Pessenda et al. 2004).

Evidence for the use of $\delta^{13}C$ as a proxy for vegetation change

There is compelling evidence for the use of $\delta^{13}C$ in documenting historical shifts between C_3 and C_4 plant communities. A number of investigations in which land use changes involving shifts between C_3 and C_4 plant communities due to deforestation and the planting of crops have provided strong evidence for use of $\delta^{13}C$ in documenting vegetation change. For example, a study in Hawaii on changes in soil C following cultivation of C_4 sugarcane on previous wildland C_3 forest found that soil $\delta^{13}C$ ranged from -19.9 to -25.4‰ from the surface to 1 m depth, while soil in which C_3 *Eucalyptus* spp. was cultivated on previous wildland forest ranged from -23.2 to -25.0‰ (Bashkin & Binkley 1998). A study in the Amazon on the effects of deforestation and pasture on SOM found that $\delta^{13}C$ values of soils under 10 year old C_4 pasture ranged from -19.1‰ at the surface to -26.1‰ at a depth of 40 cm, while corresponding C_3 forest profiles ranged from -28.5 to -22.7‰ (Desjardins et al. 1994). Furthermore, prehistoric forest clearing and crop cultivation was detected in Costa

Rica using $\delta^{13}C$ values through profiles of sediment cores which demonstrated close correspondence with pollen and charcoal fragments throughout the soil profile (Lane et al. 2004). There is thus evidence that verifiable changes in vegetation may be associated with changes in soil $\delta^{13}C$ values.

The role of decomposition in determining soil $\delta^{13}C$

It is noteworthy, however, that there are startling similarities in the patterns and values of $\delta^{13}C$ through soil profiles globally. These may have important implications for both the utility and accuracy of the method. Most soils tend to have more positive $\delta^{13}C$ values at greater depths than at shallower depths (Fig. 1.1). This inherent enrichment with depth has been well studied, and is commonly attributed to microbial decomposition of organic matter (Ågren et al. 1996; Fernandez et al. 2003). During decomposition, kinetic fractionation of ^{13}C relative to ^{12}C in an open system is thought to result in increased concentration of ^{13}C in the residual OM conserved in the soil. This kinetic fractionation has been described using the Rayleigh distillation equation in which decomposition rates of species comprising two isotopes vary and are proportional to the quantity of each species comprising the two isotopes (Krull et al. 2002). Throughout kinetic isotope fractionation by Rayleigh distillation, the reaction products at any moment are in isotopic equilibrium with the diminishing reservoir. Such an application necessitates the assumption that C isotopes are homogenously dispersed within the pool and that all constituents of soil organic carbon (SOC) decompose and donate to soil-respired CO_2 at the same rate with depth. This allows the Rayleigh calculation to be related to decomposition of an open system of SOC, from which soil-respired CO_2 diffuses to the bulk atmosphere (Ehleringer et al. 2000). By summarizing studies on modern soil from around the world under stable C_3 vegetation, Wynn et al. (2007) argued that soils exhibiting ^{13}C-enrichment follow this Rayleigh distillation process in which the ratio of ^{13}C to ^{12}C is a function of the fraction of remaining soil organic carbon, in turn driven by kinetic fractionation by decomposers. This leaves SOC ^{13}C-enriched with $\delta^{13}C$ values up to ca.

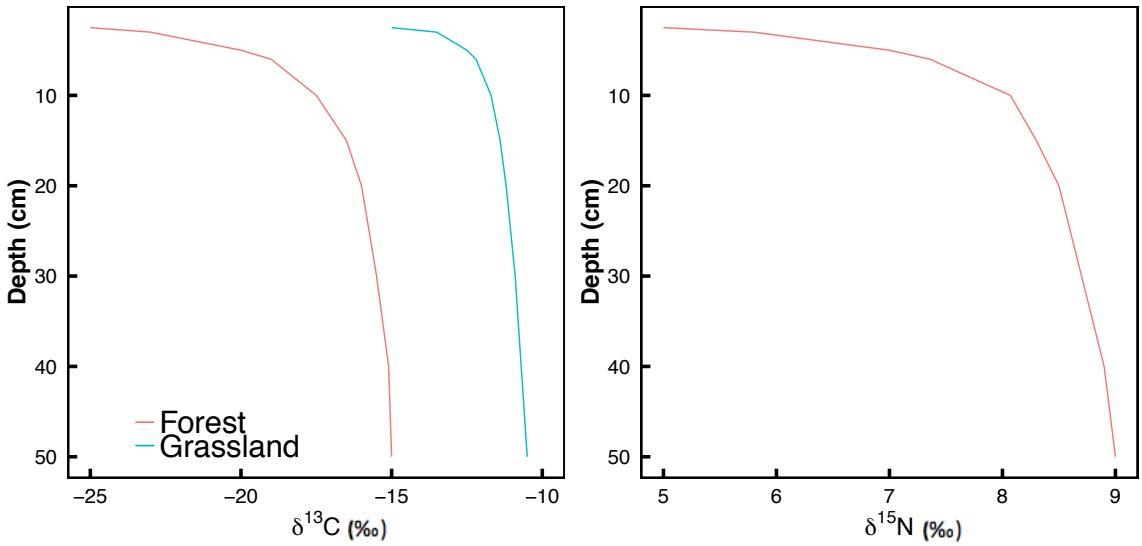


Figure 1.1: Typical soil $\delta^{13}C$ and $\delta^{15}N$ trends with soil depth. Forest represents C_3 vegetation and Grasslands represent C_4 vegetation. Note $\delta^{13}C$ and $\delta^{15}N$ x-axes are on different scales. $\delta^{13}C$ data taken from Gilson et al. (2015) and $\delta^{15}N$ data taken from February et al. (2010).

6‰ higher than the original biomass. Often the ranges of $\delta^{13}C$ values with depth are large and may overlap with the range of values commonly associated with vegetation change. For example, soil $\delta^{13}C$ values less than -20‰ have been used to indicate C_4 vegetation and values larger than -16% to indicate C_4 dominance (Gillson 2015b). In this example, an in increase in $\delta^{13}C$ of 6‰ would falsely class C_4 vegetation as C_3 while mixed stands would be very difficult to interpret. This means that interpretation of soil $\delta^{13}C$ values as indicators of historical vegetation assemblages is complicated by the role of decomposition of SOM in determining soil $\delta^{13}C$ values. Indeed, high rates of SOM decomposition would cause soil profiles to become more strongly enriched in ^{13}C with depth, compared to the surface soils. Sites with a higher vegetative turn-over rate, such as forest communities in Kenya and Mississippi, tend to have larger differences in soil $\delta^{13}C$ values at the surface relative to at depth (Krull et al. 2002; Wynn et al. 2006). Further, a study in Australia found that faster turnover of SOC occurs in tropical grassland soils relative to temperate grassland soil due to a greater amount of microbial biomass in the SOC of tropical grassland (Šantrůčková et al. 2000). This implies that for sites that have inherently high rates of decomposition, higher degrees of fractionation should be expected.

Soil organic carbon tends to become more decomposed with depth due to the general pattern of increasing age of deeply buried carbon. The age of soil C, however, often shows correspondence with clay content and aggregation which suggests that physical protection of SOC may play a more important role than decomposition (Trumbore 2000). Nevertheless, many studies have verified a cumulative deviation between $\delta^{13}C$ values of respired CO_2 and bulk SOC with age and depth (Wynn et al. 2005a; Mary et al. 1992; Šantrůčková et al. 2000). As an actively decomposing pool of SOC is made up of a mixture of new biomass and microbially processed SOC, the departure of $\delta^{13}C$-SOC from that of respired $\delta^{13}C$-CO_2 represents the amount of processing done by microbes as they favorably leave ^{12}C-depleted products in the SOC pool (Balesdent et al. 1993). This deviation amongst $\delta^{13}C$ of respired CO_2 and SOC, however, is not always found and some studies have shown little or no deviation between the two (Ekblad & Högberg 2000). While not all soils exhibit this deviation, a study using mixed C_3/C_4 grassland soils found that microbial biomass was ^{13}C-enriched by ca. 2‰, while the respired CO_2 was ^{13}C-depleted by roughly the same amount, resulting in respired CO_2 having a similarly depleted isotopic composition to SOC (Šantrůčková et al. 2000). They argued that this correspondence between $\delta^{13}C$ values of respired CO_2 and bulk surface SOC potentially covers the effect of microbial respiration. They also found that $\delta^{13}C$ values of respired CO_2 differed after various durations of incubation time, with early stages of incubation characterized by higher microbial activity (i.e. growth) and thus concluded that the degree of isotopic discrimination during metabolism is dependent on the growth stage of the microbial population.

Because this decomposition process is ongoing, ^{13}C-enrichment in decomposing organic matter changes the C isotopic composition of SOC during its different phases of decay and throughout the soil profile. Thus, ^{13}C fractionation through decomposition has important consequences for the understanding of historical vegetation changes based on soil $\delta^{13}C$, especially when SOC has experienced substantial decomposition since burial. Further, C_3 and C_4 transitions are influenced

differently by decomposition, because C_4-derived SOC may decompose more rapidly than C_3-derived SOC (Henn & Chapela 2000). This leads to the marginalization of C_4-derived SOC in the SOC pool. In order to compensate for the natural tendency of decomposition to enrich $\delta^{13}C$ values of soils and to ensure that interpretations of vegetation shifts from paleoecological studies using soil $\delta^{13}C$ values are accurate, the effects of this decomposition need to be accounted for. This can be achieved by demonstrating that the measured concentration of SOC is near its greatest concentration throughout soil formation and that decompositions has therefore had limited effects. Further, it also needs to be shown that the SOM does not include a significant proportion of ^{13}C-enriched products of microbial decomposition. The problem, however, is that SOC is often conserved in amounts representing a small portion of the original SOC as a consequence of burial mineralization, even in young paleosols (Valentine & Dalrymple 1976). Furthermore, due to generally low concentrations, SOC can be difficult to analyze without using treatments to remove mineral soil. This is a problem in itself as studies investigating both coarse and fine-textured soils discover that fine mineral particles play a noteworthy part in stabilizing ^{13}C-enriched products as a result of microbial decomposition in SOM, leaving the SOC pool progressively ^{13}C-enriched in soils hat have fine-textured particles (Bird et al. 2002). Although formation of carbonates in soil could effectively fix soil $\delta^{13}C$ values by trapping C in indurate structures, soil carbonates are also usually only formed extensively in aridland soils.

Linking soil $\delta^{15}N$ and $\delta^{13}C$

Like $\delta^{13}C$, there is the tendency for $\delta^{15}N$ to become enriched with depth. Dual isotope studies including both $\delta^{15}N$ and $\delta^{13}C$ reveal that the two ratios follow remarkably similar trajectories in space and time (Fig. 1.1; Hicks Pries et al. 2011; Ometto et al. 2006; Zeller et al. 2007; Boström et al. 2007). While a number of mechanisms are able to fractionate N isotopes, mineralization (decomposition) of organic matter by soil microbes is commonly considered the most important driver (Hobbie & Ouimette 2009). For example, Novák et al. (2003) found that with an increasing depth (0 to 20

cm below surface), $\delta^{13}C$ and $\delta^{15}N$ values of bulk soil increased on average by 0.9 and 4.2‰, respectively, in five European forest ecosystems reflecting an accumulative degree of mineralization of organic matter. Further, global soil $\delta^{13}C$ and $\delta^{15}N$ value ranges from 16 published sites also showed enrichment of both $\delta^{13}C$ and $\delta^{15}N$ as depth increased (Table 1.1). Values from soil surface compared to values at depth ranged between 0.6 to 11.01‰ for $\delta^{13}C$ and 1.2 to 6.48‰ for $\delta^{15}N$. Due to the relatively common processes involved in $\delta^{13}C$ and $\delta^{15}N$ fractionation in soil (i.e. microbial decomposition), this relationship of common N and C isotopic enrichment with depth emphasizes the need to account for decomposition processes over time when using $\delta^{13}C$ as a proxy for C_3/C_4 vegetation transitions, and provides an additional mechanism for doing so.

Vegetation turnover versus pedogenic rates

Many factors influence how long topsoil takes to form, however, it usually takes at least 100 years and frequently over 500 years. Parent material, topography, climate, organisms and time (Huggett 1998; Minasny et al. 2008) are all factors that influence soil formation. Changes in vegetation composition, however, can occur in a matter of years if conditions of increased disturbance and ecological pressure are experienced (Burrows et al. 2002; Sharp & Whittaker 2003) and even in the long term, transitions generally occur over much shorter time periods than soil formation. For example, Six et al. (2002) used $\delta^{13}C$ to estimated mean residence times of C within the upper 30 cm of the soil profile to be 80 years in fields with no-tillage, while with conventional tillage the residence time was 52 years. Further, at the global scale the mean turnover time of topsoil carbon was found to be extremely similar among croplands, grasslands and forests (Chen et al. 2010). Since pedogenesis takes significantly longer to occur than vegetation turnover, a change in dominant vegetation type from C_3 to C_4, as invoked in many studies, may be expected to be represented in the soil as a step-change of $\delta^{13}C$ values from C_3 to C_4 dominated values, although this is likely to be ameliorated by bioturbation and other mixing of soil. Soil profile values of $\delta^{13}C$ do not typically exhibit this expected step-wise pattern and rather tend to become enriched

in a more-or-less continuous manner (Bashkin & Binkley 1998; Nair et al. 2007). Bioturbation of soil layers by soil fauna causes mixing of $\delta^{13}C$ and $\delta^{15}N$ signals throughout the soil profile (Shuster et al. 2001) that might eliminate the expected step-wise pattern. Further, carbon can be advected down soil profiles such that younger C mixes with older C at depth (Kaiser & Kalbitz 2012). For example, a study on forest and grassland soil in California calculated advective transport velocities of dissolved organic carbon (DOC) and found that forest sites had velocities of 1.05 mm per year while grasslands had velocities of 0.45 mm per year (Sanderman & Amundson 2009). Over hundreds of years, C is thus transported deep into the soil profile. Additionally, plant roots constantly contribute organic matter to the soil. The contribution of root derived C into the total organic carbon (TOC) pool in the soil is large and in some cases it can be higher than the contribution of above ground residue-derived C (Kätterer et al. 2011; Kaiser & Kalbitz 2012). This is important as roots tend to be enriched in ^{13}C compared to shoots (Eissfeller et al. 2013) as well as respiring ^{13}C-depleted CO_2 (Cheng 1996), which means that they could contribute significantly to the enrichment of below-ground $\delta^{13}C$ values. Thus the variations in soil $\delta^{13}C$ and $\delta^{15}N$ with depth are the complex product of multiple contributing factors that vary within and between ecosystems. Nevertheless, the global trend of relatively monotonically increasing $\delta^{13}C$ and $\delta^{15}N$ with depth indicates that these other contributing factors are perhaps less important than microbial decomposition as the shared driver of both $\delta^{13}C$ and $\delta^{15}N$ variation with depth.

Anaplerotic CO_2 fixation by roots and soil microorganisms.

Typically, soil $\delta^{13}C$ values are strongly influenced by decomposition mediated by microbes. Firstly, kinetic discrimination against ^{13}C during respiration may result from microorganisms favorably respiring CO_2 that is ^{13}C-depleted compared to the substrate, resulting in ^{13}C enrichment of the remaining SOC (Ågren et al. 1996). Secondly, slow decomposition of resistant SOC components could lead to increased

Table 1.1: List of sites used in determining the correlation between soil $\delta^{13}C$ and $\delta^{15}N$ values through soil profiles. Variables included are mean annual temperature (MAT), mean annual precipitation (MAP), $\delta^{15}N$, $\delta^{13}C$, the dominant vegetation at the site, the Pearson correlation coefficients between $\delta^{13}C$ and $\delta^{15}N$ through soil profiles with significance values (bold where significant, $p < 0.05$). Values for $\delta^{13}C$ and $\delta^{15}N$ are include the 5 percentiles, (means) and 95 percentiles of the soil profile data.

Site	MAT (°C)	MAP (mm)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	C_3/C_4 dominant	Pearson correlation	p-value	Reference
Alaska	-4.1	405	-1.9 (0.1) 1.1	-27.0 (-24.4) -25.3	C3	0.98	**0.003**	Pries *et. al.* 2011
Amazon	24.1	2134	8.2 (9.8) 11.1	-27.6 (-26.7) -25.9	C3	0.95	**0.000**	Ometto *et. al.* 2006
Kruger - Satara OC	22.3	565	3.2 (6.1) 7.4	-13.7 (-12.3) -11.6	C4	0.94	**0.000**	February *et. al.* 2010
Kruger - Satara UC	22.3	565	4.1 (6.0) 7.0	-15.0 (-13.0) -12.0	C4	0.92	**0.000**	February *et. al.* 2010
Kruger - Pretoriuskop UC	21.0	734	2.0 (5.3) 6.9	-20.8 (-16.6) -14.5	C4	0.87	**0.000**	February *et. al.* 2010
France - Natural	9.0	1280	1.7 (3.5) 4.7	-28.4 (-27.8) -27.2	C3	0.85	**0.071**	Zeller *et. al.* 2007
Hluhluwe - Thicket	21.2	892	5.3 (6.5) 7.6	-18.0 (-16.4) -15.0	C4	0.72	**0.000**	Grey 2012
Sweden	5.8	617	2.5 (5.8) 7.5	-27.8 (-27.4) -26.8	C3	0.68	0.136	Bostrom *et. al.* 2007
Hluhluwe - Forest	21.2	892	6.8 (7.3) 7.9	-20.0 (-17.4) -15.5	C4	0.61	**0.000**	Grey 2012
Hluhluwe - Savanna	21.2	892	5.6 (6.5) 7.2	-15.0 (-13.8) -12.3	C4	0.58	**0.000**	Grey 2012
Kruger - Pretoriuskop OC	21.0	734	2.2 (5.1) 6.4	-17.6 (-14.8) -13.3	C4	0.50	**0.000**	February *et. al.* 2010
Hluhluwe - Grassland	21.2	892	7.4 (8.1) 9.0	-15.5 (-13.9) -12.7	C4	0.49	**0.001**	Grey 2012
New south wales - Grove	17.6	259	8.9 (10.0) 10.6	-22.1 (-18.8) -13.4	C4	0.46	0.297	Macdonald *et. al.* 2015
France - Plantation	9.0	1280	1.0 (3.2) 5.2	-27.2 (-26.9) -26.7	C3	0.29	0.641	Zeller *et. al.* 2007
New south wales - Inter grove	17.6	259	8.5 (9.1) 9.5	-22.8 (-18.3) -13.2	C4	0.25	0.586	Macdonald *et. al.* 2015
Karoo - Paulshoek	18.9	118	8.6 (9.2) 9.6	-21.2 (-21.0) -20.7	C3	-0.40	**0.007**	Edmund February unpublished

$\delta^{13}C$ values associated with these components as soil depth increases over time (Tu & Dawson 2005). Thirdly, microorganisms are ^{13}C-enriched by 2 to 4‰ compared to plant material (Hobbie et al. 1999) and thus influence SOM, resulting in decreasing C:N ratios with soil depth (Wallander et al. 2003), and compound-specific changes in SOM to higher $\delta^{13}C$ values in products which originate from microbes (Huang et al. 1996; Ehleringer et al. 2000). Fourthly, variable mobility (e.g. fulvic acids; Heil et al. 2000) and sorption of isotopes of dissolved organic C on soil particulates (especially clay) may contribute to soil $\delta^{13}C$ values (Craine et al. 2015).

While these processes have been shown to influence soil $\delta^{13}C$ values to varying degrees, plant roots (Cramer et al. 1993) and soil microorganisms (Miltner et al. 2004a) have also been shown to exhibit dark (i.e. non-photosynthetic) incorporation of CO_2. This is because both soil microorganisms and roots contain carboxylating enzymes, including phosphoenolpyruvate carboxylase (PEPc), which are able to incorporate dissolved inorganic carbon (DIC) into organic acids and subsequently amino acids (Ikeda et al. 1992). While PEPc shows little evidence for fractionation of CO_2, there is a direct fractionation effect of up to 9‰ against $^{12}CO_2$ during hydration by carbonic anhydrase (CA) involved in the formation of osmoregulants, however, CA has a very high affinity to DOC resulting in most if not all of the available DOC being used, leading to a net fractionation close to zero (Marin-Spiotta et al. 2014; Steiner et al. 1975; Wingate 2008). This carbon is channeled through so called "anaplerotic reactions" which are used to replenish the citric acid cycle (TCA cycle) intermediates when there is a shortage of succinyl-CoA or 2-oxoglutarate due to processes such as amino acid synthesis (Miltner et al. 2005) or organic acid exudation (Cramer et al. 1993). Inorganic C incorporated through anaplerotic reactions in microbes commonly supplies more than ca. 10% of cell carbon (Perez & Matin 1982). The importance of anaplerotic C-fixation for soil $\delta^{13}C$ values is that the inorganic C in the soil atmosphere derived from respired organic sources becomes ^{13}C-enriched by inclusion of CO_2 diffusing from the bulk atmosphere.

In the edaphic environment, the CO_2 in the soil atmosphere is a mixture of both ambient atmospheric CO_2 and ^{13}C-enriched CO_2 originating from soil and root respiration (Seuss effect; Flessa et al. 2000). The soil CO_2 therefore carries an isotopic

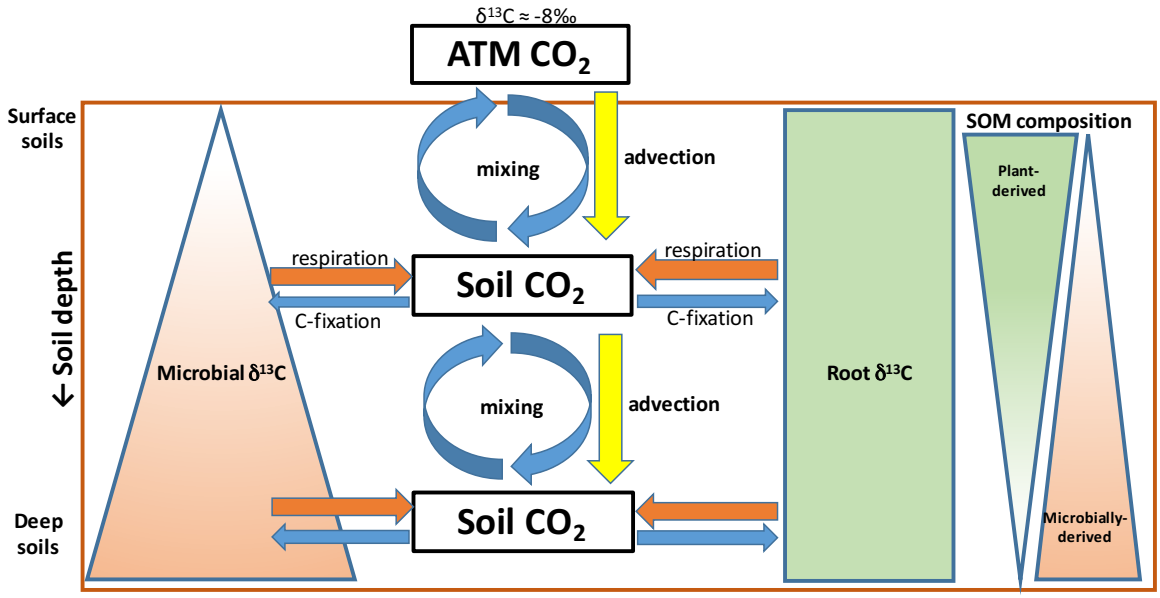


Figure 1.2: Schematic illustration of processes driving soil $\delta^{13}C$ values. In shallow soils, CO_2 from the atmosphere enters the soil through diffusion and advection where it subsequently mixes with the soil CO_2 reservoir, leading to soil CO_2 $\delta^{13}C$ values representing a mixture of both reservoirs. Soil CO_2, which is depleted of ^{12}C relative to atmospheric CO_2, is derived from both microbial and root respiration of primary production (whether C_3 or C_4). Non-fractionating anaplerotic CO_2 fixation occurs concurrently with respiration, resulting in SOM accumulating CO_2 partially derived from the atmosphere (i.e. with higher $\delta^{13}C$ values than soil derived CO_2). As CO_2 diffuses and is advected deeper in soils it mixes with CO_2 derived from deeper SOM pools, but atmospheric derived CO_2 has less influence deeper in the soil due to limits on diffusion. Thus deeper soil CO_2 $\delta^{13}C$ values are more strongly influenced by microbial and root respiratory CO_2 $\delta^{13}C$ values than by atmospheric CO_2 $\delta^{13}C$ values. Because root C is mostly derived from photosynthesis root tissue $\delta^{13}C$ values do not change strongly with depth, even when CO_2 from the soil is fixed anaplerotically (Ehleringer et al. 2000). Anaplerotic CO_2 fixation contributes a large proportion of microbial C (Miltner et al. 2004) resulting in microbial $\delta^{13}C$ values becoming more positive over time due to incorporation of atmospheric CO_2, resulting in higher $\delta^{13}C$ values of SOM. The relative contribution of microbes and vegetation to the soil SOM pool changes with depth such that shallow soils represent more plant-derived material while deeper soils are characterized by highly processed SOM of microbial origin (indicated by triangles). The same colours indicate common processes.

signature which is dependent on the mixing ratios between these two CO_2 pools (Fig. 1.2), but the soil CO_2 $\delta^{13}C$ values will always be intermediate between respired CO_2 and atmospheric CO_2 $\delta^{13}C$ values. Dark re-fixation of this pool of CO_2 by roots and microbes would lead to a contribution of ^{13}C-enriched products to the formation of SOM (Gessler et al. 2007) and increased soil bulk $\delta^{13}C$ values. This non-discriminating dark fixation would also offset respiratory release of CO_2-$\delta^{13}C$ values, consistent with the evidence showing that respiration results in preferential release of more negative $\delta^{13}C$-CO_2 (Ekblad & Högberg 2000; Breecker et al. 2014). As mixing between soil and atmospheric CO_2 plays a large role in determining the soil $^{13}CO_2$ value it would be expected that soil in which mixing is minimal, such as clay-rich soils, would show a higher enrichment of $\delta^{13}C$ in SOM at depth, consistent with the fact that SOC is increasingly ^{13}C-enriched in soils with fine-textured particles compared to course textured soils (Wynn et al. 2005b). Because of the potential for anaplerotic carbon fixation to alter SOM-$\delta^{13}C$ values, studies in which historical vegetation change from C_4 to C_3 communities is inferred using soil $\delta^{13}C$ would have to consider the effect of mixing between soil CO_2 and atmospheric CO_2. Soils in which gaseous mixing is less limited, for example by soil texture or water content, would likely show substantial ^{13}C-enrichment of SOM at depth compared to the shallower soils. This could be mistaken for evidence of a historical shift in the plant community (Balesdent and Mariotti, 1996).

Conservation implications

The problems considered above highlight the need for caution when relying on soil $\delta^{13}C$ values as a proxy for historical C_4-C_3 transitions. This is exacerbated by the consequences that studies using the technique have on conservation decisions, such as managing bush encroachment (Gillson 2015a). There are a number of caveats that should be considered to ensure that the method is reliable. Sites with inherently high decomposition rates and/or high dark CO_2 fixation rates may be problematic because these sites carry the combined influence of microbial and vegetation processes on soil $\delta^{13}C$ values. Respiration combined with anaplerotic activity results in fractionation of

soil C and the positive drift in $\delta^{13}C$ values with depth, consequently potentially masking the effect of vegetation on soil $\delta^{13}C$ values. Further, the degree of influence that decomposition combined with anaplerotic activity has on SOM at a site requires additional analyses. By analyzing $\delta^{15}N$ and its trend with soil depth, however, one can determine the relative activity of decomposing organisms because $\delta^{15}N$ serves as an independent measure of decomposition. This means that sites which show significant ^{15}N-enrichment with depth, changes in $\delta^{13}C$ are likely driven by decomposition more so than sites which exhibit little fractionation of $\delta^{15}N$. Thus, using the fact that soil $\delta^{13}C$ and $\delta^{15}N$ values through soil profiles are commonly highly correlated (Table 1.1), one can determine whether the site has the potential for quantitative problems when using $\delta^{13}C$ as a proxy for vegetation change. We suggest that the residual of the correlation between $\delta^{13}C$ and $\delta^{15}N$ may be useful for detecting vegetation changes.

Chapter 2

Correspondence between $\delta^{13}C$ and $\delta^{15}N$ in soils suggests coordinated fractionation processes for soil C and N.

Abstract

Although a number of different factors influence C and N isotopic fractionation of organic matter, the $\delta^{13}C$ and $\delta^{15}N$ values of soil organic matter both tend to increase with soil depth, following similar trajectories. This similarity has not been investigated at the global scale. As microbial decomposition increases organic matter $\delta^{13}C$ and $\delta^{15}N$ values, soil isotopic values are hypothesized to generally increase with depth across local and global scales. Soil $\delta^{13}C$ and $\delta^{15}N$ values for 16 soil depth-profile sites were used for local-scale investigation, and 5447 global single-depth sites were used for global-scale investigation of the correspondence between $\delta^{13}C$ and $\delta^{15}N$. Correlations between soil $\delta^{13}C$ and $\delta^{15}N$ with depth as well as soil $\delta^{15}N$ at the global scale were evaluated using correlative and boosted regression tree analyses in order to determine the main drivers. Strong positive correlations between $\delta^{13}C$ and $\delta^{15}N$ values through soil profiles were found at a number of sites and were found to be independent of vegetation type. Globally, soil $\delta^{13}C$ and $\delta^{15}N$ values were also found to be significantly positively correlated across a wide range of climates and biomes. The global correspondences between $\delta^{13}C$ and $\delta^{15}N$ values may suggest a mechanistic link between $\delta^{13}C$ and $\delta^{15}N$ through the process of SOM decomposition and microbial processing and highlight the importance of soil-related processes in determining isotopic signals in soils. The variability in these soil processes should be considered when interpreting soil isotopic values of $\delta^{13}C$ and $\delta^{15}N$ as indicators of ecosystem sources of soil C and N and inferring vegetation inputs.

Introduction

Soil $\delta^{15}N$ values tend to decrease with increasing mean annual precipitation (MAP) and decreasing mean annual temperature (MAT) across a broad range of climate and ecosystem types (Amundson et al. 2003). To some extent, this variation in soil $\delta^{15}N$ values is associated with vegetation inputs, given that foliar $\delta^{15}N$ values range over 35‰ across plants globally (Craine et al. 2009). Soil $\delta^{15}N$ values, however, increase with decreasing soil organic C as global soil organic C concentrations also decline with increasing MAT and decreasing MAP (Craine et al. 2015b). As a consequence, the dependence of soil $\delta^{15}N$ on MAP and MAT has been ascribed to this association of soil C with environmental variables. Furthermore, soils with greater clay concentrations often have higher soil $\delta^{15}N$ values, however, the dependence of soil $\delta^{15}N$ on soil C and clay is through fractionation associated with decomposition of soil organic matter, further linking it with environmental variables (i.e. SOM; Craine et al. 2015b).

Like soil $\delta^{15}N$, global patterns of soil $\delta^{13}C$ values are correlated with MAP and MAT (Lu et al. 2004), but also with soil texture (Sollins et al. 2009). The largest influence on soil $\delta^{13}C$ values, however, is the $\delta^{13}C$ value of the input of C to the soil organic carbon (SOC) pool, which is either directly or indirectly derived from primary productivity (Kuzyakov and Domanski 2000). As a consequence, soil $\delta^{13}C$ has been used as a proxy for historical vegetation shifts in the distribution of C_3 and C_4 vegetation (Swap et al. 2004; Gillson et al. 2004; Kuzyakov et al. 2006; Gillson 2015a). Despite these clear geographic differences, changes in soil $\delta^{13}C$ with depth do not necessarily reflect historic changes in the relative inputs of C_3 and C_4 vegetation. Turnover processes during soil development also contribute to changes in soil $\delta^{13}C$ (Cerling 1984; Balesdent et al. 1993; Qiao et al. 2014) with more decomposed SOC having higher $\delta^{13}C$ values (Boström et al. 2007). Thus both soil $\delta^{15}N$ and $\delta^{13}C$ values are, at least partially, determined by soil processes (i.e. decomposition and mineralization via microbial processing of OM), which may link the patterns of fractionation of these isotopes in the soil. If soil N and C isotope patterns are at least partially linked through common soil processes (i.e. decomposition and mineralization), then we may expect

coordinated changes in $\delta^{15}N$ and $\delta^{13}C$ values with depth through a soil profile. The complexities of C and N fractionation need to be considered, however, and are discussed further below.

The $\delta^{13}C$ values of SOM through soil profiles commonly increase by 1–3‰ as depth increases below 0.2 m relative to that of the surface litter layer (Chen et al. 2005; Boström et al. 2007). The enrichment of ^{13}C with depth has been shown to occur in tropical, temperate and boreal systems (Hobbie and Ouimette 2009). Although atmospheric $\delta^{13}CO_2$ has declined by 1.5‰ over the past 100 years, this has been shown to contribute only marginally to the enrichment of soil $\delta^{13}C$ with depth (Ehleringer et. al. 2000; Esmeijer-Liu et. al. 2012). At least four hypotheses have been suggested for C isotope fractionation through soil profiles. Firstly, kinetic discrimination against ^{13}C during respiration may result from microorganisms favorably respiring CO_2 that is ^{13}C-depleted compared to the SOM, resulting in ^{13}C-enrichment of the remaining SOC (Ågren et al. 1996). Although a number of studies show sizeable ^{13}C-depletion of the CO_2 formed (e.g. Fernandez et al. 2003), others show none or only slight fractionation (e.g. Ekblad and Högberg 2000). Secondly, microorganisms are ^{13}C-enriched by 2 to 4‰ compared to plant material (Hobbie et al. 1999) and thus influence SOM, resulting in decreasing C:N ratios with soil depth (Wallander et al. 2003), and compound-specific changes in SOM to higher $\delta^{13}C$ values more representative of microbial products (Huang et al. 1996; Ehleringer et al. 2000). Thirdly, variable mobility (e.g. fulvic acids; Heil et al. 2000) and sorption of isotopes of dissolved organic C on soil particulates (especially clay) may contribute to soil $\delta^{13}C$ profiles (Craine et al. 2015b), although some authors have questioned the significance of these mechanism (Boström et al. 2007). Finally, although preferential utilization of ^{13}C-depeleted compounds has been suggested (Boström et al. 2007), the more recalcitrant C fractions of plant biomass (e.g. lignin, lipids and cellulose) that accumulate at depth (Rovira et al. 2002) are ^{13}C-depleted relative to the whole plant (Wilson and Grinsted, 1997), and thus cannot contribute to increased ^{13}C-enrichment with depth (Wynn et al. 2006). Apart from this, some, or all, of these processes may

thus contribute to determining soil $\delta^{13}C$ values to variable extents in different ecological contexts.

As with $\delta^{13}C$, $\delta^{15}N$ values usually increases with soil depth, although sometimes maximum $\delta^{15}N$ is apparent at an intermediate depth trailed by a subsequent decrease at greater depths due to increased volatilization (Hobbie and Ouimette 2009). The degree of enrichment that $\delta^{15}N$ undergoes through a soil profile can have a much broader range than $\delta^{13}C$. In arid and semi-arid systems where soil pH is high, surface $\delta^{15}N$ values can be elevated by as much as 7‰ relative to deeper soils (Pataki et al. 2008). There are seven potentially important mechanisms that influence $\delta^{15}N$ values within soil profiles. Firstly, depletion of ^{15}N by mycorrhizal fungi and allocation of that ^{15}N-depleted N to plants (Hobbie et al. 2000) results in the accumulation of ^{15}N-enriched N resulting from mycorrhizal fungi (Hogberg 1997; Hobbie and Ouimette 2009). Secondly, depletion of ^{15}N through enzymatic hydrolysis (Silfer et al. 1992), ammonification, nitrification, or denitrification and the associated fractionation during gaseous loss of ^{14}N and preferential utilization of ^{14}N by plants, drives soil $\delta^{15}N$ values up (Handley and Raven 1992). Thirdly, mixing of soil N among different soil layers through bioturbation (Gabet et al. 2003) and trophic fractionation (i.e. faunal processes; Ponsard and Arditi 2000) could alter soil $\delta^{15}N$ profiles. Fourthly, soil texture (i.e. clay) may moderate ^{14}N gaseous loss pathways and/or the differential retention of ^{15}N-enriched SOM (Craine et al. 2015b). Fifthly, preferential microbial utilization of ^{14}N compounds could contribute to accumulation of ^{15}N-enriched compounds deeper in the soil (Boström et al. 2007). Finally, N deposition has been shown to decrease $\delta^{15}N$ values of soils because deposited N is typically depleted in ^{15}N, although this effect is relatively small (Liu et al. 2017; Esmeijer-Liu et. al. 2012).

SOM decomposition is thus common to both $\delta^{13}C$ and $\delta^{15}N$ fractionation in soil. At the global scale, climate influences decomposition through both temperature and moisture (Gholz et al. 2000). The SOM composition and nutrients concentrations (especially N) also strongly affect decomposition (Parton et al. 2007). Although most

SOM is derived from plants, only a minor portion of the annual litter and root inputs are assimilated into the stable organic matter pool, most of it once recurrent processing by soil microbes (Lerch et al. 2011). SOM transport through soils is generally downward through advection and soil development, and thus the effects of decomposition on soil $\delta^{13}C$ and $\delta^{15}N$ values are more noticeable deeper in the soil profile. With increasing depth, SOM is more highly processed by microbes (Trumbore 2009) with lower C:N ratios (Marin-Spiotta et al. 2014) and increasing $\delta^{13}C$ and $\delta^{15}N$ values (Heil et al. 2000; Billings and Richter 2006). This change in $\delta^{13}C$ and $\delta^{15}N$ is often modelled as "Rayleigh distillation", which predicts soil $\delta^{13}C$ and/or $\delta^{15}N$ values based on the soil [C]/[N] in order to account for microbial isotopic enrichment of SOM during decomposition (Mariotti et al. 1981; Baisden et al. 2002; Wynn et al. 2005; Fischer et al. 2008). This enrichment results from the kinetic fractionation during microbial processing (Dijkstra et al. 2006) with subsequent stabilization of products by fine mineral particles in soils (Wynn et al. 2006). This Rayleigh distillation model, however, only pertains to closed systems, potentially ignoring continuous inputs (Fry et al. 2006) that do occur in soils.

Although a number of different factors influence the isotopic fractionation of C and N isotopes, $\delta^{13}C$ and $\delta^{15}N$ values both increase with soil depth and commonly follow similar trajectories. We hypothesized that changes in soil $\delta^{13}C$ and $\delta^{15}N$ values are coordinated, possibly through decomposition-related processes, and that the scale of decomposition related changes in $\delta^{13}C$ may confound interpretation of soil $\delta^{13}C$ as indicative of prior C_3 or C_4 vegetation. Although the initial isotope composition of the organic matter is indisputably important, subsequent soil fractionation may result in $\delta^{13}C$ and $\delta^{15}N$ following similar trajectories in space and time. We therefore predict that changes in $\delta^{13}C$ and $\delta^{15}N$ values correspond with each other both locally through soil depths at a site and globally due to the extent of decomposition and other soil processing. In order to test these predictions, we compiled data from soil depth profiles from sixteen widely distributed sites and also conducted an analysis of global

$\delta^{13}C$ and $\delta^{15}N$ variations in surface soils in order to determine relationships between soil isotopes with climate and soil properties.

Methods

Data sources

Data for soil $\delta^{13}C$ and $\delta^{15}N$ values were acquired from literature and by contacting individual researchers known to have collected soil isotope data in the past. Soil depth-profile data included $\delta^{13}C$ and $\delta^{15}N$ for mineral soils at multiple depths at a single site. A second independent dataset included both mineral soil $\delta^{13}C$ and $\delta^{15}N$ values at a single depth at a number of geographic locations. For each site, climate data were taken from the original source and also, using the geographic coordinates, from the 50-year climatic means (1950–2000) obtained from www.worldclim.org (accessed Sep 2014) at ca. 1 km^2 resolution. Variables included were mean annual temperature (MAT), mean annual precipitation (MAP) and 17 other derived climatic variables (Supp. Table 2.1).

Potential evaporation (PET) was obtained from Trabucco and Zomer (CGIAR Consortium for Spatial Information, 2009. Accessed: http://www.csi.cgiar.org) in which PET was modelled using the method of Hargreaves et al. (1985) with data from Hijmans et al. (2015) and verified by comparison with separate data sources. From the climatic data, the monthly PET was subtracted from monthly precipitation to obtain an index of water availability (P–PET) and averaged to obtain the annual average. Normalized difference vegetation index (NDVI) data was obtained from eMODIS TERRA (US Geological Survey Earth Resources Observation and Science Center), which is corrected for molecular scattering, ozone absorption and aerosols. The NDVI data spanned between 19/12/2009 to 18/12/2012 and was at a spatial resolution of 250 m. The data was averaged to obtain monthly and annual average values using the "raster" (Hijmans et al. 2015) and "RCurl" (Lang and Lang 2016) packages in R.

The fraction of the vegetation with C_4 photosynthesis was obtained from Berry et al. (2009) in which the percentage of vegetation within each one degree by one degree grid cell of the land surface which possesses the C_4 photosynthetic pathway was determined using 'C_4 climate map' from Collatz et al. (1998), 'Continuous fields of vegetation characteristics' from DeFries et al. (2000) as well as 'Cropland fraction distribution' from Ramankutty and Foley (1998). Where necessary, the component fields were re-sampled to bring them to a common one degree by one-degree spatial resolution.

The "SoilGrids1km" global soil data product (Hengl et al. 2014), which has mean soil information at 1 km resolution for six soil depths to 1.5 m deep (ISRIC – World Soil Information 2013), was averaged across the full depth by depth weighted-averaging. The environmental data included in the models is shown in Supp. Table 2.1.

Soil depth data

Data for 9 sites, which include 4 sites in Africa (Paulshoek, Pretoriuskop, Satara, Hluhluwe) and sites in Alaska, France, Sweden, New South Wales and the Amazon in Brazil were compiled from a number of publications (Table 2.1). These made up a total of 16 different sampling groups within distinct vegetation types and included data for 79 soil profiles at multiple depths. As most sites were represented by repeated sampling of different vegetation types, the average value of the N and C isotopes at each depth for each vegetation type, as well as the confidence intervals, were determined for each site. As the ranges of $\delta^{13}C$ and $\delta^{15}N$ values through soil profiles were different in magnitude, the actual measured values were scaled using the "scale" function in R (z-transformation). This allowed both the N and C isotope patterns through the soil profiles to be plotted on the same set of axes for comparison using the 'ggplot2' package (Wickham 2009) in R. A Pearson correlation test was then performed on the scaled data. This correlation was then treated as a derived variable. As one of the locations, Hluhluwe, consisted of a number of different vegetation

types, each vegetation type at the site was plotted separately rather than averaging across the site.

Global analysis of surface soil

In order to determine the main global correlates of soil $\delta^{15}N$ values, the dataset from Craine et al. (2015b), which included soil and climatic data for sites around the globe, was re-analysed. Records that did not include a depth or mineral soil components were removed leaving a total of 5447 sites for the analysis. As the $\delta^{13}C$ and $\delta^{15}N$ values were from single depths only, the dataset was used to determine the global correlation of soil $\delta^{13}C$ and other variables with $\delta^{15}N$.

Boosted regression tree analyses

Boosted regression tree models were used to determine how differences in soil and environmental conditions influence the correlation between $\delta^{13}C$ and $\delta^{15}N$ values for soil depth-profiles, as well as the main drivers of $\delta^{15}N$ at the global scale. Boosted regression tree analysis is a form of non-linear modelling that uses machine learning (Elith et al. 2008). The modelling entails decision trees splitting the data into two homogenous groups, a process repeated many times (boosting) so as to improve the prediction of the response variable. Models are parameterized by adjusting their learning rates, tree complexity and bag fraction (Elith et al. 2008). We used a cross-validation process to identify the optimal number of trees and tree size for the model, and to guard against over-fitting (Hastie et al., 2001). Initially, the data set was randomly divided into 10 mutually exclusive subsets of equal size, 9 of which were used as a training set to create the boosted tree while the remainder was used as a test set to determine the predictive accuracy of the model. The data in the training sets were fitted using trees of different sizes (range = 2 to 10) by incrementally adding trees in sets of 50. For each combination of tree size and number of trees, the predictive accuracy of the model was determined by comparing values in the test set with those predicted by the model. This procedure was repeated 10 times so that all groups were used as cross-validation groups, and the mean predictive error calculated

across all subsets for each level of complexity. The combination of tree size and tree number that produced the lowest predictive error was chosen for all subsequent analyses. Performance was evaluated by expressing the predictive deviance of 10-fold cross validation as a percentage of the null deviance.

Two different models were used, either to explain the correlation of $\delta^{13}C$ and $\delta^{15}N$ values at the local scale across soil depths (BRTlocal), or to explain the value of $\delta^{15}N$ at the global scale for a single soil depth (BRTglobal). The climatic and soil variables listed in Supp. Table 2.1 were used as the predictor variables. The 'select07' function (Dormann et al. 2013) in R, was used to identify collinear predictors. In cases where the predictor variables were found to be strongly collinear with each other, the variable with either the strongest correlation with the response variable, or the most biologically relevant, was retained. Following an initial run (learning rate = 0.01, tree complexity = 5, bagging fraction = 0.5), a simplification procedure was implemented (Elith et al., 2008) to eliminate variables with low influence (such as NDVI and PET). Both models were run ten times using the libraries 'gbm' (Ridgeway et al. 2013) and 'dismo' (Hijmans and van Etten 2014) packages in R. Model outputs were used to ascertain the relative influence and relationship of each predictor with the correlation between $\delta^{13}C$ and $\delta^{15}N$ at the local scale or $\delta^{15}N$ at the global scale.

To account for C_3 and C_4 vegetation input into the SOM pool, global soil $\delta^{13}C$ values were analyzed for bimodality using libraries 'diptest' (Maechler and Ringach 2015) in R and cutoffs were calculated using the 'mixtools' package (Benaglia et al. 2009). $\delta^{13}C$ for C_3 and C_4 were then treated as separate sets of data on which BRT modeling for global $\delta^{15}N$ values were independently reanalyzed.

Results

Isotopic variation with soil depth

For 11 out of 16 sampling groups analyzed, the variation in average soil $\delta^{13}C$ and $\delta^{15}N$ values with depth were significantly positively correlated with each other (Fig. 2.1,

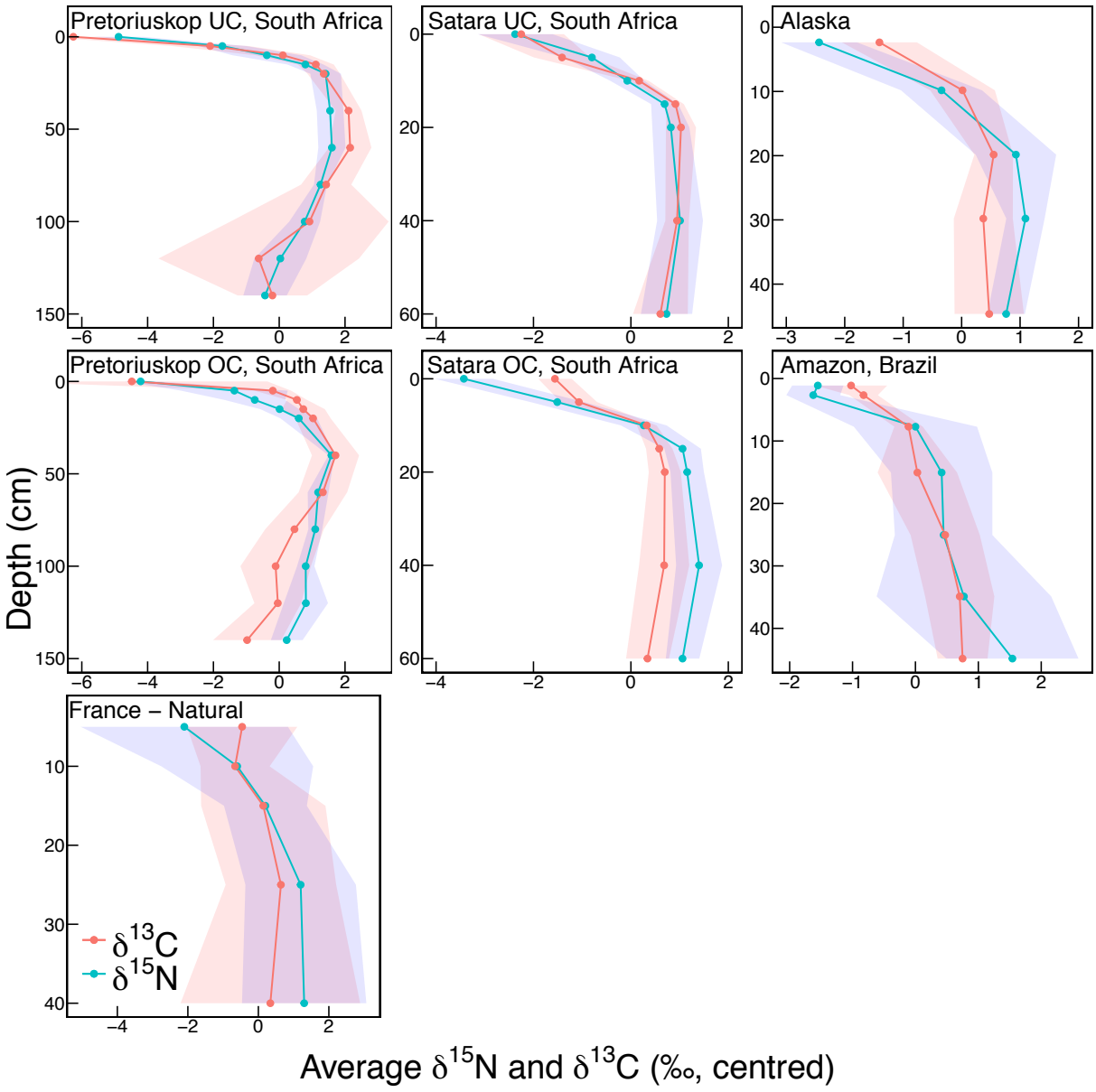


Figure 2.1: Variation with soil depth of $\delta^{13}C$ and $\delta^{15}N$ values for sites in which $\delta^{13}C$ and $\delta^{15}N$ are significantly correlated with each other (Table 2.1). The data was averaged for each depth and the confidence interval is represented by the coloured bands. The $\delta^{13}C$ and $\delta^{15}N$ data were independently centred on 0 so as to allow comparison of the variation of these within a site and thus the range of the data corresponds to that of the original data. Sites designated OC and UC are from open-canopy and under-canopy, respectively.

Table 2.1). For many of these sites, both $\delta^{13}C$ and $\delta^{15}N$ values increased with depth, with the majority of the increase occurring in the upper 10–20 cm of the profile. The

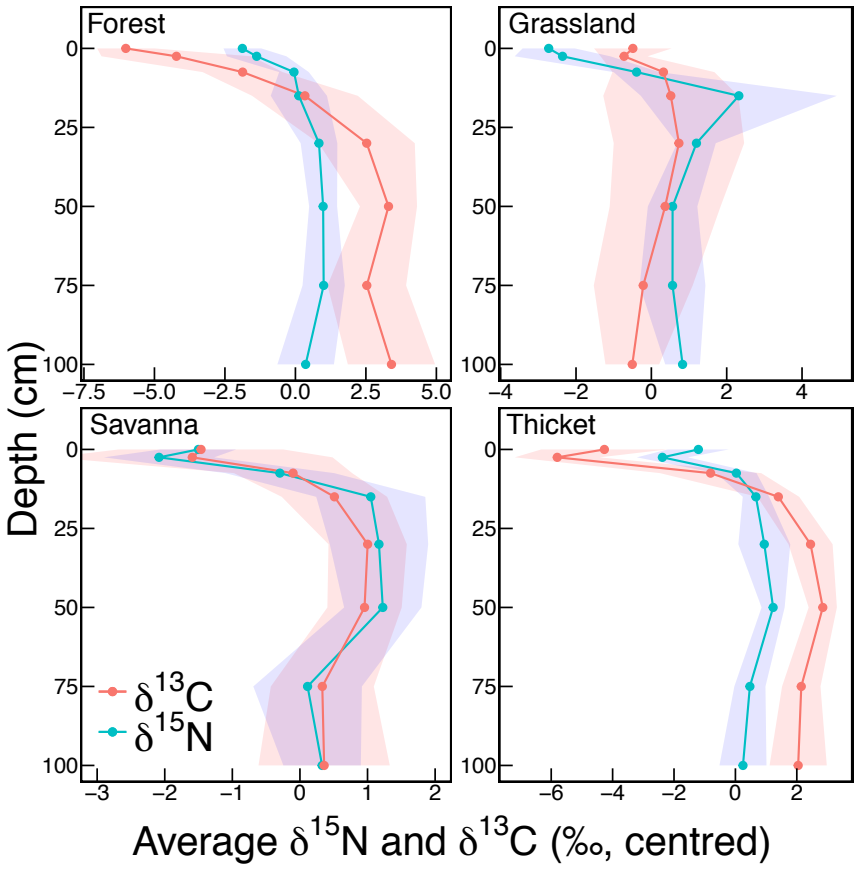


Figure 2.2: Variation with soil depth of $\delta^{13}C$ and $\delta^{15}N$ values for sites in which the dominant vegetation types differ. The data was averaged for each depth and the confidence interval represented by the coloured bands. The $\delta^{13}C$ and $\delta^{15}N$ data were independently centred on 0 so as to allow comparison of the variation of these within a site and thus the range of the data corresponds to the original data.

range of variability for both isotopes was ca. 2–8‰ through the soil profiles and this range was independent of the average $\delta^{13}C$ and $\delta^{15}N$ signature for the sites (Table 2.1). Within the relatively small geographic area of the Hluhluwe Nature reserve, the significant positive correlations between $\delta^{13}C$ and $\delta^{15}N$ values were independent of vegetation types comprising forest, grassland, savanna and thicket sites. Across all of these distinct vegetation types, $\delta^{13}C$ and $\delta^{15}N$ values increased similarly with depth (Fig. 2.2, Table 2.1). For these sites the range of variability for both isotopes was also ca. 2–8‰ with the majority of the increase in $\delta^{13}C$ and $\delta^{15}N$ values occurring within the upper ca. 20 cm of the soil. Although most sites had significant positive correlations between $\delta^{13}C$ and $\delta^{15}N$, for 5 of the 16 sampling groups, changes in average soil $\delta^{13}C$ and $\delta^{15}N$ values through the soil profiles were either not significantly

associated or negatively correlated with each other (Fig. 2.3, Table 2.1). For these sites $\delta^{13}C$ and $\delta^{15}N$ values also increased with depth, with the exception of the Paulshoek site in which $\delta^{15}N$ initially increased before subsequently decreasing below ca. 10 cm. These sites also had a wider range of $\delta^{13}C$ and $\delta^{15}N$ values than those for which there were significant correlations between $\delta^{13}C$ and $\delta^{15}N$ (Fig. 2.1, 2.2).

BRT analysis of the correlation between $\delta^{13}C$ and $\delta^{15}N$ values ranked CEC, mean diurnal temperature range, bulk density, MAT, clay and MAP as the top predictors (Fig. 2.4a), explaining 38% of the variance in the correlation between $\delta^{13}C$ and $\delta^{15}N$. Partial dependency plots, which show the effect of a variable on the response after accounting for the average effects of all other variables in the model, of the BRT analysis of the soil profile correlations between $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 2.5), showed that this was strongest at sites with CEC < 20 cmol kg^{-1} and a mean diurnal temperature range < 13°C. Sites with bulk density above 1400 kg m^3 had strong correlation between soil $\delta^{13}C$ and $\delta^{15}N$ values. The influence of clay concentration on the correlation between $\delta^{13}C$ and $\delta^{15}N$ values was generally high. A number of sites

Table 2.1: Location, site elevation and vegetation characteristics (Mucina and Rutherford, 2006) associated with the collected soils.

Soil	Latitude	Longitude	Elevation (m)	Site	Vegetation type and site characteristics
1	-33.496	18.724	114	Malmesbury	Swartland Shale Renosterveld – wheat
2	-33.970	18.446	119	Newlands	Peninsula Shale Fynbos - pine plantation
3	-33.968	18.447	126	Newlands	Peninsula Shale Fynbos - natural
4	-34.002	18.392	137	Orangekloof	Southern Afrotemperate Forest - granite
5	-33.990	18.393	343	Orangekloof	Southern Afrotemperate Forest - sandstone
6	-33.991	18.387	359	Orangekloof	Southern Afrotemperate Forest - sandstone
7	-33.196	20.647	888	Matjiesfontein	Koedoesberge-Moordenaars Karoo
8	-32.405	24.174	693	Aberdeen	Eastern Lower Karoo
9	-32.766	25.749	630	Cookhouse	Bedford Dry Grassland
10	-34.003	18.386	88	Orangekloof	Southern Afrotemperate Forest- sandstone

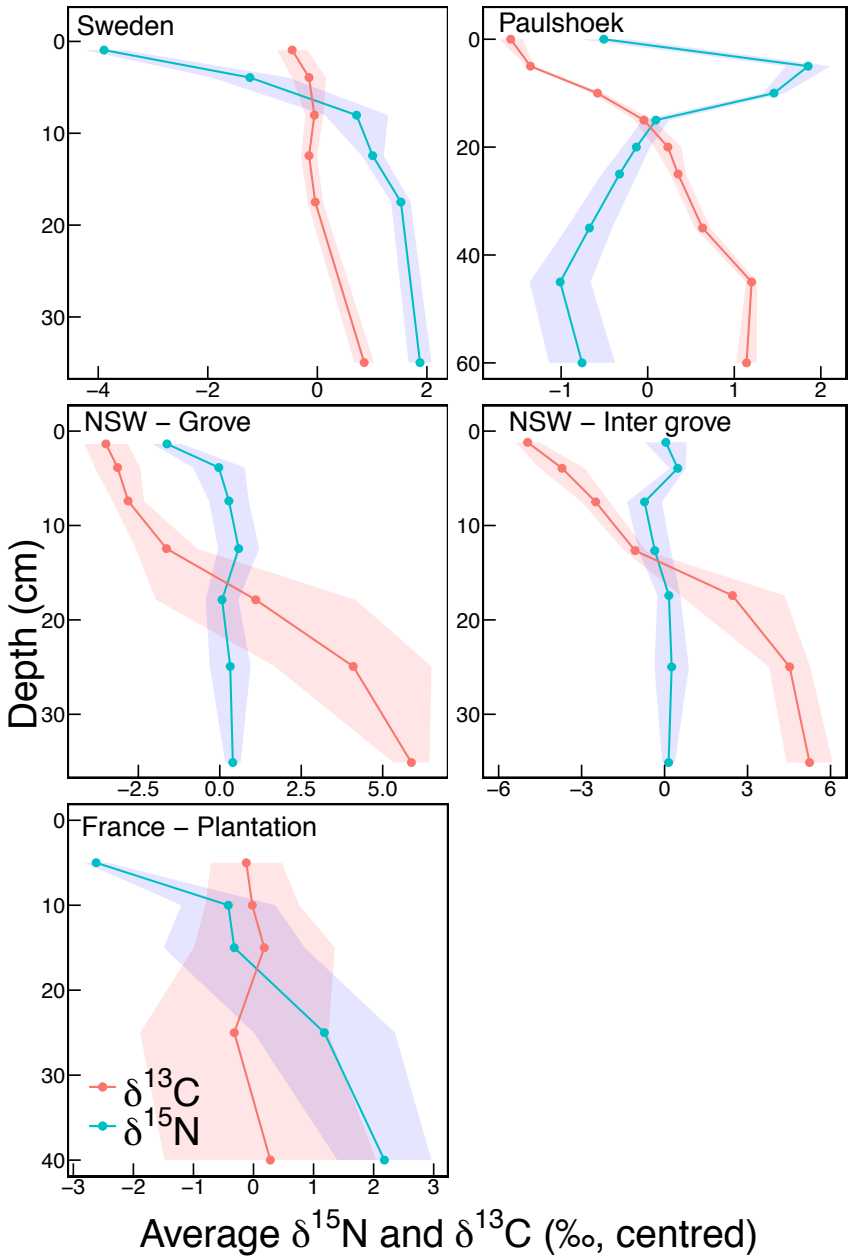


Figure 2.3: Variation with soil depth of $\delta^{13}C$ and $\delta^{15}N$ values for sites in which $\delta^{13}C$ and $\delta^{15}N$ are poorly correlated with each other. The data was averaged for each depth and the confidence interval represented by the coloured bands calculated from the standard error. The $\delta^{13}C$ and $\delta^{15}N$ data were independently centred on 0 so as to allow comparison of the variation of theses within a site. The range of the data corresponds to the original data.

with clay concentrations between 30 and 35%, however, had a relative low influence of clay on the correlation. These sites were arid, receiving < 500 mm mean annual precipitation and had a relatively poor correlation compared to mesic sites (i.e.

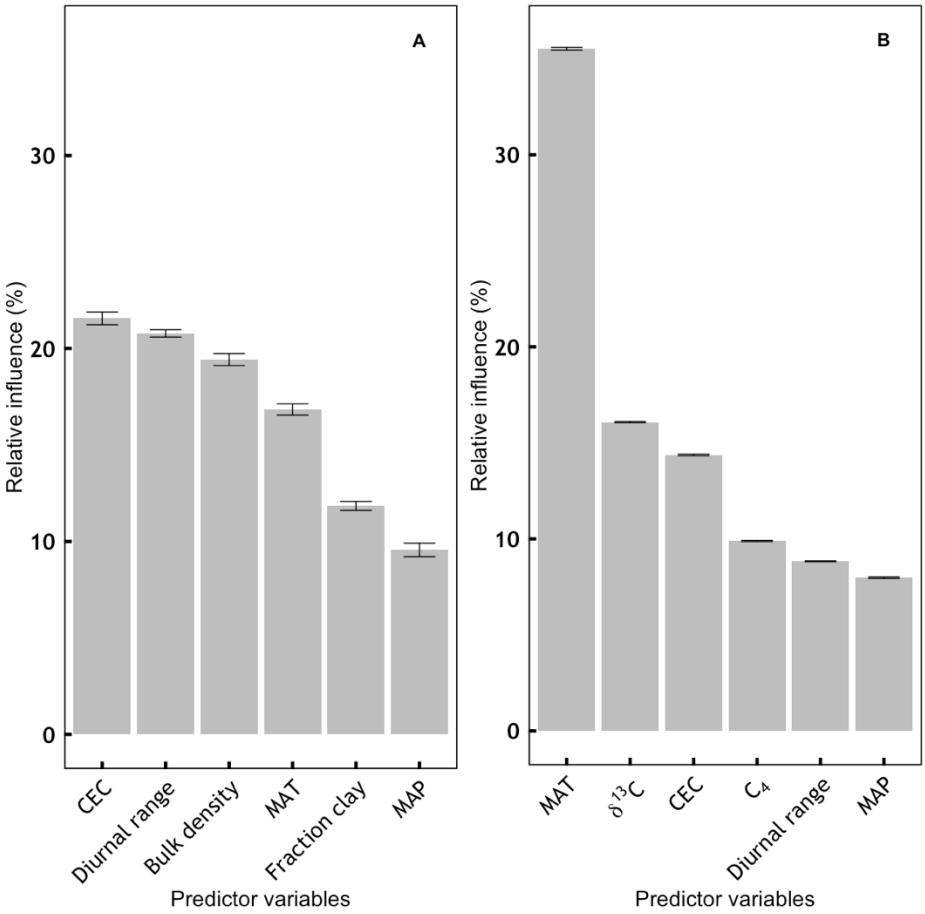


Figure 2.4: Relative influence of variables in determining the correlation between global soil $\delta^{13}C$ and $\delta^{15}N$ as determined by BRT analysis as well as the relative influence of variables in determining the global soil $\delta^{15}N$ as determined by BRT analysis. Values are the mean ± SE of 10 runs of each model. Error bars represent standard error.

between 500 and 1000 mm) with a moderate influence in hydric sites (>1000 mm). The correlation between $\delta^{13}C$ and $\delta^{15}N$ values was stronger at sites with MAT > 19°C (Supp. Fig. 2.4f).

Global geographic variation

Globally, soil $\delta^{15}N$ values of surface soils were significantly positively correlated with $\delta^{13}C$, MAT and the prevalence of C_4 photosynthetic vegetation and negatively correlated with CEC and diurnal T range (Table 2.2). Geospatial variation in global

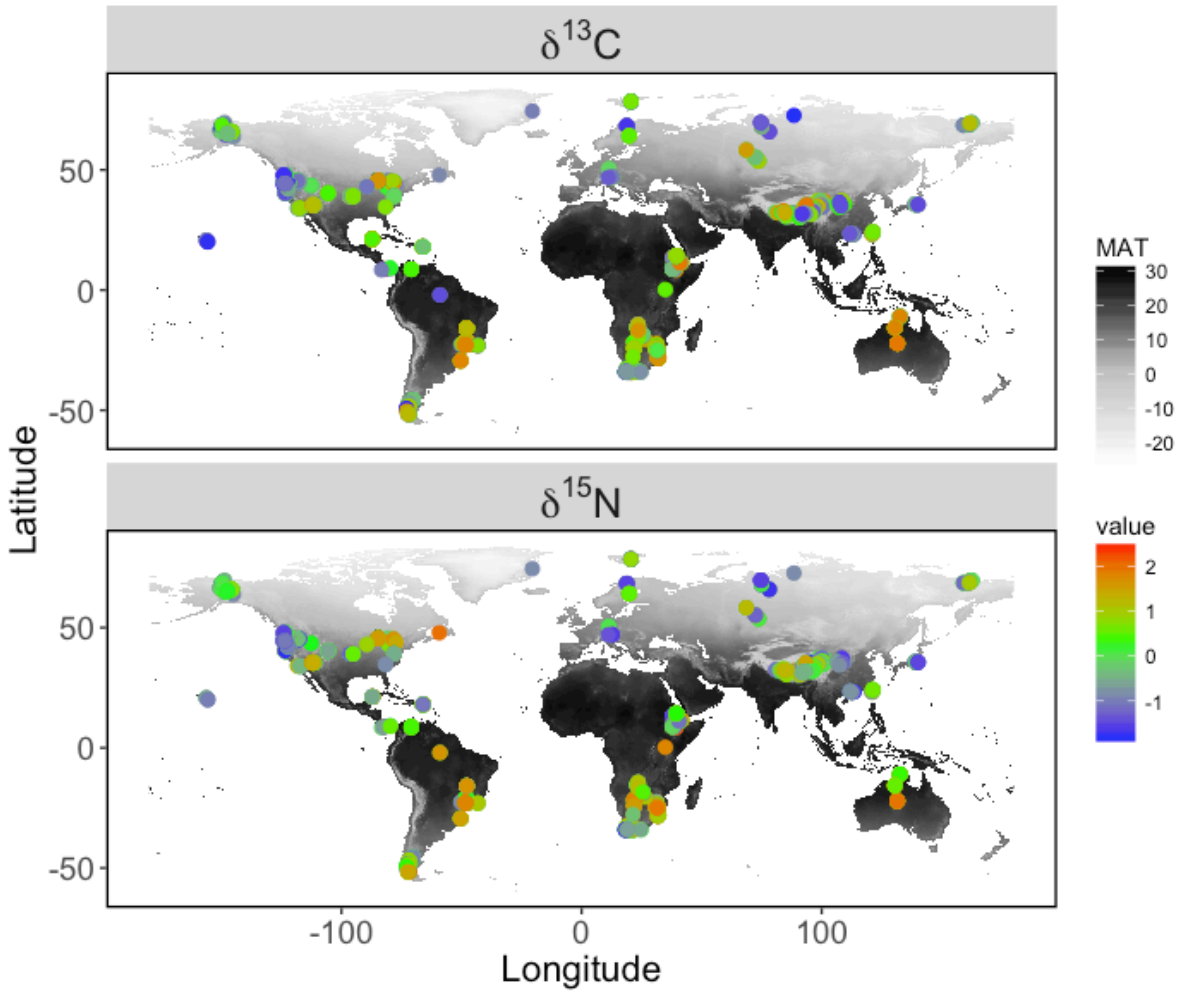


Figure 2.5: The global variation in soil $\delta^{13}C$ and $\delta^{15}N$. The color of the points represents the site averages of $\delta^{13}C$ and $\delta^{15}N$ values standardized and centered to range between -1 and 1. Background fill colour represents mean annual temperature.

$\delta^{13}C$ and $\delta^{15}N$ values that were spatially averaged over 0.1° corresponded relatively well with each other at high latitudes (> 50°) where both $\delta^{13}C$ and $\delta^{15}N$ values were more negative compared to sites located nearer the equator (Fig. 2.5). Sites in which $\delta^{13}C$ values were relatively high (Fig. 2.5) were from more arid regions such as Southern Africa, Australia and North America and in which C_4 grass communities exist. BRT analysis of global soil $\delta^{15}N$ values (BRTglobal) ranked MAT, $\delta^{13}C$, CEC, C_4, diurnal range and MAP as the top predictors of soil $\delta^{15}N$ (Fig. 2.4b), which explained

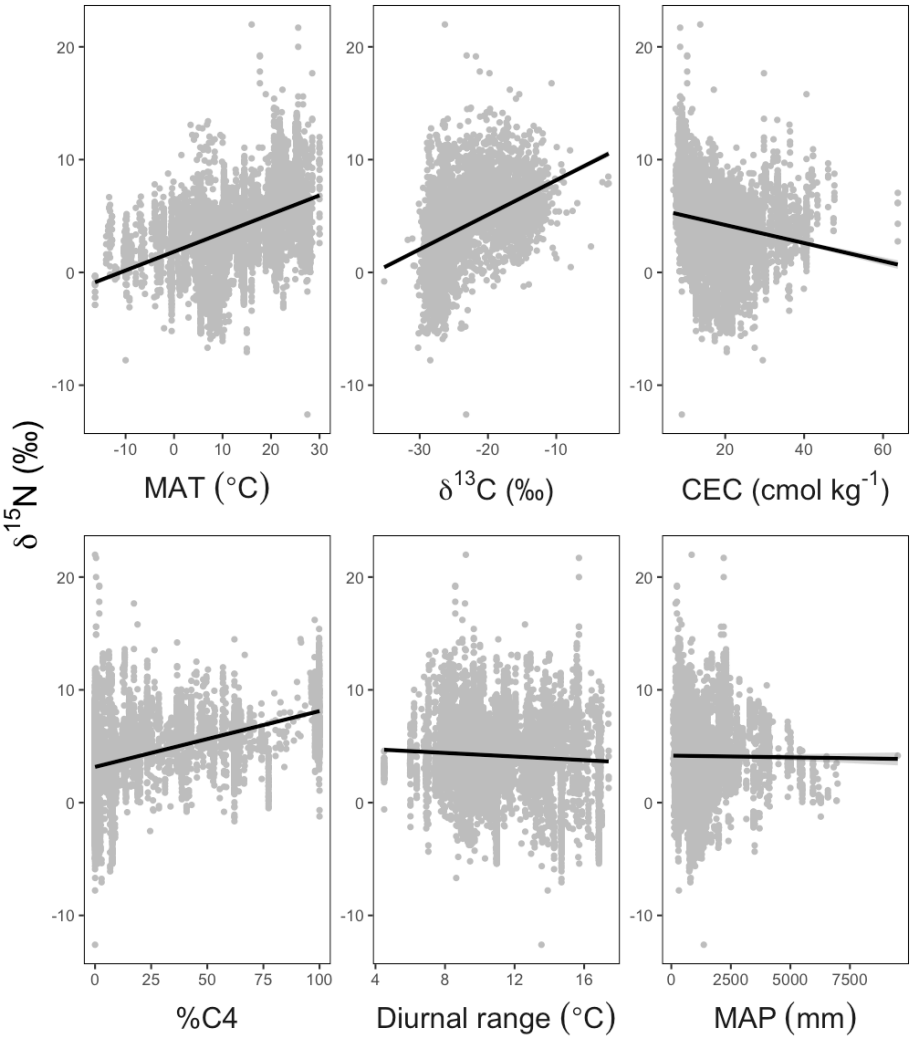


Figure 2.6: Bivariate analysis of the top six predictors of global soil $\delta^{15}N$ against global soil $\delta^{15}N$. Lines indicate linear model function.

62% of the variation in $\delta^{15}N$ values. The partial dependency plots for the BRTglobal (Supp. Fig. 2.5) showed that as MAT increased, $\delta^{15}N$ values also increased. Sites with $\delta^{13}C$ values below ca. -30‰ had low $\delta^{15}N$ values, which increased rapidly with increased $\delta^{13}C$ values up until ca. -20‰, above which changes in $\delta^{15}N$ values were relatively small. Therefore, much of the change in $\delta^{15}N$ values associated with $\delta^{13}C$ values occurred in a range of $\delta^{13}C$ values considered to be characteristic of C_3 dominated sites (Supp. Fig 2.2). Sites with CEC values > 10 cmol kg^{-1} had relatively low soil $\delta^{15}N$ values. $\delta^{15}N$ values were also low for sites with < 75% C_4 vegetation. Soil $\delta^{15}N$ values were reduced with increases in mean diurnal temperature range and generally with increased MAP (Supp. Fig. 2.5f).

Global $\delta^{15}N$ values predicted from the full BRTglobal model, including both C_3 and C_4 sites, were strongly correlated with observed global $\delta^{15}N$ values (Supp. Fig. 2.1). There was, however, a degree of under-prediction of $\delta^{15}N$ values at low observed $\delta^{15}N$ values and over-prediction at high observed $\delta^{15}N$ values. Global soil $\delta^{13}C$ values were bimodal with two ranges of $\delta^{13}C$ values having peaks at -26.36‰ and -17.58‰, indicating that there were a number of sites dominated by either predominantly C_3 or C_4 plants (Supp. Fig. 2.2). BRT's predicting global soil $\delta^{15}N$ based on a subset of sites that were predominantly C_3 dominated ranked MAT, $\delta^{13}C$, CEC, bulk density, diurnal T range and MAP as top predictors (Supp. Table 2.2). The BRT developed for C_4 dominated sites ranked MAT, CEC, bulk density, MAP, diurnal range and $\delta^{13}C$ as top predictors. Although soil $\delta^{13}C$ was found to be a strong predictor of $\delta^{15}N$ for C_3 sites, it was a weak predictor in C_4 dominated sites.

Table 2.2: Bivariate ranged major axis (RMA) analysis results of top six predictors of global soil $\delta^{15}N$ with correlation coefficients (r) shown with p-values (bold where significant). All variables used in the prediction of global soil $\delta^{15}N$ are shown in Supp. Table 2.1.

Predictor variables	n	r	p-value	intercept	slope
MAT	7461	0.48	**< 0.001**	1.13	0.21
$\delta^{13}C$	5501	0.48	**< 0.001**	14.16	0.43
CEC	7328	-0.2	**< 0.001**	7.07	-0.15
$\%C_4$	7415	0.43	**< 0.001**	2.99	0.05
Diurnal T range	7456	-0.1	**< 0.001**	5.82	-0.15
MAP	7474	-0.01	0.579	4.22	< 0.00

Discussion

This study suggests that either common or coordinated processes may contribute to fractionation of soil C and N isotopes. The link between soil $\delta^{13}C$ and $\delta^{15}N$ values may inform understanding of these processes due to this coordination of soil processes determining both C and N isotope fractionation. Our results suggest that although the initial isotope composition of the organic matter is indisputably important,

subsequent fractionation via soil processes, such as decomposition and related processes, may result in correlations between $\delta^{13}C$ and $\delta^{15}N$ values in geographic space and commonly following similar trajectories with soil depth. More positive $\delta^{13}C$ and $\delta^{15}N$ values with soil depth (Fig. 2.1) must result from increasing fractionation or more prolonged fractionation in deeper soils relative to shallower soils.

The importance of the vegetation characteristics in determining C isotopic composition is apparent from the bimodal distribution of soil $\delta^{13}C$ values associated with C_3 (-22‰ to -32‰; Troughton 1979) and C_4 (-9.2‰ to -19.3‰; Hattersley 1982) vegetation (Supp. Fig. 2.2) whereas the variation in $\delta^{13}C$ within the C_3 and C_4 groupings is caused by climatic and geographical factors (Damesin et al. 1997). Likewise, global variation in soil $\delta^{15}N$ values (Fig. 2.5) is associated with variation in foliar $\delta^{15}N$ that varies with MAP, MAT, N availability, foliar N concentration and with the degree of N_2 fixation (Craine et al. 2009). Organic matter enters soils in a diversity of ways and this influences the initial isotopic signature of soil C and N (Eissfeller et al. 2013). The majority of SOM, however, enters the soil as plant-derived detritus, where it is utilized by soil microbes (Berg and McClaugherty 2008) and decomposer fauna (Hättenschwiler and Gasser 2005). Consequently, the isotopic values of the dominant vegetation and the variation in $\delta^{13}C$ and $\delta^{15}N$ values, both between and within species (Damesin et al. 1997; Craine et al. 2015a), strongly influence SOM isotopic composition.

Unlike for C, however, there are also strong ecosystem feedbacks between soil and vegetation N in determining ecosystem $\delta^{15}N$ values, because soil $\delta^{15}N$ also partially determines plant $\delta^{15}N$. Despite this dependence of SOM isotopic composition on that of OM and vegetation, the variations in $\delta^{13}C$ (range: -27.8 to -12.4‰) and $\delta^{15}N$ (range: -0.1 to 10.1‰) with depth in soil profiles were often strongly correlated with each other (Table 2.1). Likewise, geospatial variation in global $\delta^{13}C$ and $\delta^{15}N$ values also corresponded relatively well across a wide range of climates and biomes (Fig. 2.5). For example, C_3 and C_4 dominated sites showed similar patterns of $\delta^{13}C$ and $\delta^{15}N$

enrichment through soil profiles (Fig. 2.2), although the range of values was smaller with C_4 vegetation.

The correspondence between the increases of $\delta^{13}C$ and $\delta^{15}N$ values with depth is probably through processing of SOM, which is further supported by the most influential predictors in the BRT model for the correlation between $\delta^{13}C$ and $\delta^{15}N$ values through soil profiles (Fig. 2.4a), which themselves are related to microbial activity. Furthermore, soil $\delta^{13}C$ values were also strong determinants of $\delta^{15}N$ globally (regardless of soil and ecosystem type) while the remaining top predictors of $\delta^{13}C$ could be related to SOM decomposition (Fig. 2.4b). Processing of SOM is determined by characteristics of the SOM, such as the C and N composition (Fernandez et al. 2003), as well as by environmental factors including soil temperature, moisture and aeration (Gholz et al. 2000; Zhang et al. 2008). The reason for the positive correlation between MAT and both $\delta^{15}N$ and $\delta^{13}C$ values could therefore be due to microbial activity increasing with increasing temperature. Mean diurnal temperature range (e.g. Li et al. 2011), CEC and soil fertility (Sikora 2013) may also be linked to SOM decomposition through soil microbial processes. Although favorable moisture conditions stimulate decomposer communities (Cotrufo et al. 2013), MAP was not significantly correlated with either $\delta^{13}C$ or $\delta^{15}N$ values at the global scale (Fig. 2.4b, Table 2.2). This is likely because many ecosystem properties depend on MAP obscuring clear relationships. For example, Craine et. al. (2015a) related variation in global soil $\delta^{15}N$ to variation in clay concentrations.

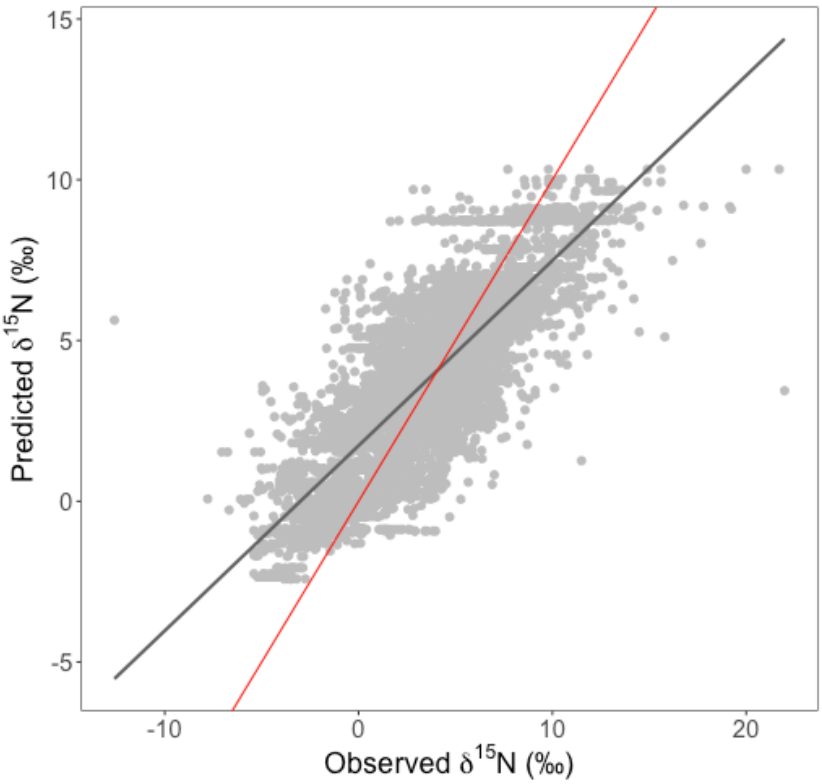
Despite strong global geographic correspondence between $\delta^{13}C$ and $\delta^{15}N$ and correspondence over soil depth (11 of 16 sites), some sites had non-significant (New South Wales, France, Sweden) or negative (Paulshoek) correlations between $\delta^{13}C$ and $\delta^{15}N$ (Fig. 2.3, Table 2.1). These sites indicate the complexity of the relationship between soil $\delta^{13}C$ and $\delta^{15}N$, and dependence on other factors. For example, the New South Wales sites had a large proportion of N_2-fixing microbes in the surface soil (Macdonald et. al. 2015) resulting in $\delta^{15}N$ being close to 0‰. The non-significant

Swedish and French sites were both associated with plantations (Boström et al. 2007; Zeller et. al. 2007), whereas a corresponding natural site in France showed a significant relationship (Fig. 2.1). Paulshoek exhibited a maximum soil $\delta^{15}N$ value at intermediated depths, which is indicative of N-loss during nitrification and denitrification (Hobbie et al. 2009). This is not surprising as Paulshoek is arid with high soil temperatures and sporadic rainfall (Table 2.1) and these conditions increase nitrification/denitrification rates (Craine et al. 2015). Thus despite the general global relationship between $\delta^{13}C$ and $\delta^{15}N$, this correspondence does vary depending on local biotic, disturbance and environmental influences.

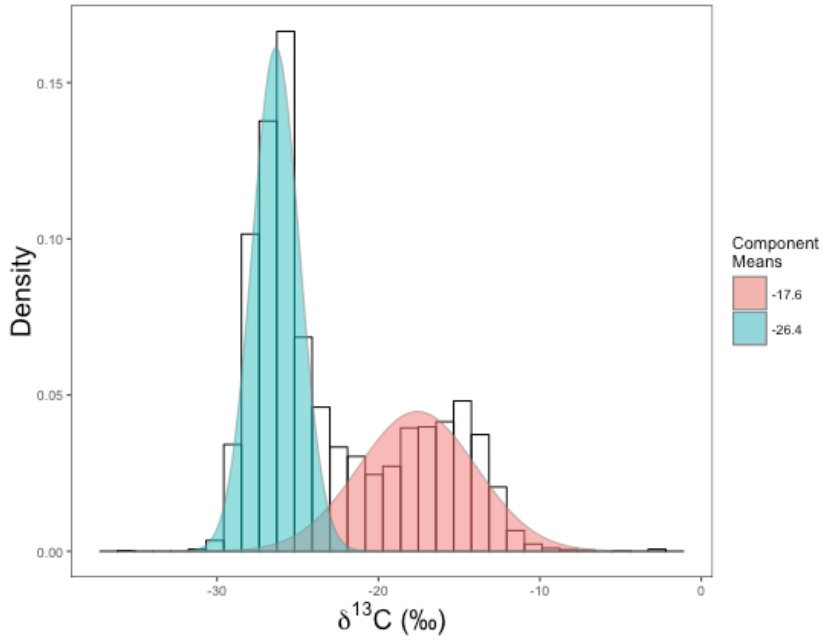
As a consequence of a link between soil $\delta^{13}C$ and $\delta^{15}N$, interpretation of soil $\delta^{13}C$ values as indicators of historical vegetation assemblages is complicated by the role of soil processes in determining soil $\delta^{13}C$ values, as also shown by Wynn et. al. (2005). The ranges of $\delta^{13}C$ values with depth are commonly large (up to 11.0 ‰, Supp. Fig. 2.3) which overlaps the range of values commonly associated with vegetation change. For example, $\delta^{13}C$ values between -16 and -20‰ have been used to indicate mixed C_3 and C_4 vegetation and > -16% to indicate C_4 dominance (Gillson 2015b). From our study, however, whilst the minimum $\delta^{13}C$ values of soils with C_3 and C_4 vegetation reflect the isotopic signature of the vegetation inputs, the maximum $\delta^{13}C$ values are indistinguishable. Since the maximum $\delta^{13}C$ values of soils supporting C_3 vegetation overlap with the minimum $\delta^{13}C$ values of C_4 vegetation, interpretation of intermediate $\delta^{13}C$ values (i.e. < ca. -15 ‰) as indicating historical vegetation characteristics should be approached with caution. Furthermore, in order to prove that prehistoric $\delta^{13}C$ SOC values are indeed representative of ancient vegetation assemblages in samples of deep SOC, one must establish that the fraction of SOC remaining in the sample is very close to the original highest concentration during soil formation and that fractionation has not been great (Wynn et al. 2006). This is because Rayleigh distillation and mixing processes vary with environmental and soil properties, with particularly strong effects associated with fine mineral particles (i.e. clay) in fine

grained soils (Krull and Skjemstad 2003; Wynn et al. 2005) and should not be assumed to be constant everywhere.

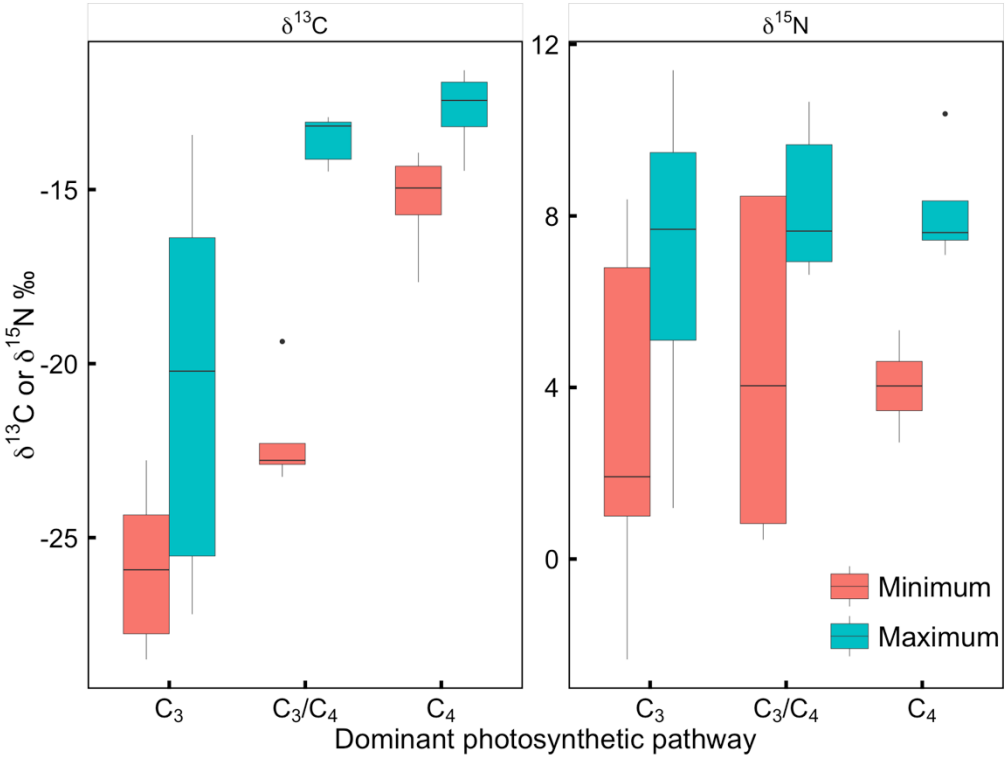
Supplementary Material



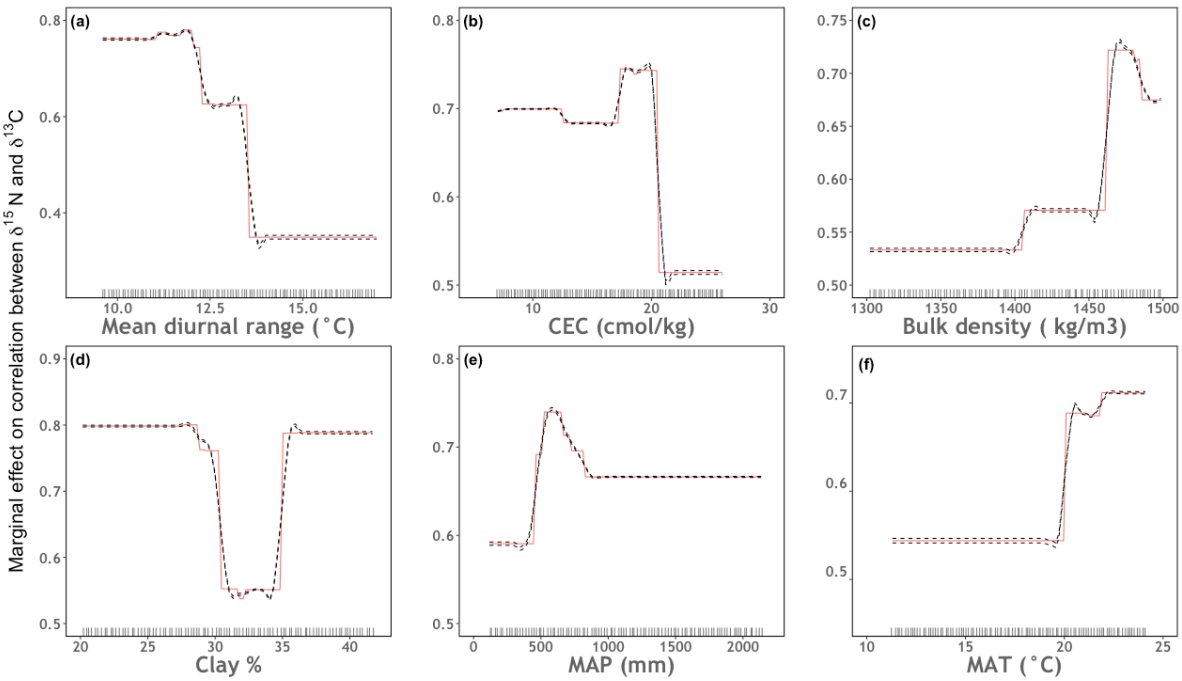
Supp. Fig. 2.1: Observed $\delta^{15}N$ against predicted d15N at the global scale based on the global BRT model. Black line indicates the y=x for the observed $\delta^{15}N$ values while the red line represents the fitted function for predicted $\delta^{15}N$ (y = -0.5x + 1.1).



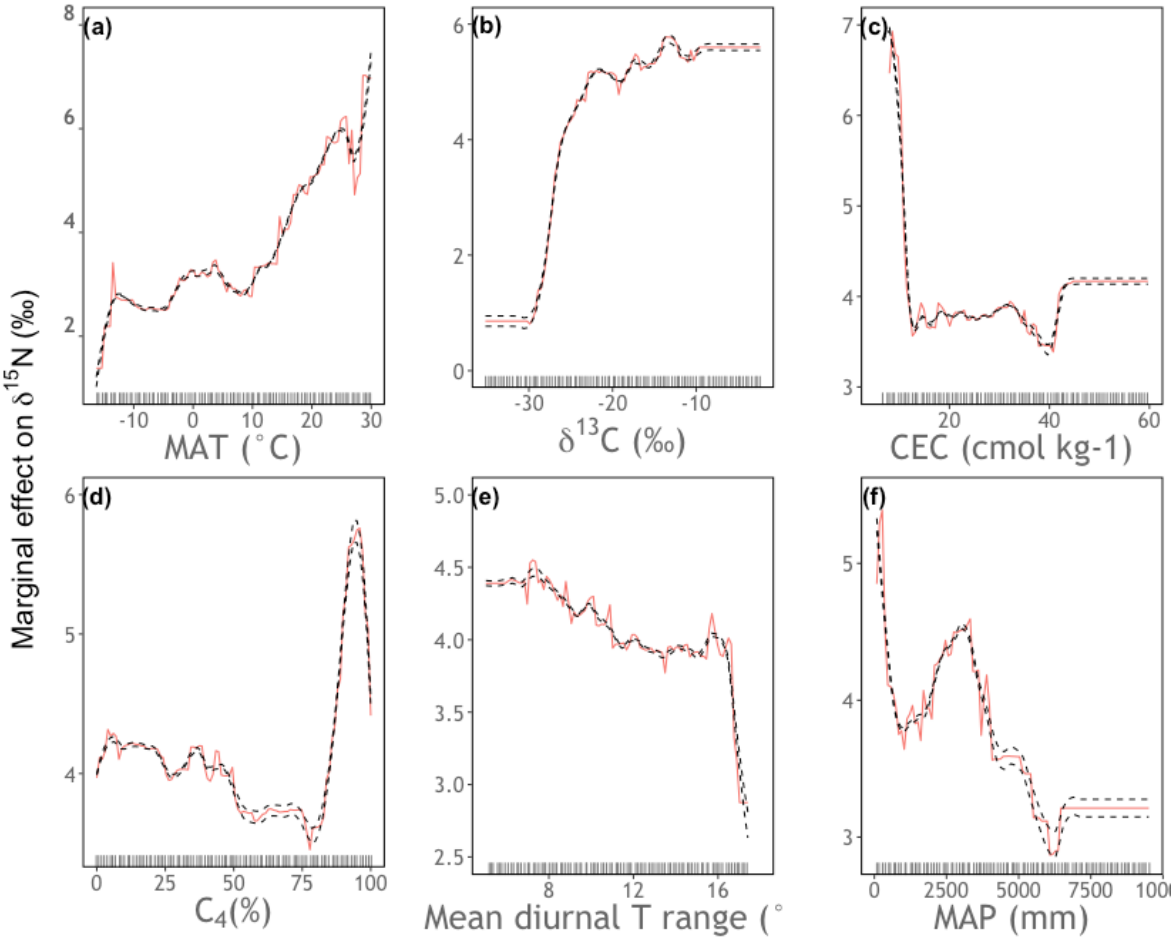
Supp. Fig. 2.2: Test for bimodality of soil $\delta^{13}C$ data. Red ribbon represents normal distribution of C_4 sites while the blue ribbon represents normal distribution for C_3 sites.



Supp. Fig. 2.3: Minimum and maximum soil $\delta^{13}C$ and $\delta^{15}N$ for dominant photosynthetic pathway for all local soil profile sites used in this study.



Supp. Fig. 2.4: The partial dependence of the correlation between $\delta^{13}C$ and $\delta^{15}N$ on the six most influential environmental predictors. The fitted function represents the modeled relationship between the correlation coefficient of $\delta^{13}C$ and $\delta^{15}N$ and an environmental predictor once the average effects of all other predictors were accounted for. the Solid line represents the mean of 10 runs of the final, simplified BRT model. Dashed lines represents the 95% CI. A flattening of the line indicates that an increase in the predictor has no further effect on the fitted function.



Supp. Fig 2.5: The partial dependence of $\delta^{15}N$ on the six most influential environmental predictors. The fitted function represents the modeled relationship between $\delta^{15}N$ and an environmental predictor once the average effects of all other predictors were accounted for. Solid line represents the mean of 10 runs of the final, simplified BRT model. Dashed lines represents the 95% CI. A flattening of the line indicates that an increase in the predictor has no further effect on the fitted function.

Supp. Table 2.1: Climatic and soil variables used in the investigation.

WorldClim Bioclimatic variables	ISRIC SoilGrids	Other
Mean Annual Temperature (MAT)	Soil organic carbon content	NDVI
Mean Diurnal Range	pH index measured in water solution	$\%C_4$
Isothermality	Bulk density (fine earth)	Potential evapotranspiration (PET)
Temperature Seasonality	Cation Exchange Capacity of soil (CEC)	
Max Temperature of Warmest Month	Weight percentage of the sand particles (0.05–2 mm)	
Min Temperature of Coldest Month	Weight percentage of the silt particles (0.0002–0.05 mm)	
Temperature Annual Range	Weight percentage of the clay particles (<0.0002 mm)	
Mean Temperature of Wettest Quarter	Volumetric percentage of coarse fragments (>2 mm)	
Mean Temperature of Driest Quarter		
Mean Temperature of Warmest Quarter		
Mean Temperature of Coldest Quarter		
Mean Annual Precipitation (MAP)		
Precipitation of Wettest Month		
Precipitation of Driest Month		
Precipitation Seasonality		
Precipitation of Wettest Quarter		
Precipitation of Driest Quarter		
Precipitation of Warmest Quarter		
Precipitation of Coldest Quarter		

Supp. Table 2.2: Relative influence of predictor variables on global soil $\delta^{15}N$ for C_3 and C_4 dominated sites as determined by BRT analysis.

Sites	Predictor variable	Relative influence (%)
C_3	MAT	27
	$\delta^{13}C$	22
	CEC	22
	Bulk density	9.2
	Diurnal range	6.4
	MAP	5.8
C_4	MAT	53
	CEC	19
	Bulk density	8.8
	MAP	7.4
	Diurnal range	7.2
	$\delta^{13}C$	1.4

Chapter 3

The contribution of microbial anaplerotic CO_2 fixation to determining soil $\delta^{13}C$ values.

Abstract

In soils both $\delta^{13}C$ and $\delta^{15}N$ values of soil organic matter (SOM) tend to increase with soil depth. This is at least partially because decomposition by soil microorganisms isotopically fractionates both C and N compounds in the soil. Soil microbes are, however, also capable of fixing N_2 and CO_2 from the soil atmosphere. Apart from surface photosynthetic CO_2 fixation, dark anaplerotic (i.e. non-photosynthetic) fixation of CO_2 is especially important for provision of C-skeletons for amino acid synthesis. We hypothesized that these N_2 and CO_2 fixing processes can contribute to determining SOM $\delta^{13}C$ and $\delta^{15}N$ values. Soils from 10 sites across South Africa differing in soil properties were incubated in the dark for 3 d under continuous exposure to $^{13}CO_2$- and $^{15}N_2$-enriched atmospheres with varying soil moisture (10, 50 and 100% of field capacity) and temperature (4, 25, 40°C). There was no evidence of significant N_2 fixation in any treatment. Significant soil anaplerotic CO_2 fixation, however, occurred in all soils. Highest rates of anaplerotic CO_2 fixation occurred in soils at 50% field capacity and 25°C, suggesting a link with microbial biotic activity. Soils with low C and N concentrations and low C:N ratios exhibited the highest rates of CO_2 fixation in soils, indicating a link between anaplerotic CO_2 fixation rates and soil nutrient status. The higher rates of CO_2 fixation in soils with low nutrients may indicate that soil microbes rely increasingly on anaplerotic fixation as SOM-N declines, forcing greater reliance on de novo amino acid synthesis, and thus anaplerotic CO_2 fixation. The ubiquitous occurrence of anaplerotic $^{13}CO_2$ fixation in

these soils indicates that anaplerotic fixation is likely important in contributing to determining soil $\delta^{13}C$ values. Diffusion of low $\delta^{13}C$ bulk atmospheric CO_2 (ca. -10‰) into the soil atmosphere (<< -10‰) will drive soil CO_2 $\delta^{13}C$ towards ca. -10‰, and constant anaplerotic CO_2 fixation will result in SOM $\delta^{13}C$ also tending towards -10‰ in more highly processed SOM deeper in the soil.

Introduction

Both $\delta^{13}C$ and $\delta^{15}N$ values of soil organic matter (SOM) tend to increase as soil depth increases in tropical, temperate and boreal systems (Hobbie & Ouimette 2009). While a number of processes have been suggested to play a part in this trend, soil microorganisms play a key role in the fractionation of C and N compounds which enter the soil environment (Wynn et al. 2006; Hobbie & Ouimette 2009). Indeed, many of the biogeochemical processes in soils are microbially mediated and a wide range of autotrophic and heterotrophic microorganisms are involved in below-ground soil processes that include mineralization, oxidation and assimilation of carbon (C) and nitrogen (N) into forms available to the plants (Curiel Yuste et al. 2007). Microbial mineralization proceeds through decomposition of more complex molecules into simpler forms. During decomposition, organic N is converted into forms that are readily available to plants (e.g. NO_3^-) while C is lost as CO_2 during soil respiration (Knoepp & Swank 1998; Schimel & Bennett 2004).

The processes of C and N mineralization are highly spatially and temporally heterogeneous at the global scale, and are regulated by a number of factors, including climate and soil characteristics (Knoepp & Swank 1998; Chen et al. 2013). Microbial mineralization involves biochemical reactions that are sensitive to the activation energies, leading to these processes having a higher affinity for lighter isotopes, such as ^{12}C and ^{14}N. This has been suggested to lead to the fractionation of C and N as lighter isotopes are progressively used up as mineralization progresses, leaving SOM ^{13}C- and ^{15}N-enriched (Price & Sowers 2004). Kinetic fractionation of soil C by

decomposition is, however, controversial as a number of studies have found contradictory evidence for the role of respiration in fractionation. For example, several studies have demonstrated no kinetic fractionation during decomposition (Ekblad & Högberg 2000; Ekblad & Nordgren 2002; Lin & Ehleringer 1997), while others have demonstrated a significant fractionation of respired CO_2 during decomposition (Fernandez et al. 2003; Šantrůčková et al. 2000). While mineralization has been suggested to enrich SOM with ^{13}C and ^{15}N, there is little evidence. Microbes, however, are able to fix both CO_2 and N_2 and incorporate the C and N into biological mass, resulting in microbes exhibiting a $\delta^{13}C$ and $\delta^{15}N$ value closer to that of the source CO_2 and N_2 (Ehleringer et al. 2000; Craine et al. 2015).

Soil is generally a net source of CO_2, although some microorganisms photosynthetically fix CO_2 in surface soils, and this may be an important process in determining the $\delta^{13}C$ value of soils (Macdonald et al. 2015). Microbes are commonly considered to have a negligible positive contribution to soil C, however, because the C input from plants into the SOM pool is commonly large, but is approximately balanced by the mineralization of the same SOM (Chadwick et al. 1994). There are a large number of soil organisms from the archaeal and bacterial domains that are capable of dark anaplerotic CO_2 fixation in a wide range of aerobic to anaerobic conditions (Saini et al. 2011). Anaplerotic CO_2 fixation refers to the process of replenishing TCA cycle intermediates (Atomi 2002). Anaplerotic reactions are important in all heterotrophic organisms as they play the role of regenerating the intermediates of the citric acid cycle (TCA cycle) which have been withdrawn for use in the biosynthesis of amino acids and other compounds (Feisthauer et al. 2008). This links anaplerotic reactions to microbial biomass and growth (Miltner et al. 2005) with up to 10% of the total cell C derived from anaplerotic CO_2 fixation (Perez & Matin 1982). Anaplerotic reactions typically use the enzyme phosphoenolpyruvate carboxylase (PEPc) to incorporate dissolved inorganic carbon in the form of bicarbonate into the TCA cycle where organic acids are formed and used for biosynthesis (Taybi et al. 2004). PEPc, however, also functions in plant roots where it

incorporates a significant amount of soil derived CO_2 into organic and amino acids (Cramer et al. 1993).

Since anaplerotic CO_2 fixation is common in microbes and in plant roots, it is surprising that its potential role in influencing soil $\delta^{13}C$ values has not been considered. The $\delta^{13}C$ and $\delta^{15}N$ values of CO_2 and N_2 in the soil are not constant because they are influenced by the degree of mixing between soil and bulk atmospheric reservoirs of these gasses (Susfalk et al. 2002; Clough et al. 2003). For example, shallow soils have a larger component of atmospheric gasses, whereas in deeper soil the mixing of soil- with atmospheric-gasses is limited, leading to greater recycling of soil gasses (Susfalk et al. 2002). Both waterlogging (Greenway 2006) and soil texture (Wynn et al. 2005) play a role in determining the extent of mixing of soil gasses. While the mixture of gasses and their consequent isotopic characters may be irrelevant for respiration occurring during decomposition, it is relevant for processes, such as N_2 fixation and anaplerotic CO_2 fixation, that incorporate these gasses into biological structures.

There are a number of factors which may influence the rates of microbial soil anaplerotic CO_2 fixation. For example, soil additions such as reduced sulphur compounds and H_2 have been shown to increase soil CO_2 fixation by acting as electron acceptors (Stein et al. 2005; Dong & Layzell 2001). Furthermore, the addition of readily degradable organic C (e.g. manure) increases soil heterotrophic CO_2 fixation (Wu et al. 2015). Since microbial abundance in soil is strongly linked to both moisture and temperature (Fierer et al. 2009), these factors are also likely to strongly influence the anaplerotic CO_2 fixation rate. For example, Tiwari et al. (1987) demonstrated that both waterlogged and air-dried soils evolve significantly less CO_2 (through respiration) than soils at field capacity as microbial activity decreases. Similarly, extreme temperatures decrease soil respiration rates and the highest rates are found to occur in soils between 15 and 30°C (Lloyd & Taylor 1994). This would mean that

extreme soil moisture and temperature conditions would likely suppress anaplerotic CO_2 fixation, as microbes are far less active.

We hypothesized that N_2 fixation and anaplerotic CO_2 fixation are ubiquitous in soils and that this would result in changes in soil $\delta^{13}C$ and $\delta^{15}N$. We also hypothesized that these fixation rates would vary with environmental circumstances that modify the prevalence of soil microbes. Furthermore, soils in which nutrients C and N are less available may exhibit higher rates of anaplerotic CO_2 fixation compared to soils which are nutrient rich. We determined the effect of soil microbial N_2- and anaplerotic CO_2-fixation on soil $\delta^{13}C$ and $\delta^{15}N$ values under varying soil conditions in order to ascertain whether assimilation of inorganic sources of C and N influence $\delta^{13}C$ and $\delta^{15}N$ fractionation. To achieve this, we collected 10 soils from different environments and exposed them to atmospheres enriched with $^{13}CO_2$ and $^{15}N_2$ and monitored the incorporation of these isotopes into soil organic matter with varying soil moisture and temperature.

Methods

In order to determine the effects of soil conditions on both CO_2 and N_2 fixation I supplied $^{13}CO_2$ and $^{15}N_2$ to soils sampled from a variety of edaphic circumstances. The effects of soil moisture, temperature as well as microbial activity on fixation rates of CO_2 and N_2 were then determined from changes in $\delta^{13}C$ and $\delta^{15}N$ of the soil to provide estimates of the responses of anaplerotic CO_2 fixation and N_2 fixation, respectively to these variables. We used sterilized soils to provide a control for microbial fixation of CO_2 and N_2.

Field sampling

Soil (A-horizon, 0–15 cm) was collected using a soil auger (8.25 cm diameter) at 10 locations across South Africa (Table 3.1). Surface litter and organic matter accumulation was scraped off the collection site before cores were collected. Soils

were processed within 24 h or undisturbed cores were kept at 4°C for up to 7 d prior to processing.

Field capacity

Approximately 100 ml of oven dried soil (40°C for 24 h) were weighed into funnels with a wad of glass wool in the neck. Funnels with soil were placed into graduated cylinders and 100 ml of water was poured over the soil and allowed to drain for at least 1 h or until dripping stopped. The volume of water retained in the soil was determined by subtracting the water collected in the graduated cylinder from 100 in order to represent field capacity expressed as a percentage (w/w).

Soil particle size analysis

Soil particle size distributions were determined using a Malvern Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK) on 1 mm sieved soils. The soils were suspended in water and organic material that floated to the surface was removed by hand. Each sample was subjected to 180 s ultrasonic dispersal to ensure complete disaggregation of particles. The soils were then introduced to the laser diffractometer with a Malvern Hydro 2000G wet dispersion unit. The. The proportion of the soil particles in each size class were recorded and plotted. These size classes were then summed into categories representing clay, silt and sand, according to the Wentworth grain size chart (Wentworth 1933).

X-ray florescence (XRF) analysis

Sieved and dry-ashed soil were milled in a mortar and pestle to a fine powder. The powder was placed into sample cups with a polypropylene bottom and assessed in a SPECTRO XEPOS X-Ray Fluorescence (XRF) analyzer (SPECTRO, AMATEK materials analysis division, Kleve, Germany). Measurements were conducted in a helium atmosphere using a silicon drift detector. The instrument was calibrated by using a certified standard GBW07312 (National Research Center for CRMs, Beijing, China),

for which elemental concentrations were obtained from NOAA Technical memorandum NOS ORCA 68 (1992).

$^{13}CO_2$ and $^{15}N_2$ labeling of soil samples

Four replicate soil samples (10 g) from each of the 10 sites were adjusted to 50% field capacity and placed into 20 ml glass vials. The vials were fitted with 1 000 µl Eppendorf tubes with their caps removed. Two sets of 10 soils were sealed using Suba-seals (Sigma-Alderich, Germany). Two sets of 10 soils were closed with Suba-seal plugs and autoclaved for 90 min so that soils lacking microbial activity can be compared to soils in which microbial activity is present. Sodium bicarbonate (0.344 g $NaH^{13}CO_3$, 98 atom % ^{13}C, Sigma-Alderich, Germany) was diluted with 10 ml of distilled water. This was estimated to be sufficient to raise the CO_2 concentration in the sealed glass vial to 50 000 ppm. In order to introduce $^{13}CO_2$ label, 0.1 ml of sodium bicarbonate stock was injected into the Eppendorf tube and CO_2 released with 1 ml of 0.1 M lactic acid solution, also injected through the septum into the Eppendorf. The $^{15}N_2$ label was introduced by injecting 0.2 ml of labeled $^{15}N_2$ gas (ICON supplier details, 99 atom %) through the septum using a hypodermic needle and gas syringe. In order to prevent pressure from increasing during both $\delta^{13}C$ and $\delta^{15}N$ label addition, each vial was equipped with a second pressure relief needle inserted through the septum with a 1 m length of tubing attached. The pressure relief needle was removed once the pressure had equalized with atmospheric pressure. The samples were then incubated in the dark at 25°C for 3 d. After the 3 d of incubation, the soils were analysed.

Six replicate vials were each filled with 10 g of soil from each site. Of these some were adjusted to 100%, 50% and 10% field capacity in order to test soil soil moisture effects on CO_2 and N_2 fixation rates. After field capacity adjustments, the $^{13}CO_2$ and $^{5}N_2$ isotopes were introduced to the vials as described above. The samples were then incubated in the dark at 25°C for 3 days, after which the soils were analysed. In another set of vials the $^{13}CO_2$ and $^{5}N_2$ isotopes were introduced to the vials maintained at 50% field capacity, as described above, and then incubated at 4°C, 25°C, 40°C for 3

d in the dark and then analysed in order to test soil soil temperature effects on CO_2 and N_2 fixation rates.

Coralloid roots were harvested from *Encephalartus latifrons* Lehm. which is known to contain N_2-fixing bacterioids (Spratt 1915) and placed into 20 ml vials with damp paper towel lining the bottom of the vials. $^{13}CO_2$ and $^{5}N_2$ isotopes were introduced to some of the vials, as described above, while the other vials received no label. These vials were then incubated in the dark at 25°C for 3 d.

Soil $\delta^{13}C$ and $\delta^{15}N$

Following experimental treatments, soils were ground to a fine powder using a mortar and pestle. Soil extracts were then acidified with 5 ml 0.1M HCl in order to remove any unfixed dissolved inorganic C and dried in an oven at 40°C for 24 h. Soil $\delta^{13}C$ and $\delta^{15}N$ were determined using mass spectrometry. Approximately 2 mg of each sample was weighed into a tin capsule (5x9 mm, Säntis Analytical, Teufen, Switzerland). The samples were then combusted in a Flash 2000 organic elemental analyser and the gases passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit (all from Thermo Scientific, Bremen, Germany). Eight in-house standards and one IAEA (International Atomic Energy Agency) standard (USGS25) were used to calibrate the results.

Statistical analysis

All statistical analyses were performed in R 3.2.3 (R Core Team 2015). An ANOVA was used to determine significant differences between enriched soils, control and sterile treatments followed by a post-hoc Tukey test. A Linear Mixed Effects model (LME) followed by a post-hoc Tukey test was used to assess significance between both CO_2- and N_2-fixation rates in control soils versus soils under different treatments. Predicted values generated from the linear model were plotted rather than raw data.

Results

Soil variability

Most of the soils were collected from within the Cape Florisitc Region, but in diverse settings representing both open-canopy nutrient poor vegetation (Fynbos, shale-derived soils) and closed canopy Afrotemperate forests both on relatively nutrient-rich granitic (4) and nutrient-poor sandstone-derived soils (5, 6). Within this region we also collected soil from a shale-derived Renosterveld site (1). Further afield, samples were taken from shale-derived Karoo soils as well as grassland sites. These samples exhibited strong variability in both physical and chemical properties. For example, while clay was ubiquitously low in these samples, silt content ranged between ca. 25% and 70% (Supp Fig. 3.1).

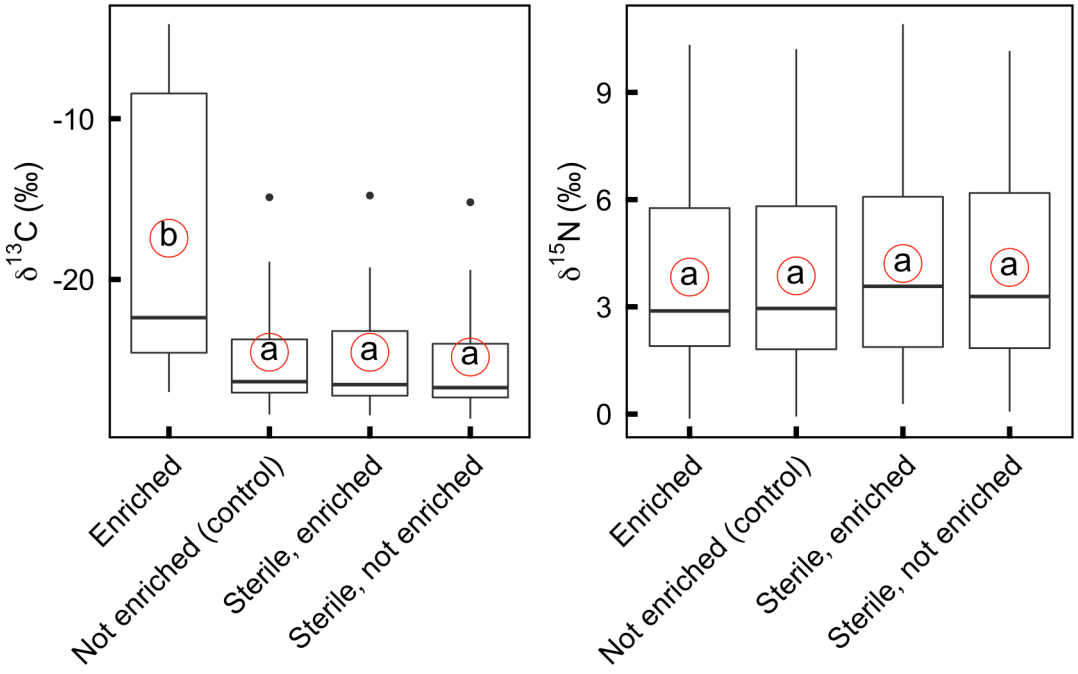


Figure 3.1: Soil $\delta^{13}C$ and $\delta^{15}N$ values (‰) for soils that were either sterilized or unsterilized and either supplied with $^{13}CO_2$ or $^{15}N_2$ or atmospheric air and incubated at 25˚C and 50% field capacity. The same letters indicate a non-significant difference ($p > 0.05$) as calculated by a Tukey post-hoc test following a one-way ANOVA ($\delta^{13}C$: $F = 3.73$, $df = 3$, $p = 0.02$; $\delta^{15}N$: $F = 0.03$, $df = 3$, $p = 0.99$).

Table 3.1: Location, site elevation and vegetation characteristics (Mucina and Rutherford, 2006) associated with the collected soils.

Soil	Latitude	Longitude	Elevation (m)	Site	Vegetation type and site characteristics
1	-33.496	18.724	114	Malmesbury	Swartland Shale Renosterveld – wheat
2	-33.970	18.446	119	Newlands	Peninsula Shale Fynbos - pine plantation
3	-33.968	18.447	126	Newlands	Peninsula Shale Fynbos - natural
4	-34.002	18.392	137	Orangekloof	Southern Afrotemperate Forest - granite
5	-33.990	18.393	343	Orangekloof	Southern Afrotemperate Forest - sandstone
6	-33.991	18.387	359	Orangekloof	Southern Afrotemperate Forest - sandstone
7	-33.196	20.647	888	Matjiesfontein	Koedoesberge-Moordenaars Karoo
8	-32.405	24.174	693	Aberdeen	Eastern Lower Karoo
9	-32.766	25.749	630	Cookhouse	Bedford Dry Grassland
10	-34.003	18.386	88	Orangekloof	Southern Afrotemperate Forest- sandstone

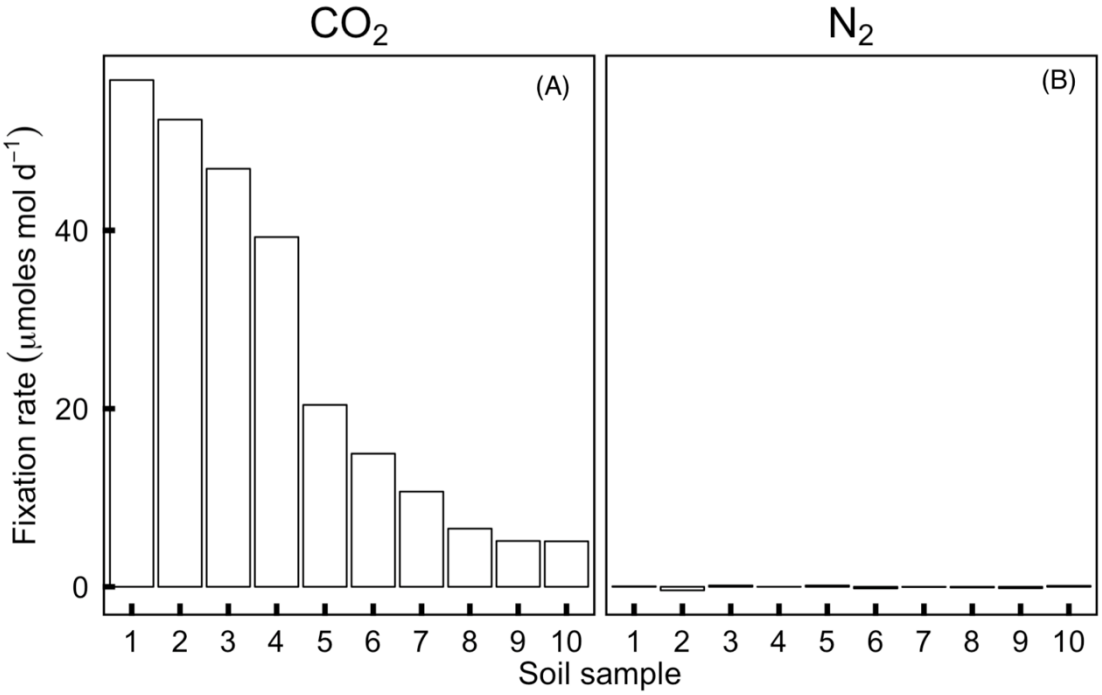


Figure 3.2: Anaplerotic $^{13}CO_2$ incorporation rates (µmoles ^{13}C mol C^{-1} d^{-1}) for soils enriched with $^{13}CO_2$ and N_2-fixation rates (µmoles ^{15}N mol N^{-1} d^{-1}) for soils enriched with $^{15}N_2$. Note that soil numbers are ordered from largest fixation rates to lowest (Table 3.1).

N_2 fixation

Coralloid roots collected from E. latifrons and exposed to 99 atom% $^{15}N_2$ were significantly ^{15}N enriched ($\delta^{15}N$ increased by 32.2‰) compared to the controls. This

indicates that N_2-fixation took place in this tissue that is known to contain blue-green algae (Spratt 1915). Since these samples were incubated in the dark, this N_2 fixation occurred in the absence of light. Despite this positive result, there was no evidence of N_2 fixation by the soils that were incubated under similar conditions (Fig. 3.1b). The $\delta^{15}N$ values of these soils were indistinguishable from the atmospheric $\delta^{15}N$ values. There was also no evidence of significant N_2 fixation when the N incorporation was expressed relative to soil N content (Fig. 3.2b).

Anaplerotic CO_2 fixation

Coralloid roots of E. spp. exposed 98 atom % $^{13}CO_2$ were significantly enriched in ^{13}C ($\delta^{13}C$ increased by 7.5‰) relative to roots in atmospheric CO_2, indicating that dark anaplerotic fixation of $^{13}CO_2$ occurred in these roots. There were no significant differences in $\delta^{13}C$ values between soils that were not supplied with $^{13}CO_2$ and those that were sterilized (whether exposed to $^{13}CO_2$ or not). Therefore, unsterilized soils that were not supplied with $^{13}CO_2$ were used as controls. In contrast, unsterilized soils exposed to $^{13}CO_2$ had significantly more positive $\delta^{13}C$ values than the other treatments (Fig. 3.1, Supp. Table 3.1) indicating incorporation of $^{13}CO_2$ by these soils. Despite the overall uptake of $^{13}CO_2$ by the soils, there was a large degree of variability between the extent of $^{13}CO_2$ incorporation between the soils (Fig. 3.2). The rate of anaplerotic activity (expressed per soil weight) was positively linearly related to the soil C concentration (Supp Fig. 3.2). The incorporation of $^{13}CO_2$ was also expressed relative to the soil C content because the capacity of microbial anaplerotic activity for enrichment of large pools of soil C is likely to be limited (Fig. 3.2). The range of this incorporation rate was between 5.1 and 57 μmol mol^{-1} C d^{-1}. Anaplerotic $^{13}CO_2$ fixation rates were significant linearly related to K and Fe, but to no other elemental constituent of the soils (Fig. 3.3). The field capacity of the soil had a significant negative linear relationship with anaplerotic $^{13}CO_2$ fixation rate. Likewise, the C:N ratios in the soils were strongly negative correlated with anaplerotic $^{13}CO_2$ fixation rate (Fig. 3.4). Soil C and N concentrations were also both strongly correlated with

anaplerotic $^{13}CO_2$ fixation rate with high rates of fixation occurring in soils with low C and N concentrations (Fig. 3.4).

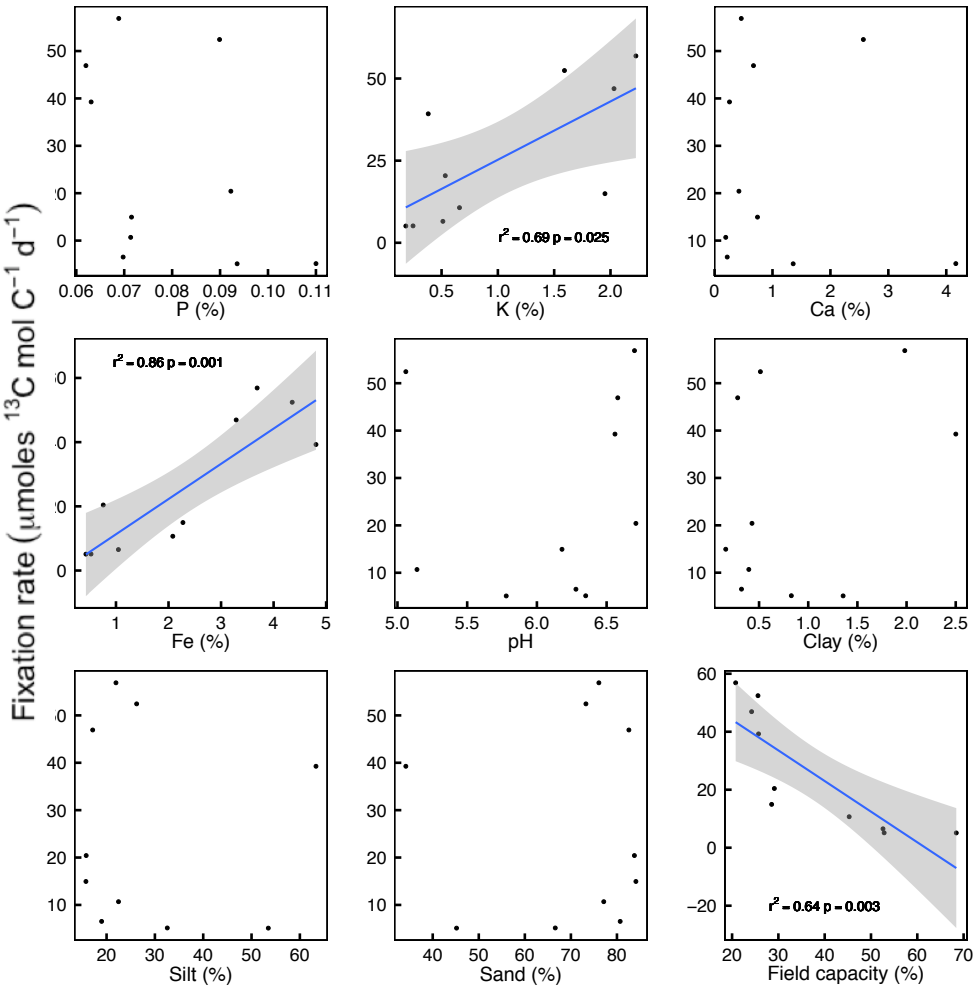


Figure 3.3: Anaplerotic $^{13}CO_2$ fixation rate (µmoles C^{13} mol C^{-1} d^{-1}) against a number of soil variables for soils supplied with $^{13}CO_2$ for 3 d. Significant linear fits are indicated by regression lines and 95% confidence interval is represented by the grey bands. K(%): F = 7.52, df = 8, p = 0.025; Fe(%): F = 22.95, df = 8, p = 0.001; Field capacity(%): F = 16.7, df = 8, p = 0.003.

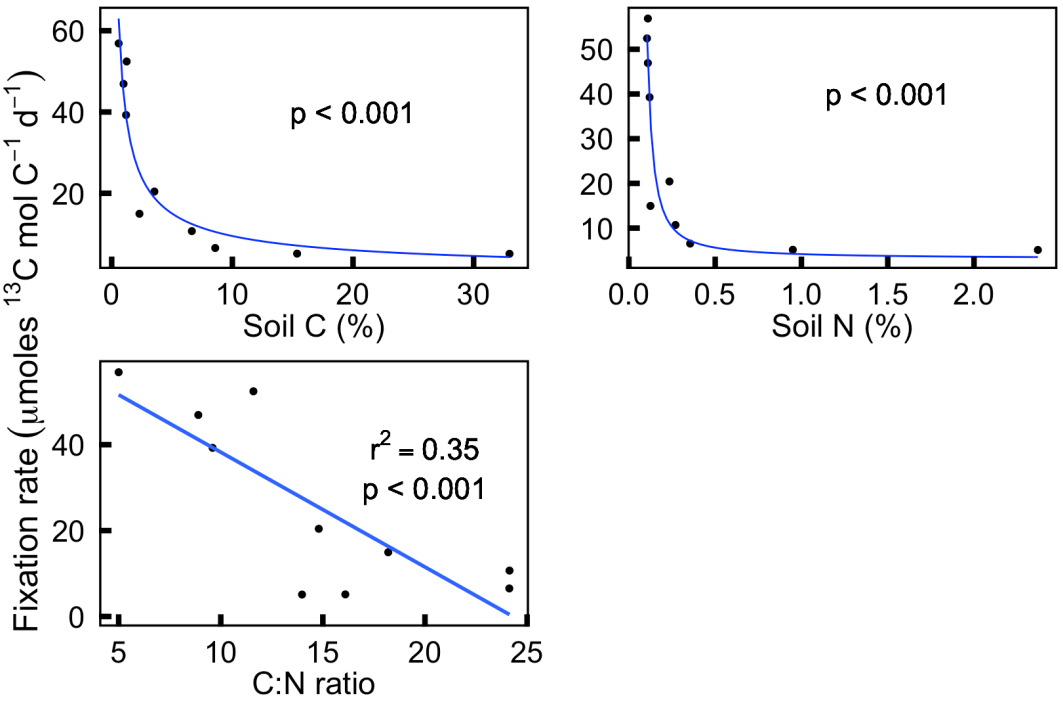


Figure 3.4: Anaplerotic $^{13}CO_2$ incorporation rate (µmoles C^{13} mol C^{-1} d^{-1}) plotted against soil C % (w/w) N % (w/w) and C/N ratios for ^{13}C enriched soils used in the experiment. Soil C was fitted with $43.6(x^{-0.663})$, soil N% with $3.09(1.35^{(1/x)})$ and soil C/N ratios by -2.67x +64.99.

Soil moisture and temperature effects on CO_2- and N_2- fixation

Anaplerotic $^{13}CO_2$ fixation rate did not differ significantly from that of soils not exposed to $^{13}CO_2$ when maintained at 10% (w/w) of measured soil field capacity (Fig. 3.5, Supp. Table 3.1). In contrast, the rate of anaplerotic $^{13}CO_2$ fixation was significantly higher than in soils not exposed to $^{13}CO_2$ at 50% and 100% of field capacity, indicating strong dependence of anaplerotic fixation on water availability. Consistent with the lack of evidence of N_2-fixation, there was no evidence of a significant effect of soil moisture content on $^{15}N_2$–fixation (Fig. 3.5, Supp. Table 3.1).

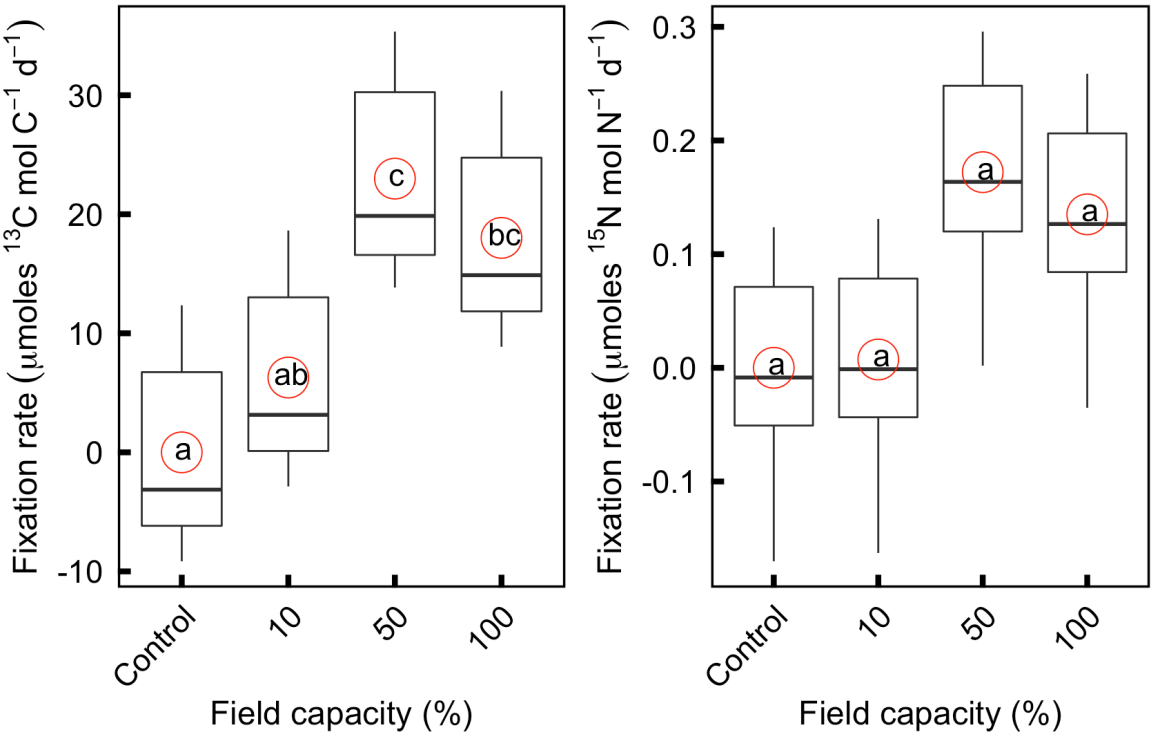


Figure 3.5: Anaplerotic $^{13}CO_2$ incorporation (µmoles C^{13} mol C^{-1} d^{-1}) and $^{15}N_2$–fixation (µmoles N^{15} mol N^{-1} d^{-1}) rates for control (not enriched) and $^{13}CO_2$ or $^{15}N_2$ enriched soils at 10, 50 and 100% field capacity at 25˚C. The same letters indicate a non-significant difference ($p > 0.05$) as calculated by a Tukey post hoc test on a LME model results (Statistics are summarized in Supp. Table 3.1).

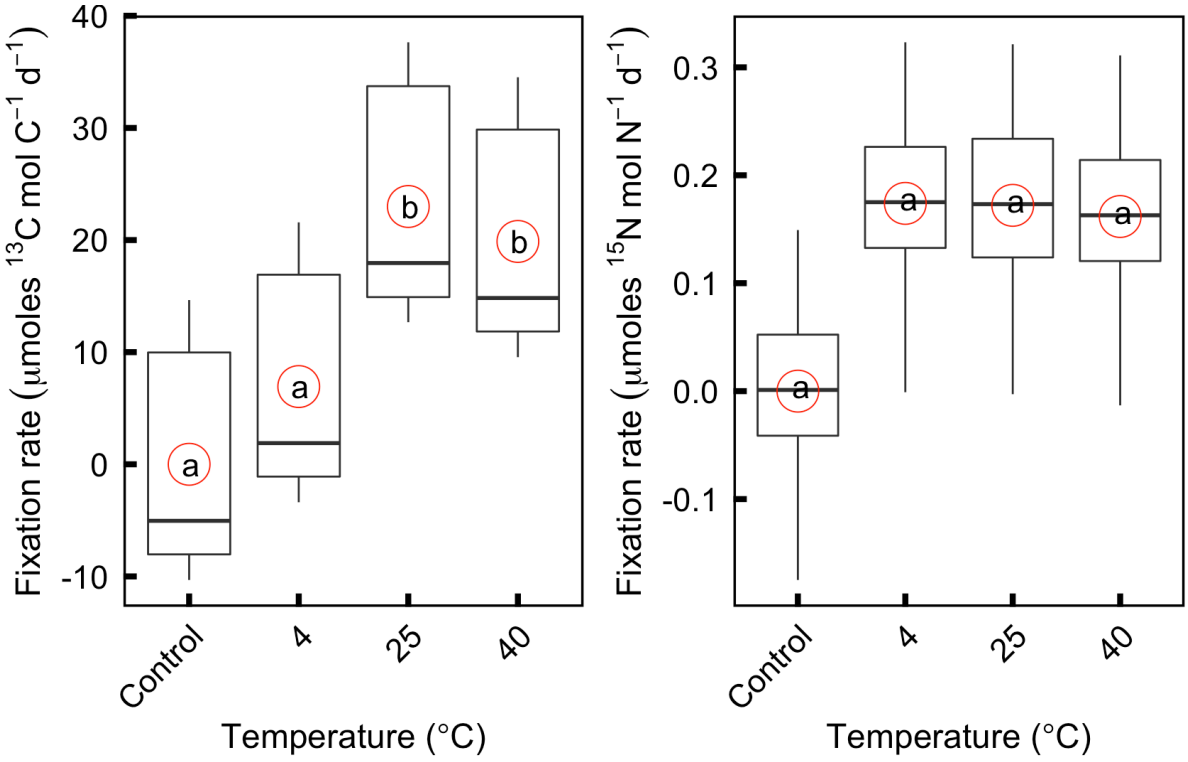


Figure 3.6: Anaplerotic $^{13}CO_2$ incorporation (µmoles C^{13} mol C^{-1} d^{-1}) $^{15}N_2$–fixation (µmoles N^{15} mol N^{-1} d^{-1}) rates for control (not enriched) and $^{13}CO_2$ or $^{15}N_2$ enriched soils incubated at 4, 25 and 40˚C and 50% field capacity. The same letters indicate a non-significant difference ($p > 0.05$) as calculated by a Tukey post hoc test on a LME model results (Statistics are summarized in Supp. Table 3.1).

Anaplerotic $^{13}CO_2$ fixation rate in soils maintained at 4°C did not differ significantly from soils not exposed to $^{13}CO_2$ (Fig. 3.6, Supp. Table 3.1). In contrast the rate of anaplerotic $^{13}CO_2$ fixation was significantly higher than in soils not exposed to $^{13}CO_2$ at both 25°C and at 40°C. There was no evidence of a significant temperature effect on soil $^{15}N_2$–fixation (Fig. 3.6, Supp. Table 3.1).

Discussion

We found evidence for dark CO_2-fixation by soil microorganisms in a number of soils with varying characteristics, but not for N_2-fixation. Although soil surface fixation of CO_2 by RubisCO is likely (Nowak et al. 2015), this only pertains to fixation in the upper surface soils (Wu et al. 2013). Considering that the soils in this investigation were incubated in the dark, there was no possible influence of autotrophic CO_2-fixation by

microbes. The detected CO_2 fixation is therefore attributable to dark anaplerotic CO_2 fixation. All soils that were exposed $^{13}CO_2$ incorporated ^{13}C label to varying degrees (Fig. 3.2), as did the roots of E. spp. The lack of evidence of N_2 fixation could have been due to insensitivity of our technique, but the fact that we successfully detected dark $^{15}N_2$ fixation in E. spp suggests that if the rates of fixation were high we should have been able to detect these. Therefore, we conclude that rates of N_2–fixation in the soils we surveyed were low. No doubt, other soils may have greater rates of N_2 fixation and when this does occur, it is likely that it would be strongly associated with anaplerotic CO_2 fixation to supply C-skeletons for amino acid synthesis (Cramer et al. 1993). The observation that soil microbes are capable of anaplerotic CO_2 fixation is not novel (Miltner et al. 2005), but here we present evidence for the importance of this in partially determining changes in soil $\delta^{13}C$ values along with the extent of SOM decomposition.

The measured variability in the rates of anaplerotic $^{13}CO_2$ fixation must be linked to soil properties and the soil microbial biomass. For example, increased microbial incorporation of soil CO_2 with H_2 or reduced S compounds added to soils has been attributed to changes in the microbial community and decomposition of complex molecules (Stein et al. 2005; Dong & Layzell 2001). The ability of soil microbes to mineralize and decompose organic matter is linked to a range of temperatures (Curiel Yuste et al. 2007)) and moisture levels (Davidson & Janssens 2006) in which they can function. If the soil is too dry or waterlogged, the soil microbes will either dry out or become aerobically limited causing them to either become dormant if moisture is too low or die. In our experiments in which a small sample of soil (ca. 10 g) was incubated, waterlogging imposed diffusion limitations (e.g. at 100% field capacity) are likely to be of minor importance. However, under wet conditions soils with high field capacity may limit gaseous diffusion, thereby limiting microbial activity and biomass and possibly explaining why anaplerotic CO_2 fixation was negatively related to soil field capacity (Fig. 3.5). We found evidence that anaplerotic CO_2 fixation was sensitive to temperature with no evidence of fixation at 4°C.

Since soil microbial communities are strongly determined by the edaphic properties of the soils (Fierer et al. 2009), it is likely that these also determine the rates of microbial anaplerotic CO_2 fixation. However, we found relatively weak association of anaplerotic fixation with soil chemical characteristics, apart from K, Fe, C and N. The variability in anaplerotic fixation was most strongly associated with soil C and N, which are in turn dependent on vegetation and faunal inputs to the soil. The lack of significant association between soil elements other than K, Fe, C and N is, however, not too surprising since the relationship between microbial communities and elemental characteristics is complex, being necessarily influenced by the entire functioning of the ecosystem as well as disturbance events (e.g. fire, anthropogenic disturbance). Soil microbial biomass is generally correlated with SOC across multiple biomes (Fierer et al. 2009). Although anaplerotic activity is high in soils with high SOC (Supp. Fig. 3.2) and thus high microbial biomass, the contribution of this fixed C is low, because it comprises a small proportion of SOC. When expressed per C, the rates of anaplerotic activity were high in low SOC soils (Fig. 3.4). SOC enters the soil through a number of inputs that include vegetation, and thus plant functional types significantly influence the amount and distribution of SOC in the soil profile (Jobbágy & Jackson 2000) through both above- and below-ground (i.e. root) inputs. SOC generally decreases with depth because organic C enters the soil from above-ground inputs, but subsoil horizons are characterized by high mean residence times (i.e. thousand years) and is highly processed (Rumpel & K gel-Knabner 2010). This means that continuous recycling of SOC and cummulative effects of anaplerotic activity are likely to be especially important for C deeper in soils.

Soil C:N ratios are commonly used as an indicator of the extent of decomposition rates (Wang et al. 2015). During decomposition microbes respire organic C while organic N is recycled to a greater extent (Miltner et al. 2004) and thus low C:N ratios are associated with more highly processed SOM deeper in soils (Tian et al. 2010). The negative association of soil C:N with rates of anaplerotic CO_2 fixation is therefore consistent with soils in which decomposition is further advanced having higher

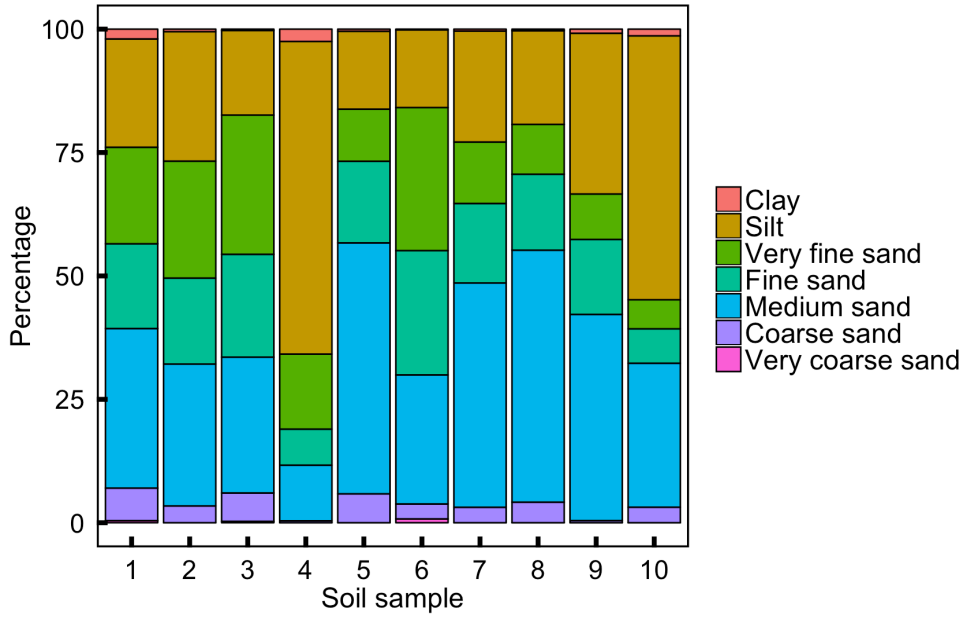
anaplerotic activity. More highly processed SOM is likely to have limited availability of organic N compounds that can be directly utilized, requiring greater de novo synthesis of amino compounds that would depend on anaplerotic CO_2 fixation (Rovira & Vallejo 2002).

Soil inorganic C (SIC) in the soil atmosphere originates from diffusion or advection of inorganic C into the soil from the bulk atmosphere or from in situ microbial, faunal and floral respiration (Flessa et al. 2000). Although much of this respired CO_2 is lost into the bulk atmosphere, soil CO_2 concentrations can typically be 40 x higher than the bulk atmosphere (Enoch & Dasberg 1971) and generally increase with depth. The concentration of SIC is closely related to SOC which governs microbial respiration (MI et al. 2008) as well as soil texture and soil water content governing CO_2 diffusion. CO_2 in the soil atmosphere is thus a mixture of atmospheric CO_2 and microbially- and root-derived respiration (Flessa et al. 2000), with an isotopic composition dependent on the mixing ratios between these two pools. Dark re-fixation of this pool of CO_2 by roots and microbes would lead to a contribution of ^{13}C-enriched products to the formation of SOM (Gessler et al. 2007) and increased soil bulk $\delta^{13}C$ values. Assuming direct proportionality between the rates of anaplerotic CO_2 fixation we measured with 98 atom% $^{13}CO_2$ and those with atmospheric enrichment, we estimated that anaplerotic CO_2 fixation could drive a 1.2‰ change in soil $\delta^{13}C$ values per annum. We speculate that this, coupled with large atmospheric CO_2 inputs close to the soil surface, could partially explain the rapid change in $\delta^{13}C$ within the top 20 cm of the soil. Lower in the soil profiles, however, atmospheric CO_2 is likely less accessible, but continual recycling of C between microbial biomass CO_2 is likely to accumulate atmospheric isotopic characteristics, resulting in older SOM at depth that may be thousands of years old (Rumpel & Kögel-Knabner 2010) not reflecting the original $\delta^{13}C$ value of the input material. This means that anaplerotic CO_2 fixation has the potential to contribute significantly to the common pattern of increasing $\delta^{13}C$ with depth within soil profiles (Wynn et al. 2006).

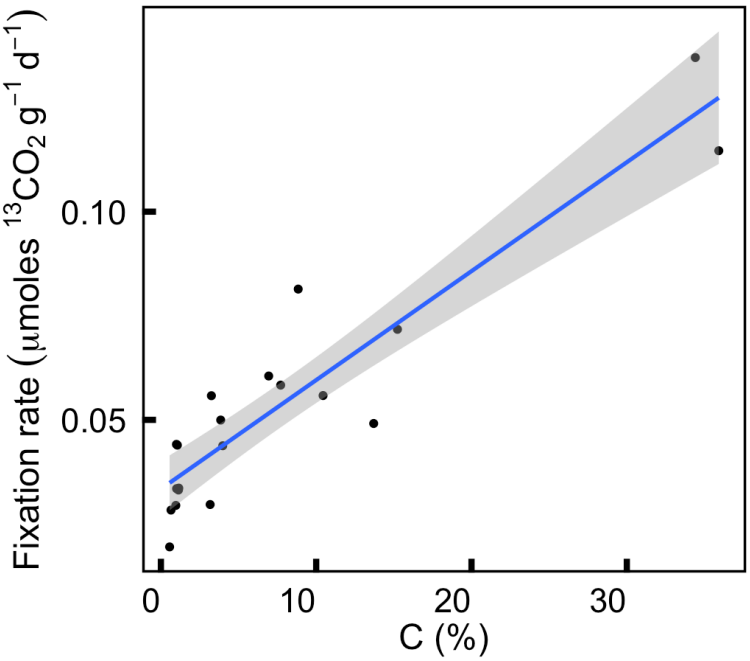
Conclusion

The evidence for significant fractionation of C during respiration is contradictory (Supp. Table 3.2), with some reports of strong discrimination against $^{13}CO_2$ (e.g. Fernandez et al. 2003; Šantrůčková et al. 2000) and others of little discrimination (e.g. Ekblad & Högberg 2000; Ekblad & Nordgren 2002; Lin & Ehleringer 1997). Anaplerotic CO_2 fixation has been widely reported for both microbes and plants and we have presented evidence that this incorporates atmospheric $^{13}CO_2$ at significant rates into 10 diverse soils. Although $\delta^{13}C$ values of C inputs to soils are indisputably important, anaplerotic CO_2 fixation could potentially contribute to determining the $\delta^{13}C$ variation of SOM with depth. Because it is experimentally difficult to dissociate respiratory CO_2 flux from concurrent anaplerotic CO_2 fixation, measurements of discrimination against respiratory $^{13}CO_2$ production may in fact sometimes include the anaplerotic fixation of CO_2 and its effects on $\delta^{13}C$ of CO_2.

Supplementary Material



Supp. Fig. 3.1: Comparison of the proportion of the soil volume (%) comprising different particle sizes (upper limit of each class in µm: clay <4, silt <62, very fine sand <125, fine sand <250, medium sand <500, coarse sand <1000, very coarse sand <2000) between soil samples. Soil sample number corresponds to soil samples in Table 3.1.



Supp. Fig. 3.2: $^{13}CO_2$ fixation rate (µmoles g^{-1} soil dry weight d^{-1}) against soil C (%, w/w) for soils maintained at 25˚C and at 50% field capacity. Note that in contrast to Figs 2, 3, 4, 5 and 6, here $^{13}CO_2$ fixation rate is expressed per gram of soil. Regression line indicates significant linear fit and grey bad represents the 95% confidence interval ($F = 107.1$, $df = 18$, $p < 0.001$).

Supp. Table 3.1: Summary statistics of the linear mixed effects modelling (Batjes 1996; Bouwman et al. 1997) used to assess significance of soil $^{13}CO_2$ and $^{15}N_2$ fixation rates between treatments with varying field capacity and varying temperature (Fig. 3.5, 3.6). Bold p-values indicate statistical significance, $df_1 = 3$.

Treatment	Fixed effect	Estimate	S.E.	df	t-value	p
Field capacity-$^{13}CO_2$	Not enriched (intercept)	-6.283	4.485	37	-1.304	0.200
	100%, enriched	11.733	1.269	37	2.434	**0.019**
	50%, enriched	16.710	4.174	37	4.004	**< 0.001**
	10%, enriched	6.283	4.819	37	1.401	0.169
Field capacity -$^{15}N_2$	Not enriched (intercept)	0.079	0.063	37	1.239	0.223
	100%, enriched	0.127	0.100	37	1.276	0.209
	50%, enriched	-0.022	0.095	37	-0.235	0.815
	10%, enriched	-0.080	0.089	37	-0.903	0.372
Temperature-$^{13}CO_2$	Not enriched	-24.127	4.460	27	-5.409	**< 0.001**
	40°C, enriched	-4.252	4.460	27	-0.953	0.348
	25°C, enriched	24.127	4.217	27	5.722	**< 0.001**
	4°C, enriched	-17.195	4.460	27	-3.855	**< 0.001**
Temperature-$^{15}N_2$	Not enriched (intercept)	-0.172	0.068	27	-2.541	**0.015**
	40°C, enriched	-0.010	0.068	27	-0.153	0.879
	25°C, enriched	0.172	0.048	27	3.559	**0.001**
	4°C, enriched	0.002	0.068	27	0.027	0.978

Supp. Table 3.2: Summary of published values for ^{13}C fractionation between SOM and respired CO_2 and between microbial biomass and respired CO_2. The values presented are the differences between the $\delta^{13}C$ values of the CO_2 and the source. Negative values indicate fractionation against $^{13}CO_2$ release. The reference for each data item is indicated. Simple mean of the differences across all studies is shown.

Sample	CO_2-SOM (‰)	Reference	Sample*	CO_2-Microbial (‰)	Reference
Bulk soil	-0.30	Breeker et al. (2014)	Tropical soil a	0.30	Santruckova et al. (2000a)
Bulk soil	0.10	Breeker et al. (2014)	Tropical soil b	5.50	Santruckova et al. (2000a)
Bulk soil	0.40	Breeker et al. (2014)	Temperate soil a	-2.30	Santruckova et al. (2000a)
Bulk soil	0.00	Breeker et al. (2014)	Temperate soil b	3.00	Santruckova et al. (2000a)
Litter layer	0.00	Bostrom et al. (2007)	Bulk soil	4.27	Qian et al. (1997)
0–2 cm	0.50	Bostrom et al. (2007)	Bulk soil	4.06	Qian et al. (1997)
Roots	1.80	Cheng (1996)	Bulk soil	2.62	Qian et al. (1997)
Bulk soil	-5.30	Cheng (1996)	Bulk soil	-3.27	Santruckova et al. (2000b)
Bulk soil	0.11	Qian et al. (1997)	Bulk soil	-5.72	Santruckova et al. (2000b)
Bulk soil	-0.38	Qian et al. (1997)	Bulk soil	-3.25	Santruckova et al. (2000b)
Bulk soil	-2.17	Qian et al. (1997)	Bulk soil	-4.06	Santruckova et al. (2000b)
Bulk soil	-0.17	Santruckova et al. (2000b)	Bulk soil	-3.29	Santruckova et al. (2000b)
Bulk soil	-3.82	Santruckova et al. (2000b)	Bulk soil	-1.83	Santruckova et al. (2000b)
Bulk soil	-0.55	Santruckova et al. (2000b)	Bulk soil	-0.38	Santruckova et al. (2000b)
Bulk soil	-0.36	Santruckova et al. (2000b)	Bulk soil	-0.71	Santruckova et al. (2000b)
Bulk soil	-1.89	Santruckova et al. (2000b)	Bulk soil	-0.92	Santruckova et al. (2000b)
Bulk soil	-0.53	Santruckova et al. (2000b)	Bulk soil	-2.98	Santruckova et al. (2000b)
Bulk soil	0.42	Santruckova et al. (2000b)	Bulk soil	-2.36	Santruckova et al. (2000b)
Bulk soil	-2.11	Santruckova et al. (2000b)	Bulk soil	-2.57	Santruckova et al. (2000b)
Bulk soil	1.48	Santruckova et al. (2000b)	Bulk soil	-1.33	Santruckova et al. (2000b)
Bulk soil	-4.28	Santruckova et al. (2000b)	Bulk soil	-1.03	Santruckova et al. (2000b)
Bulk soil	-0.56	Santruckova et al. (2000b)	Bulk soil	-1.33	Santruckova et al. (2000b)
Bulk soil	0.03	Santruckova et al. (2000b)	Bulk soil	-3.18	Santruckova et al. (2000b)
Bulk soil	0.57	Santruckova et al. (2000b)	Bulk soil	-2.77	Santruckova et al. (2000b)
Bulk soil	1.07	Santruckova et al. (2000b)	Bulk soil	-2.26	Santruckova et al. (2000b)
Bulk soil	-0.43	Santruckova et al. (2000b)	Bulk soil	-2.15	Santruckova et al. (2000b)
Bulk soil	0.02	Santruckova et al. (2000b)	Bulk soil	-2.77	Santruckova et al. (2000b)
Bulk soil	0.53	Santruckova et al. (2000b)	Bulk soil	-0.10	Santruckova et al. (2000b)
Bulk soil	1.04	Santruckova et al. (2000b)	Bulk soil	0.65	Werth et al. (2006)
Bulk soil	1.15	Santruckova et al. (2000b)	Bulk soil	3.67	Werth et al. (2006)
Bulk soil	0.23	Santruckova et al. (2000b)			
Bulk soil	1.60	Santruckova et al. (2000b)			
Bulk soil	1.38	Werth et al. (2006)			
Bulk soil	5.49	Werth et al. (2006)			
Mean	**-0.15**		**Mean**	**-0.88**	

* a = 10 day incubation, b = 40 day incubation.

Chapter 4

General Discussion and Synthesis

Soil $\delta^{13}C$ and $\delta^{15}N$ values tend to increase with depth (Chapter 1: Fig. 1.1) and for $\delta^{13}C$ this has been commonly attributed to vegetation changes (C_3/C_4 shifts). The similarity in $\delta^{13}C$ and $\delta^{15}N$ profiles (Chapter 2: Fig. 2.1 and 2.2), however, suggests that microbial decomposition may play an important role because both carbon (C) and nitrogen (N) are also thought to be fractionated through microbial processing (Chen et al. 2005; Hobbie & Ouimette 2009). There is some evidence supporting the claim that microbial decomposition plays a more important role than vegetation changes in determining soil $\delta^{13}C$ values through soil profiles. For example, the increase in $\delta^{13}C$ and $\delta^{15}N$ valued with depth is common, and often highly correlated (Boström et al. 2007; Hobbie & Ouimette 2009), and has been shown to occur across a wide range of climates and biomes (Chapter 2: Fig. 2.1, 2.2 and 2.5; Table 2.1). An analysis of the factors driving the strength of the correlation between soil $\delta^{13}C$ and $\delta^{15}N$ values with depth suggests that it is relatively independent of vegetation type (Chapter 2: Fig. 2.2). Instead, microbial influence is strong because soil and climatic variables, which potentially influenced microbial biomass and function, were best able to explain the observed correlations (Chapter 2: Fig. 2.4a). Further, the same analysis showed that the global drivers of soil $\delta^{15}N$ strongly relate to soil $\delta^{13}C$ values as well as with factors possibly related to microbial processing (Chapter 2: Fig. 2.4b and 2.6). The analysis also showed that sites in which the correlation between $\delta^{13}C$ and $\delta^{15}N$ broke down (Chapter 2: Fig 2.3), were as a result of other soil processes such as volatilization and N_2-fixation being particularly high. It is indisputable that vegetation and faunal inputs to soils do strongly influence the $\delta^{13}C$ and $\delta^{15}N$ values, especially of the surface soils, and that decomposition related processes influence the changes in these values over time and with depth. In this synthesis I address the question of whether the soil isotope values can be disambiguated so that vegetation inputs can be discerned.

While the correspondence between $\delta^{13}C$ and $\delta^{15}N$ can be related to decomposition, the factors that determine soil $\delta^{13}C$ and $\delta^{15}N$ are extremely complex and microbial decomposition and vegetation shifts are not the only processes that drive the fractionation of the isotopes with depth. For $\delta^{15}N$ there was no evidence found in this study of an influence of N_2 fixation. Despite this there are circumstances in which microbial N_2 is important and it has been shown to fractionate N between -2 and 2 ‰ (Hogberg 1997). For $\delta^{13}C$, a problem with the explanation that microbial decomposition and respiration fractionate both soil organic matter (SOM) and CO_2, respectively, is that kinetic fractionation of soil C by decomposition is controversial, and a number of studies have found contradictory evidence for the role of respiration in fractionation (Chapter 3: Supp. Table 3.2). For example, several studies have demonstrated no kinetic fractionation during decomposition (Ekblad & Högberg 2000; Ekblad & Nordgren 2002; Lin & Ehleringer 1997; Bernoux et al. 1998; Amundson et al. 1998; Ågren et al. 1996; Wynn, Bird & Wong 2005a; Bowen & Beerling 2004; Cleveland et al. 2004), while others have demonstrated a significant fractionation of respired CO_2 during decomposition (Fernandez et al. 2003; Šantrůčková et al. 2000). There are, however, processes such as anaplerotic CO_2 fixation and gaseous mixing which might play an important role in determining soil $\delta^{13}C$ values with depth. Anaplerotic CO_2 fixation allows soil microbes and roots to assimilate CO_2 from the soil atmosphere and incorporate the C into cellular biomass by providing C intermediates depleted in the TCA-cycle (Miltner et al. 2004). An analysis of ten soils from across South Africa demonstrated this as soil microbes were shown to incorporate labeled $\delta^{13}C$ into biomass under varying conditions (Chapter 3: Fig. 3.2). The anaplerotic CO_2 fixation rate is strongly related to soil nutrients (Chapter 3: Fig. 3.3 and 3.4), moisture (Chapter 3: Fig 3.3 and 3.5) and temperature (Chapter 3: Fig 3.6) with highest rates found in soils that were at 50% field capacity (w/w) and 25°C, which are within favorable conditions for microbial biomass and growth. Further, high rates of anaplerotic CO_2 fixation were found in soils with low soil C, N and C/N ratios. This suggests that microbial and root anaplerotic CO_2 fixation

may have an influence on soil $\delta^{13}C$ values and previous work has demonstrated that up to 10% of the microbial cell C may be derived from anaplerotic reactions (Perez & Matin 1982). It is clear that anaplerotic activity has the potential to contribute C to the SOM pool, but in this synthesis I discuss (below) the assessment of the quantitative contribution of this source of C to the SOM pools in the soil.

Soil CO_2 is comprised of a mixture between atmospheric CO_2 and the soil CO_2 pool, which includes CO_2 from autotrophic and heterotrophic respiration. The mixture of the two pools greatly influences the $\delta^{13}C$-CO_2 value and there is less incorporation of atmospheric CO_2 into the deeper soil pools (Cerling 1984). Diffusion of low $\delta^{13}C$ bulk atmospheric CO_2 (ca. -10‰) into the soil atmosphere (<< -10‰) will drive soil CO_2 $\delta^{13}C$ towards ca. -10‰, and constant anaplerotic CO_2 fixation will result in SOM $\delta^{13}C$ also tending towards -10‰ in more highly processed SOM deeper in the soil. This would lead to the pattern of $\delta^{13}C$ enrichment with depth which may potentially mask other soil processes and limit the appropriateness of paleo-interpretations. In order to account for this, the extent of anaplerotic CO_2 fixation and gaseous mixing would need to be determined for any given site. This leaves the question (discussed below) of how to establish a reliable way of determining the amount of anaplerotic C incorporated into SOM as well as the result of this C having a different $\delta^{13}C$ value to the bulk SOM.

Measurement of anaplerotic activity

Direct measurements of anaplerotic activity in situ is difficult because it requires differentiation of the respiratory efflux of CO_2 from the refixation of respired CO_2 and the potential uptake of CO_2 from the soil atmosphere. There are, however, a number of ways in which anaplerotic CO_2 fixations' contribution to SOM can be determined. For example, similar to the method used in the experimental investigation in Chapter 3, one can use labeled isotope analysis to determine fixation rates and to quantify anaplerotic C inputs (Chapter 3: Fig. 3.2, 3.3 ,3.4, 3.5 and 3.6; see also Nowak et al. (2015):

$$\text{Anaplerotic C [mg]} = \frac{^{13}C_{\text{after labeling}}}{^{12}C_{\text{after labeling}}} * C_{\text{sample}}[\text{mg}] - \frac{^{13}C_{\text{before labeling}}}{^{12}C_{\text{before labeling}}} * C_{\text{sample}}[\text{mg}] \quad (1)$$

The $^{13}C/^{12}C$ ratio can be obtained from the measured $\delta^{13}C$ as follows:

$$\frac{^{13}C}{^{12}C}\text{sample} = \left(\frac{\delta^{13}C_{\text{measured}}}{1000} + 1\right) * 0.011237 \quad (2)$$

This method has been applied to soil samples in vitro and the results showed significant photosynthetic activity of CO_2 assimilating microbes in the top 10 cm, as $\delta^{13}C$ values of SOM were shifted by up to 2 ‰ towards more negative values. While the method has been shown to produce good results for both anaplerotic (Chapter 3) and photosynthetic fixation in soils (Nowak et al. 2015), soil disturbance alters the CO_2 concentration in the soil, alters the soil moisture properties and may influence soil texture and bulk density. It is therefore desirable to attempt to measure anaplerotic activity in situ. This can be complicated to do as it would require the controlled introduction of labeled $^{13}CO_2$ which can be problematic at different depths. Here I suggest that this could be achieved using a simple method in which a pipe is inserted into the soil and labeled CO_2 is allowed to flow through it for a time (Fig. 4.1). This would introduce a uniform labeled-CO_2 concentration while displacing prior soil CO_2. This is likely to influence soil properties to some extent. From my experience of introducing $^{13}CO_2$ to soil sample in the laboratory, however, such a labelling study could be completed in a few days prior to removal of the soil and extraction for analysis.

An alternate method which does not use ^{13}C-labeled isotopes would be to use natural labels such as C_3 and C_4 derived sucrose as used previously (Ekblad & Högberg 2000). For example, one could bury sucrose depots in the soil in water- and gas-permeable sachets and analyze the contribution of the added sucrose to soil SOM $\delta^{13}C$ values.

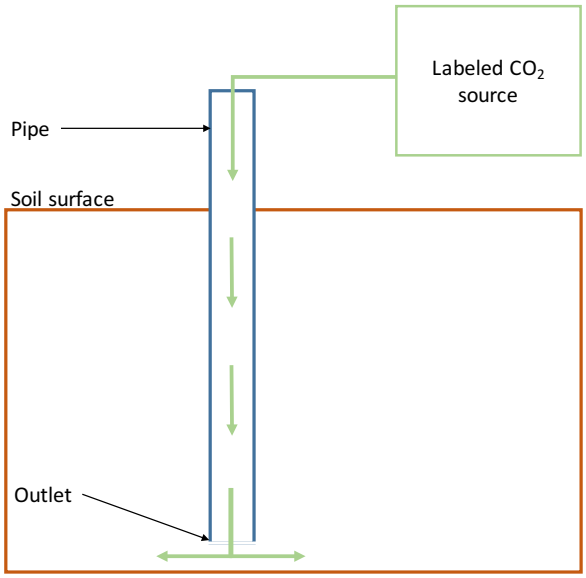


Figure 4.1: Method for the controlled introduction of labeled $^{13}CO_2$ into a soil depth profile. A pipe is inserted into the soil compartment and labeled CO_2 is allowed to flow through the pipe into the soil for a duration. Green arrows indicate flow of labeled CO_2.

This is because the sucrose would be used for respiration by the soil microbes leading to respired CO_2-$\delta^{13}C$ values close to that of the sucrose which may be of either C_3 or C_4 origins (Ekblad et al. 2002), essentially labeling the soil atmosphere. Any subsequent change in SOM-$\delta^{13}C$ values could be due to anaplerotically fixed CO_2. The soil CO_2-$\delta^{13}C$ could be monitored after sucrose additions and soils collected and SOM-$\delta^{13}C$ determined. The advantages of using natural labels is that they are easily obtained and inexpensive, however, the method likely has relatively low sensitivity compared to ^{13}C-enrichment techniques (due to more subtle changes in the $\delta^{13}C$ of CO_2) and it could be difficult to produce consistent labeled-CO_2 concentrations as microbial respiration is not consistent (Fierer et al. 2009).

While label experiments have been useful in determining anaplerotic CO_2 contributions to SOM, it is also possible to estimate this contribution without the use of labeled isotopes by applying mixing equations which uses two isotopic signatures (δ_1 and δ_2) to partition the contributions (f_a and f_b) of two sources (a and b) to a mixture (m) (Fry 2006):

$$\delta_m = f_a\delta_1 + f_b\delta_2$$
$$1 = f_a + f_b \quad (3)$$

There are, however, a number of assumptions that need to be made for the mixing equation to be used. One would need to assume that the fractionation during respiration is nil and that roots, mycelia and other sources of respiratory CO_2 are not present. While it is possible to solve isotope mixing models with multiple components with more than one isotopic species, the problem for doing this with anaplerotic CO_2 fixation is that there is no other isotope. As an example of using this method, we used the data of Boström et al. (2007), in which they determined whether fractionation during respiration could be responsible for the increase in soil $\delta^{13}C$ values with depth. These authors collected soil samples at multiple depths and removed root material and allowed samples to equilibrate and dissipate photosynthetic sources of respiratory CO_2. As a consequence, root and mycelial respired CO_2 from these sources are likely negligible. Further, when sampling soil CO_2, soil samples were placed in a closed atmosphere that was initially CO_2-free and the air recycled through the system allowing respired CO_2 to accumulate. Boström et al. (2007) concluded that there was no evidence for fractionation during SOM respiration. They interpreted their data as indicating changes in SOM $\delta^{13}C$ as a consequences of historical changes in $\delta^{13}C$ of CO_2 that could have influenced the $\delta^{13}C$ of the SOM. In contrast, by assuming no fractionation during respiration, I used their data to calculate the potential anaplerotic CO_2 fixation contribution for each depth in their sampled soils (to a depth of 35 cm). For each sample, I adjust Eqn. 3 in order to determine the proportion of SOM (f_a) at depth n+1 that is derived from anaplerotic CO_2 fixation:

$$\text{SOM-}\delta^{13}C_{\text{depth n+1}} = (f_a)\text{SOM-}\delta^{13}C_{\text{depth n}} + (f_b)\ CO_2\text{-}\delta^{13}C_{\text{depth n+1}} \quad (4)$$

This is useful as it can give an indication of anaplerotic activity without the use of introduced isotopic labels. The results indicate that the portion of SOM which is derived from the anaplerotic fixation of CO_2 is high in the surface soils but declines

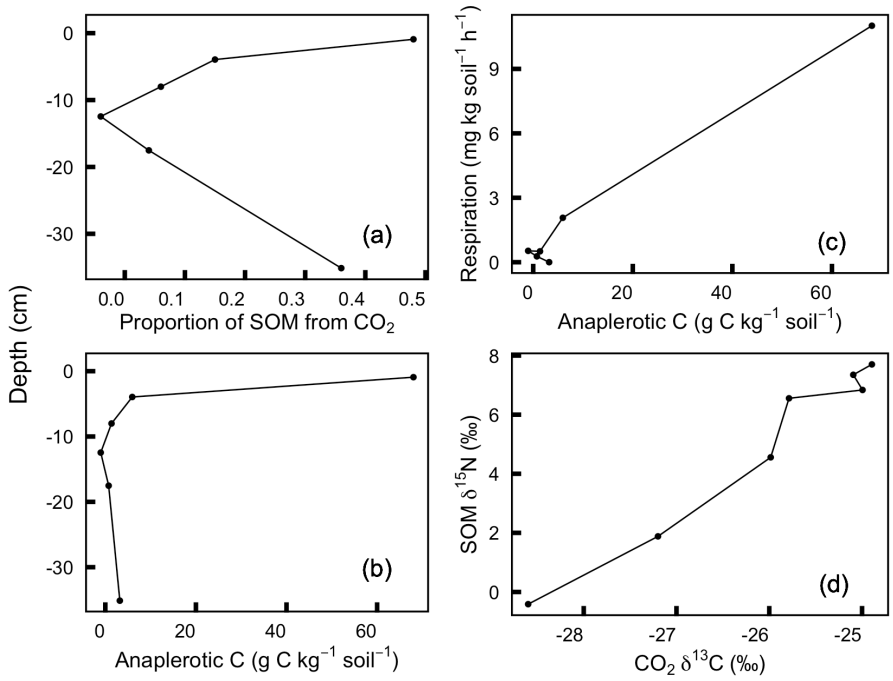


Figure 4.2: a) Proportion of SOM carbon derived from CO_2 with depth (using Eqn. 3), b) amount of anaplerotic carbon (g C kg^{-1} $soil^{-1}$) with depth, c) amount of anaplerotic carbon (g C kg^{-1} $soil^{-1}$) against respiration rate (mg kg $soil^{-1}$ h^{-1}), d) CO_2-$\delta^{13}C$ value against SOM-$\delta^{15}N$ value. Data recalculated from Bostrom et al. (2007).

until about 12 cm, where after it increases again (Figure 4.2a). What is perhaps more useful is that the estimated proportion can then be normalized for soil C in order to determine the amount of anaplerotic C in the soil at any given depth (Fig. 4.2b). By doing this we find that the highest amounts of anaplerotic C is in the upper ca. 5 cm of soil and deeper horizons appear to contain little anaplerotic C in comparison. Given the interpretation of the results in Chapter 3, this is likely due to higher microbial biomass occurring in the upper soil surface layers compared to deeper layers (Kuzyakov & Blagodatskaya 2015), thus allowing more anaplerotic C to accumulate.

This data allows further examination of the relationship between anaplerotic CO_2 fixation and other variables. For example, the relationship between respiration and anaplerotic C concentrations in soil indicates that as respiration increases, so does the contribution of anaplerotic CO_2 fixation to SOM (Fig. 4.2c). This is not surprising given that conditions found to enhance microbial biomass and growth (i.e. 25°C and 50% field capacity) also increase anaplerotic CO_2 fixation rates (Chapter 3: Fig. 3.3,

3.4, 3.5 and 3.6). Further, the strong association between soil CO_2-$\delta^{13}C$ values and SOM-$\delta^{15}N$ values from the same data set (Fig. 4.2d) is not unexpected given that microbially respired CO_2 will carry $\delta^{13}C$ values similar to that of SOM (assuming zero fractionation) and globally there is a strong correlation between SOM $\delta^{13}C$ and $\delta^{15}N$ values (Chapter 2: Fig. 2.1, 2.2 and 2.5; Table 2.1). By using an independent data set from literature to test our hypothesis of anaplerotic CO_2 fixation contributing to soil SOM-$\delta^{13}C$ values, the congruency of explanations demonstrated above further emphasize the importance of the process. In order to determine whether a site has significant anaplerotic CO_2 fixation, however, one would need to perform a labeled isotope analysis or one could use the method modified from (Boström et al. 2007) to determine proportional contributions. Given the general relationships found between anaplerotic CO_2 fixation rates and microbial growth parameters, however, a model could possibly serve to estimate anaplerotic CO_2 fixation and its influence on soil C isotopes.

Disambiguating vegetation versus microbial soil $\delta^{13}C$ values

The question arises as to whether the relatively consistent changes in soil $\delta^{13}C$ and $\delta^{15}N$ with depth globally can be estimated given soil properties. A number of models for estimating soil $\delta^{13}C$ with depth exist (Amundson et al. 1998; Wynn, Bird & Wong 2005b; Bowen & Beerling 2004; Cleveland et al. 2004). However, few take soil CO_2 into account which limits the ability to incorporate anaplerotic CO_2 fixation into the model structure. For example, Wynn et al. (2005b) demonstrated that SOM $\delta^{13}C$ values can be predicted using Rayleigh distillation modelling in which the ratio of ^{13}C to ^{12}C is a function of the fraction of remaining soil organic carbon, which is in turn driven by kinetic fractionation by decomposers. The Rayleigh distillation model, however, only pertains to closed systems, potentially ignoring continuous inputs and CO_2 mixing (Fry 2006) that does occur in soils and may underestimate the role of microbial processes in soil, as we argue was the case in the work by Gillson (2015). In contrast,

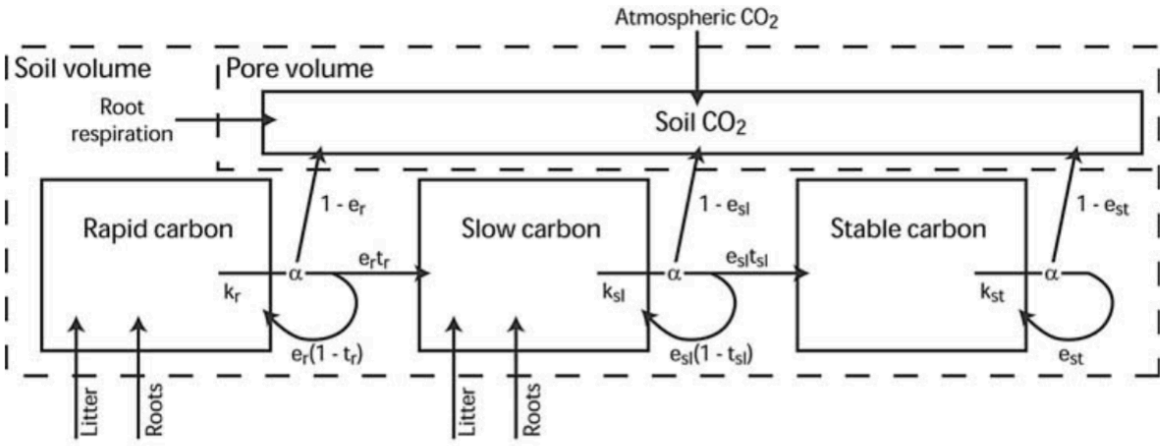


Figure 4.3: Bowen and Beerling: model for soil organic carbon and CO_2 reservoirs and fluxes assuming kinetic fractionation during microbial respiration. Parameters described in Table 4.1, see text for description. $k_r/k_{sl}/k_{st}$ = microbial consumption constants, $e_r/e_{sl}/e_{st}$ = efficiency of microbial assimilation, t_r/t_{sl} = transformation constant for decomposed SOM, α = fractionation factor for microbial respiration, $x_{1/2/3}$ = proportion of anaplerotically fixed CO_2.

Bowen and Beerling's (Bowen & Beerling 2004) Integrated model for soil organic carbon and CO_2, (Fig. 4.3, Table 4.1) is based on the "Generalized Compact Model" of Elzein and Balesdent (1995) and the soil CO_2 production-diffusion model of Cerling (1984), both of which produce good agreement with observations.

In this model, organic matter is added to soil from plants from both above- and below-ground. SOM is then divided into three pools based on the recalcitrance of the SOM and turnover time (rapid C, slow C and stable C), which both increase with depth. SOM is removed from each pool by soil microbes ($k_{r/sl/st}$), which respire some of the C to produce CO_2 (1- $e_{r/sl/st}$) and assimilate the rest as new biomass ($e_{r/sl/st}$). Microbial biomass is then returned to a SOM pool, with some carbon transformed to a more stable form ($t_{r/sl/st}$) while the rest is recycled to the SOM pool of origin (1- $t_{r/sl/st}$). The vertical movement of SOM in the model is based on advection and takes into consideration that processes that mix and distribute OM within soils decrease with depth. The soil respired CO_2 flux (1- $e_{r/sl/st}$), which also includes the products of root

Table 4.1: Model independent parameters and values. Subscript r, sl and st represents the rapid, slow and stable carbon pools respectively.

Symbol	Parameter	Unit	Value[a]
$C_{r,veg}/C_{sl,veg}$	litter C input	mol $m^{-2}yr$	24/6
ζ_r/ζ_{sl}	depth-integrated root C production	mol $m^{-2}yr$	12/18
Z	depth scaling term for root production	m	0.15
δ_{Veg}	plant carbon isotope composition	‰	- 26.6
C_{Atm}	atmospheric C concentration	mol m^{-3}	0.01185
δ_{Atm}	atmospheric carbon isotope composition	‰	-6.8
a_0	zero-depth advection constant for SOM	m yr^{-1}	0.004
τ	rate of decrease of *advection constant (a)* with depth	1 m^{-1}	0.5
d	diffusion constant for CO_2 in soil	m^2 yr^{-1}	81.7
ε	soil porosity	-	0.45
$k_{r/sl/st}$	microbial consumption constants	1 yr^{-1}	0.2/0.01/0.001
$e_{r/sl/st}$	efficiency of microbial assimilation	-	0.4/0.4/0.2
α	fractionation factor for microbial respiration	-	0.999
$t_{r/sl}$	transformation constant for decomposed SOM	-	0.45/0.15
L	basal depth of soil	m	1.5
$x_{1/2/3}$	proportion of anaplerotically fixed CO_2	-	-[b]

[a]Values taken from Bowen et al. (2004).
[b]Values can be estimated as described in text.

respiration, is estimated using Cerling (1984) model for the production and mixing of soil CO_2. This model takes into account the porosity of the soil and as well as the fact that CO_2 within soils is a mixture of atmospheric CO_2 and root- and microbially respired CO_2, which changes with depth. SOM-$\delta^{13}C$ values are determined in the model by applying the assumption that only microbial respiration is isotopically fractionating such that respired CO_2 is ^{13}C-depleted. This fractionation factor (α) is defined as the ratio of the respired C to the pool of C consumed by microbes. Similarly, soil CO_2-$\delta^{13}C$ values are determined using the respiration fractionation factor, soil porosity as well as mixing between respiration and atmospheric CO_2 pools.

While the model has been shown to produce good results (Bowen & Beerling 2004), it assumes that microbial respiration fractionates SOM. Further, it does not take into account the effects of anaplerotic CO_2 fixation from soil microbes and roots which

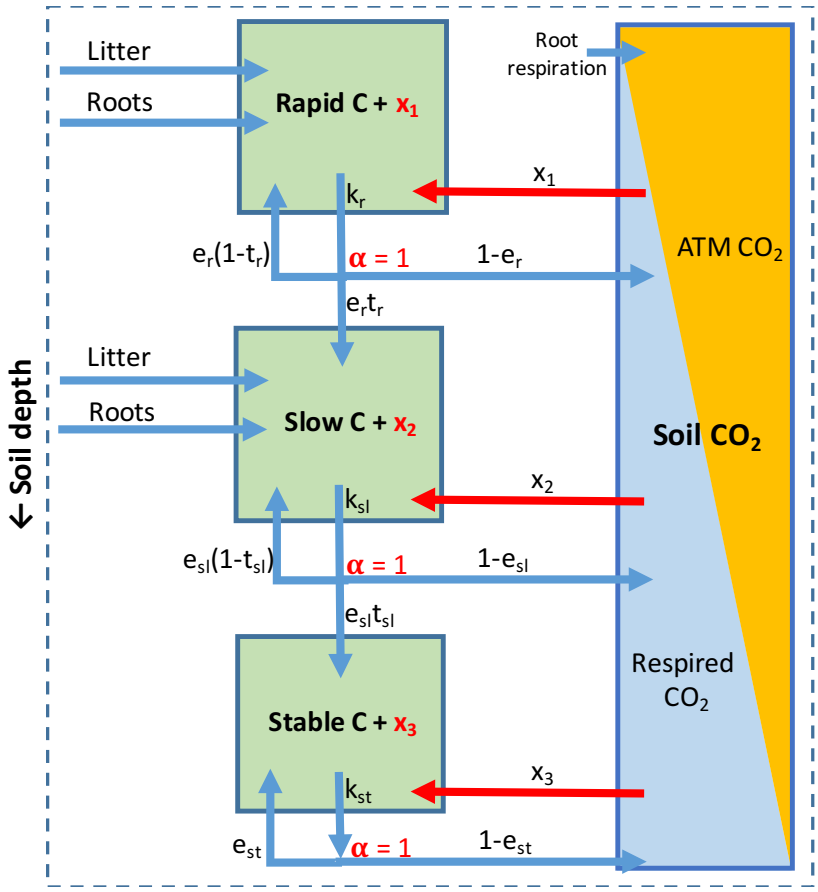


Figure 4.4: Adaptation of Bowen and Beerling: model for soil organic carbon and CO_2 assuming zero kinetic fractionation during microbial respiration and including anaplerotic CO_2 fixation as a parameter. Parameters described in Table 4.1, see text for details. $k_r/k_{sl}/k_{st}$ = microbial consumption constants, $e_r/e_{sl}/e_{st}$ = efficiency of microbial assimilation, t_r/t_{sl} = transformation constant for decomposed SOM, ∂ = fractionation factor for microbial respiration, $x_{1/2/3}$ = proportion of anaplerotically fixed CO_2.

would add C with $\delta^{13}C$ values similar to that of the CO_2 source and would change with depth. By adapting the model (Fig. 4.4, Table 4.1) and assuming a zero respiratory fractionation factor (i.e. α =1) a new source of fractionated C needs to be introduced (Fig. 4.4: $x_{1/2/3}$) in order to produce the observed ^{13}C-enrichment with depth. This source of C could be as a result of anaplerotic CO_2 fixation, however, the likely rates of the anaplerotic C would need to be quantified in order to determine its potential influence on the final SOM-$\delta^{13}C$ value. This can be done using the methods discussed above. By adapting Bowen and Beerling's (Bowen & Beerling 2004) "Integrated model for soil organic carbon and CO_2" in order to account for anaplerotic CO_2 fixation by soil microbes and roots (Fig. 4.4), we can estimate soil $\delta^{13}C$ values of SOM and how

they change with depth. Use of such a model would allow one to determine whether a site is likely to contain SOM which is isotopically representative of the vegetation it was derived from, or whether soil processes such as anaplerotic CO_2 fixation and mineralization have played a large role so that soil SOM is no longer a reliable paleo-indicator. In order to apply this model (Fig. 4.4) to a specific site, one would need to parameterize the model with data from that site. These parameters are outlined in Table 4.1 and there are a number of ways in which they can be determined. For example, one could measure the parameters directly. Some of the parameters are likely to vary among environments and could be estimated as done by Bowen & Beerling (2004). These authors estimated some parameters from the literature while others were estimated by correcting a starting model using an iterative least squares approach.

In contemplation of whether SOM-$\delta^{13}C$ values at a site can be used as reliable paleo-indicators, there are a number of approaches one could take. Given the strong global correlation between soil $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 4.2d; Chapter 2: Fig. 2.1, 2.2, 2.5 and 2.6; Table 2.1 and 2.2) and its relation to microbial activity (Chapter 2: Fig. 2.4a), as well as the finding that anaplerotic CO_2 fixation is also related to microbial activity (Fig. 4.2c; Chapter 3: Fig. 3.3, 3.4, 3.5 and 3.6; Supp. Fig. 3.2), one could determine whether a sites SOM has likely undergone processes such as decomposition and anaplerotic CO_2 fixation by determining a sites soil $\delta^{13}C$ and $\delta^{15}N$ correlation coefficient. If the the correlation is strong it is probably not appropriate to use soil $\delta^{13}C$ values as paleo-indicators as this is probably indicative of high rates of microbial processing. Another approach one could use would be to parameterize the model using site specific data and then predict a soil $\delta^{13}C$ profile as a result of anaplerotic CO_2 fixation using Eqn. 4 (discussed above). The SOM-$\delta^{13}C$ values predicted from the model could then be compared to that of measured SOM values and if the measured $\delta^{13}C$ values are significantly different to model predictions, this could indicate

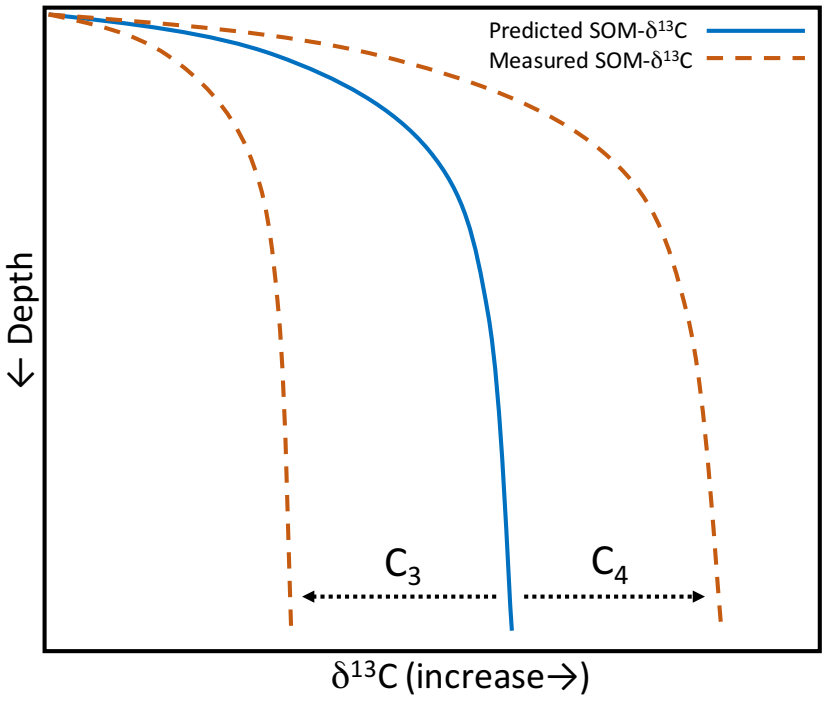


Figure 4.5: Determination of the suitability of SOM-$\delta^{13}C$ as a paleo-indicator of vegetation change (i.e. C_3 - C_4 transitions) given a SOM-$\delta^{13}C$ depth profile predicted using the adapted model (Fig. 4.4) of Bowen and Beerling. See text for details.

additional fractionation took place. For example, if a site is dominated by C_3 vegetation and the measured SOM-$\delta^{13}C$ values at depth are more positive than predicted values, then it is possible that the difference is due to the presence of ^{13}C-depleted C_4 derived SOM. However, if a C_4 dominated site is identified to have more negative $\delta^{13}C$ values at depth than predicted, we might expect the difference is due to ^{13}C-enriched C_3 derived SOM (Fig. 4.5).

Conclusion

The implications of mineralization and anaplerotic CO_2 fixation on soil $\delta^{13}C$ values, especially with depth, are that if these processes contribute significantly to the $\delta^{13}C$ signature of bulk soil at depth (as demonstrated by anaplerotic CO_2 fixation) one cannot determine whether the $\delta^{13}C$ value of the soil at depth is indicative of a past vegetation assemblies (i.e. C_3/C_4 transitions). This also casts doubt on the use of deep SOM as a paleobarometry indicator (Bowen & Beerling 2004) as well as any other

processes in which SOM-$\delta^{13}C$ is used as a proxy. In order to account for these processes, modeling exercises can help in concert with direct measurements of in situ anaplerotic CO_2 fixation using labels or the collection of soil CO_2-$\delta^{13}C$ and SOM-$\delta^{13}C$ data. By taking anaplerotic CO_2 fixation by soil microbes and roots into account, we provide a better understanding of soil $\delta^{13}C$ reliability in paleo-studies.

References

Affek, H.P. & Yakir, D., (2014). The Stable Isotopic Composition of Atmospheric CO_2 2nd ed., Elsevier Ltd.

Ågren, G. I., Bosatta, E., & Balesdent, J. (1996). Isotope discrimination during decomposition of organic matter: a theoretical analysis. Soil Science Society of America Journal, 60(4), 1121-1126.

Amundson, R., Austin, A. T., Schuur, E. A., Yoo, K., Matzek, V., Kendall, C., Uebersax, A., & Baisden, W. T. (2003). Global patterns of the isotopic composition of soil and plant nitrogen. *Global biogeochemical cycles*, *17*(1).

Amundson, R., Stern, L., Baisden, T., & Wang, Y. (1998). The isotopic composition of soil and soil-respired CO_2. *Geoderma*, *82*(1), 83-114.

Archer, S., Boutton, T. W., & Hibbard, K. A. (2001). Trees in grasslands: biogeochemical consequences of woody plant expansion. Global biogeochemical cycles in the climate system, 115-138.

Baisden, W. T., Amundson, R., Brenner, D. L., Cook, A. C., Kendall, C., & Harden, J. W. (2002). A multiisotope C and N modeling analysis of soil organic matter turnover and transport as a function of soil depth in a California annual grassland soil chronosequence. *Global Biogeochemical Cycles*, *16*(4).

Balesdent, J., Girardin, C., & Mariotti, A. (1993). Site-Related^(13) C of Tree Leaves and Soil Organic Matter in a Temperate Forest. *Ecology*, *74*(6), 1713-1721.

Bashkin, M. A., & Binkley, D. (1998). Changes in soil carbon following afforestation in Hawaii. Ecology, 79(3), 828-833.

Batjes, N. H. (1996). Total carbon and nitrogen in the soils of the world. European journal of soil science, 47(2), 151-163.

Benaglia, T., Chauveau, D., Hunter, D., & Young, D. (2009). mixtools: An R package for analyzing finite mixture models. *Journal of Statistical Software*, *32*(6), 1-29.

Berg B, McClaugherty C. Plant litter. Decomposition, humus formation, carbon sequestration. 2nd Ed Springer. 2008.

Still, C. J., Berry, J. A., Collatz, G. J., & DeFries, R. S. (2009). ISLSCP II C4 vegetation percentage. *ISLSCP Initiative II Collection: Data Set*.

Bernoux, M. et al., 1998. The use of stable carbon isotopes for estimating soil organic matter turnover rates. *Geoderma,* 82(1-3), pp.43–58.

Berry JA, Collatz GJ, DeFries RS (2009) ISLSCP II C4 vegetation percentage.

Billen, G., Garnier, J., & Lassaletta, L. (2013). The nitrogen cascade from agricultural soils to the sea: modelling nitrogen transfers at regional watershed and global scales. *Phil. Trans. R. Soc. B*, 368(1621), 20130123.

Billings, S. A., & Richter, D. D. (2006). Changes in stable isotopic signatures of soil nitrogen and carbon during 40 years of forest development. *Oecologia*, *148*(2), 325-333.

Bird, M., Santruckova, H., Lloyd, J., & Lawson, E. (2002). The isotopic composition of soil organic carbon on a north–south transect in western Canada. *European Journal of Soil Science*, 53(3), 393-403.

Birks, H. H., & Birks, H. J. B. (2000). Future uses of pollen analysis must include plant macrofossils. *Journal of biogeography*, 27(1), 31-35.

Bond, W. J., & Midgley, G. F. (2000). A proposed CO_2-controlled mechanism of woody plant invasion in grasslands and savannas. *Global Change Biology*, 6(8), 865-869.

Boström, B., Comstedt, D., & Ekblad, A. (2007). Isotope fractionation and ^{13}C enrichment in soil profiles during the decomposition of soil organic matter. *Oecologia*, 153(1), 89-98.

Bouwman, A. F., Lee, D. S., Asman, W. A. H., Dentener, F. J., Van Der Hoek, K. W., & Olivier, J. G. J. (1997). A global high-resolution emission inventory for ammonia. *Global biogeochemical cycles*, 11(4), 561-587.

Bouwman, A.F., Beusen, A.H.W., Griffioen, J., Van Groenigen, J.W., Hefting, M.M., Oenema, O., Van Puijenbroek, P.J.T.M., Seitzinger, S., Slomp, C.P. and Stehfest, E., (2013). Global trends and uncertainties in terrestrial denitrification and N2O emissions. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 368(1621), 20130112.

Bowen, G. J., & Beerling, D. J. (2004). An integrated model for soil organic carbon and CO2: Implications for paleosol carbonate pCO_2 paleobarometry. *Global Biogeochemical Cycles*, *18*(1).

Breecker, D. O., Bergel, S., Nadel, M., Tremblay, M. M., Osuna-Orozco, R., Larson, T. E., & Sharp, Z. D. (2015). Minor stable carbon isotope fractionation between

respired carbon dioxide and bulk soil organic matter during laboratory incubation of topsoil. *Biogeochemistry*, 123(1-2), 83-98.

Burrows, N. D., Ward, B., & Cranfield, R. (2002). Short-term impacts of logging on understorey vegetation in a jarrah forest. *Australian Forestry*, 65(1), 47-58.

Cerling, T. E. (1984). The stable isotopic composition of modern soil carbonate and its relationship to climate. *Earth and Planetary science letters*, 71(2), 229-240.

Chadwick, O. A., Kelly, E. F., Merritts, D. M., & Amundson, R. G. (1994). Carbon dioxide consumption during soil development. *Biogeochemistry*, 24(3), 115-127.

Chen, Q., Shen, C., Sun, Y., Peng, S., Yi, W., Li, Z. A., & Jiang, M. (2005). Spatial and temporal distribution of carbon isotopes in soil organic matter at the Dinghushan Biosphere Reserve, South China. *Plant and Soil*, *273*(1), 115-128.

Chen, S., Huang, Y., Zou, J., Shen, Q., Hu, Z., Qin, Y., Chen H. & Pan, G. (2010). Modeling interannual variability of global soil respiration from climate and soil properties. *Agricultural and Forest Meteorology*, 150(4), 590-605.

Cheng, W. (1996). Measurement of rhizosphere respiration and organic matter decomposition using natural 13 C. *Plant and Soil*, 183(2), 263-268.

Cleveland, C.C. et al., 2004. Composition, Dynamics, and Fate of Leached Dissolved Organic Matter in Terrestrial Ecosystems: Results from a Decomposition Experiment. *Ecosystems*, 7(3), pp.1–12.

Clough, T. J., Rolston, D. E., Stevens, R. J., & Laughlin, R. J. (2003). N_2O and N_2 gas fluxes, soil gas pressures, and ebullition events following irrigation of 15NO3−-labelled subsoils. *Soil Research*, 41(3), 401-420.

Collatz GJ, Berry JA, Clark JS. Effects of climate and atmospheric CO_2 partial pressure on the global distribution of C4 grasses: present, past, and future. *Oecologia*. 1998 May 1;114(4):441-54.

Coombes, P., Chiverrell, R. C., & Barber, K. E. (2009). A high-resolution pollen and geochemical analysis of late Holocene human impact and vegetation history in southern Cumbria, England. *Journal of Quaternary Science*, 24(3), 224-236.

Cotrufo MF, Wallenstein MD, Boot CM, et al (2013) The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Glob Change Biol* 19:988–995. doi: 10.1111/gcb.12113

Cowell, C. M. (1998). Historical change in vegetation and disturbance on the Georgia Piedmont. *The American midland naturalist,* 140(1), 78-89.

Craine JM, Brookshire ENJ, Cramer MD, et al (2015a) Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant Soil* 1–28. doi: 10.1007/s11104-015-2542-1

Craine JM, Elmore AJ, Aidar MPM, et al (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytologist* 183:980–992. doi: 10.1111/j.1469-8137.2009.02917.

Craine, J. M., Elmore, A. J., Wang, L., Augusto, L., Baisden, W. T., Brookshire, E. N. J., Cramer, M.D., Hasselquist, N.J., Hobbie, E.A., Kahmen, A. & Koba, K. (2015). Convergence of soil nitrogen isotopes across global climate gradients. *Scientific reports*, 5.

Cramer, M. D., Lewis, O. A. M., & Lips, S. H. (1993). Inorganic carbon fixation and metabolism in maize roots as affected by nitrate and ammonium nutrition. *Physiologia Plantarum*, 89(3), 632-639.

Curiel Yuste, J., Baldocchi, D. D., Gershenson, A., Goldstein, A., Misson, L., & Wong, S. (2007). Microbial soil respiration and its dependency on carbon inputs, soil temperature and moisture. *Global Change Biology*, 13(9), 2018-2035.

Damesin, C., Rambal, S., & Joffre, R. (1997). Between-tree variations in leaf $\delta^{13}C$ of Quercus pubescens and Quercus ilex among Mediterranean habitats with different water availability. *Oecologia*, 111(1), 26-35.

Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 440(7081), 165.

Davis, M. B., & Shaw, R. G. (2001). Range shifts and adaptive responses to Quaternary climate change. *Science*, 292(5517), 673-679.

DeFries RS, Hansen MC, Townshend JR, Janetos AC, Loveland TR. A new global 1-km dataset of percentage tree cover derived from remote sensing. *Global Change Biology*. 2000 Feb 1;6(2):247-54.

Desjardins, T., Andreux, F., Volkoff, B., & Cerri, C. C. (1994). Organic carbon and ^{13}C contents in soils and soil size-fractions, and their changes due to deforestation and pasture installation in eastern Amazonia. *Geoderma*, 61(1-2), 103-118.

Dijkstra, P., Ishizu, A., Doucett, R., Hart, S. C., Schwartz, E., Menyailo, O. V., & Hungate, B. A. (2006). 13 C and 15 N natural abundance of the soil microbial biomass. *Soil Biology and Biochemistry*, *38*(11), 3257-3266.

Dong, Z., & Layzell, D. B. (2001). H2 oxidation, O_2 uptake and CO_2 fixation in hydrogen treated soils. *Plant and Soil*, 229(1), 1-12.

Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, Marquéz JR, Gruber B, Lafourcade B, Leitão PJ, Münkemüller T. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*. 2013 Jan 1;36(1):27-46.

Ehleringer, J. R., Buchmann, N., & Flanagan, L. B. (2000). Carbon isotope ratios in belowground carbon cycle processes. *Ecological Applications*, 10(2), 412-422.

Eissfeller, V., Beyer, F., Valtanen, K., Hertel, D., Maraun, M., Polle, A., & Scheu, S. (2013). Incorporation of plant carbon and microbial nitrogen into the rhizosphere food web of beech and ash. *Soil Biology and Biochemistry*, 62, 76-81.

Ekblad, A., & Nordgren, A. (2002). Is growth of soil microorganisms in boreal forests limited by carbon or nitrogen availability?. *Plant and Soil*, *242*(1), 115-122.

Ekblad, A., & Högberg, P. (2000). Analysis of $\delta^{13}C$ of CO_2 distinguishes between microbial respiration of added C4-sucrose and other soil respiration in a C3-ecosystem. *Plant and Soil*, *219*(1), 197-209.

Ekblad, A., Nyberg, G. & Högberg, P., 2002. ^{13}C-discrimination during microbial respiration of added C3-, C4- and 13C-labelled sugars to a C3-forest soil. *Oecologia*, 131(2), pp.245–249.

Elith, J., Leathwick, J. R., & Hastie, T. (2008). A working guide to boosted regression trees. *Journal of Animal Ecology*, *77*(4), 802-813.

Elzein, A., & Balesdent, J. (1995). Mechanistic simulation of vertical distribution of carbon concentrations and residence times in soils. *Soil Science Society of America Journal*, 59(5), 1328-1335.

Engler, R., Randin, C. F., Thuiller, W., Dullinger, S., Zimmermann, N. E., Araújo, M. B., Pearman, P.B., Le Lay, G., Piedallu, C., Albert, C.H. and Choler, P. (2011). 21st century climate change threatens mountain flora unequally across Europe. *Global Change Biology*, 17(7), 2330-2341.

Enoch, H., & Dasberg, S. (1971). The occurrence of high CO2, concentrations in soil air. *Geoderma*, 6(1), 17-21.

Esmeijer-Liu, A. J., Kürschner, W. M., Lotter, A. F., Verhoeven, J. T., & Goslar, T. (2012). Stable carbon and nitrogen isotopes in a peat profile are influenced by early stage diagenesis and changes in atmospheric CO_2 and N deposition. *Water, Air, & Soil Pollution*, 223(5), 2007-2022.

Eswaran, H., Van Den Berg, E., & Reich, P. (1993). Organic carbon in soils of the world. *Soil science society of America journal*, 57(1), 192-194.

February, E. C., & Higgins, S. I. (2010). The distribution of tree and grass roots in savannas in relation to soil nitrogen and water. *South African Journal of Botany*, 76(3), 517-523.

Feisthauer, S., Wick, L. Y., Kästner, M., Kaschabek, S. R., Schlömann, M., & Richnow, H. H. (2008). Differences of heterotrophic $^{13}CO_2$ assimilation by Pseudomonas knackmussii strain B13 and Rhodococcus opacus 1CP and potential impact on biomarker stable isotope probing. *Environmental microbiology*, 10(6), 1641-1651.

Fensham, R. J., & Fairfax, R. J. (2002). Aerial photography for assessing vegetation change: a review of applications and the relevance of findings for Australian vegetation history. *Australian Journal of Botany*, 50(4), 415-429.

Fernandez, I., Mahieu, N., & Cadisch, G. (2003). Carbon isotopic fractionation during decomposition of plant materials of different quality. *Global Biogeochemical Cycles,* 17(3).

Fierer, N., Strickland, M. S., Liptzin, D., Bradford, M. A., & Cleveland, C. C. (2009). Global patterns in belowground communities. *Ecology letters*, 12(11), 1238-1249.

Fischer, V., Joseph, C., Tieszen, L. L., & Schimel, D. S. (2008). Climate controls on C3 vs. C4 productivity in North American grasslands from carbon isotope composition of soil organic matter*. Global Change Biology*, 14(5), 1141-1155.

Flessa, H., Ludwig, B., Heil, B., & Merbach, W. (2000). The Origin of Soil Organic C, Dissolved Organic C and Respiration in a Long-Term Maize Experiment in Halle, Germany, Determined by 13C Natural Abundance. *Journal of Plant Nutrition and Soil Science*, 163(2), 157-163.

Flinn, K. M., & Vellend, M. (2005). Recovery of forest plant communities in post-agricultural landscapes. *Frontiers in Ecology and the Environment*, 3(5), 243-250.

Freudenthal, T., Wagner, T., Wenzhöfer, F., Zabel, M., & Wefer, G. (2001). Early diagenesis of organic matter from sediments of the eastern subtropical Atlantic: evidence from stable nitrogen and carbon isotopes. *Geochimica et Cosmochimica Acta*, 65(11), 1795-1808.

Fry, B. (2006). Stable isotope ecology (Vol. 521). New York: Springer.

Gabet, E. J., Reichman, O. J., & Seabloom, E. W. (2003). The effects of bioturbation on soil processes and sediment transport. *Annual Review of Earth and Planetary Sciences*, *31*(1), 249-273.

Gessler, A., Keitel, C., Kodama, N., Weston, C., Winters, A.J., Keith, H., Grice, K., Leuning, R. and Farquhar, G.D., (2007). $\delta^{13}C$ of organic matter transported from the leaves to the roots in Eucalyptus delegatensis: short-term variations and relation to respired CO_2*. Functional Plant Biology*, 34(8), 692-706.

Gholz, H. L., Wedin, D. A., Smitherman, S. M., Harmon, M. E., & Parton, W. J. (2000). Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. *Global Change Biology*, *6*(7), 751-765.

Gillson, L. (2015). Evidence of a tipping point in a southern African savanna?. *Ecological complexity*, *21*, 78-86.

Gillson, L., Waldron, S., & Willis, K. J. (2004). Interpretation of soil $\delta^{13}C$ as an indicator of vegetation change in African savannas. *Journal of Vegetation Science*, 15(3), 339-350.

Greenway, H., Armstrong, W., & Colmer, T. D. (2006). Conditions leading to high CO_2 (> 5 kPa) in waterlogged–flooded soils and possible effects on root growth and metabolism. *Annals of Botany*, 98(1), 9-32.

Haberle, S. G., & Maslin, M. A. (1999). Late Quaternary vegetation and climate change in the Amazon Basin based on a 50,000 year pollen record from the Amazon Fan, ODP Site 932. *Quaternary Research*, 51(1), 27-38.

Handley, L. L., & Raven, J. A. (1992). The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant, Cell & Environment*, *15*(9), 965-985.

Hargreaves GL, Hargreaves GH, Riley JP (1985) Irrigation Water Requirements for Senegal River Basin. Journal of Irrigation and Drainage Engineering-Asce 111:265–275.

Hashimoto, S., Carvalhais, N., Ito, A., Migliavacca, M., Nishina, K., & Reichstein, M. (2015). Global spatiotemporal distribution of soil respiration modeled using a global database. *Biogeosciences*, 12, 4121-4132.

Hastie, T., Friedman, J., & Tibshirani, R. (2001). Model assessment and selection. In The elements of statistical learning (pp. 193-224). Springer New York.

Hättenschwiler, S., & Gasser, P. (2005). Soil animals alter plant litter diversity effects on decomposition. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(5), 1519-1524.

Hattersley, P. W. (1982). δ13 values of C4 types in grasses. *Functional Plant Biology*, *9*(2), 139-154.

Heil, B., Ludwig, B., Flessa, H., & Beese, F. (2000). 13C and 15N distributions in three spodic dystric cambisols under beech and spruce. *Isotopes in environmental and health studies*, 36(1), 35-47.

Hengl, T., de Jesus, J. M., MacMillan, R. A., Batjes, N. H., Heuvelink, G. B., Ribeiro, E., Samuel-Rosa, A., Kempen, B., Leenaars, J.G., Walsh, M.G. & Gonzalez, M. R. (2014). SoilGrids1km—global soil information based on automated mapping. *PLoS One*, *9*(8), e105992.

Henn, M. R., & Chapela, I. H. (2000). Differential C isotope discrimination by fungi during decomposition of C3-and C4-derived sucrose. *Applied and Environmental Microbiology*, 66(10), 4180-4186.

Hicks Pries, C.E., Schuur, E.A.G. & Crummer, K.G., (2011). Holocene Carbon Stocks and Carbon Accumulation Rates Altered in Soils Undergoing Permafrost Thaw. *Ecosystems*, 15(1), 162–173.

Hijmans RJ, van Etten J, Cheng J, Mattiuzzi M, Sumner M, Greenberg JA, Lamigueiro OP, Bevan A, Racine EB, Shortridge A, Hijmans MR. Package 'raster'. R package. 2015 Dec 18.

Hijmans RJ, Van Etten J. raster: Geographic data analysis and modeling. R package version 2.2-31. URL http://CRAN. R-project. org/package= raster

Hobbie E, Werner RA. Intramolecular, compound-specific, and bulk carbon isotope patterns in C3 and C4 plants: a review and synthesis. *New Phytologist*. 2004 Feb 1;161(2):371-85.

Hobbie EA, Macko SA, Shugart HH (1999) Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. *Oecologia* 118:353–360.

Hobbie EA, Macko SA, Williams M. (2000) Correlations between foliar $\delta^{15}N$ and nitrogen concentrations may indicate plant-mycorrhizal interactions. *Oecologia*. 122(2):273-83.

Hobbie, E. A., & Ouimette, A. P. (2009). Controls of nitrogen isotope patterns in soil profiles. *Biogeochemistry*, 95(2-3), 355-371.

Högberg, P. (1998). Tansley Review No. 95: 15N natural abundance in soil–plant systems. *New Phytologist*, 139(3), 595-595.

Houlton, B. Z., Sigman, D. M., & Hedin, L. O. (2006). Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. *Proceedings of the National Academy of Sciences*, *103*(23), 8745-8750.

Huang, Y., Bol, R., Harkness, D. D., Ineson, P., & Eglinton, G. (1996). Post-glacial variations in distributions, 13 C and 14 C contents of aliphatic hydrocarbons and bulk organic matter in three types of British acid upland soils. *Organic Geochemistry*, 24(3), 273-287.

Huggett, R. J. (1998). Soil chronosequences, soil development, and soil evolution: a critical review. *Catena*, 32(3), 155-172.

Ikeda, M., Mizoguchi, K., & Yamakawa, T. (1992). Stimulation of dark carbon fixation in rice and tomato roots by application of ammonium nitrogen. *Soil Science and Plant Nutrition*, 38(2), 315-322.

Jobbágy, E. G., & Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological applications*, 10(2), 423-436.

Kaiser, K., & Kalbitz, K. (2012). Cycling downwards–dissolved organic matter in soils. *Soil Biology and Biochemistry*, 52, 29-32.

Kätterer, T., Bolinder, M. A., Andrén, O., Kirchmann, H., & Menichetti, L. (2011). Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. *Agriculture, Ecosystems & Environment*, 141(1), 184-192.

Knoepp, J. D., & Swank, W. T. (1998). Rates of nitrogen mineralization across an elevation and vegetation gradient in the southern Appalachians. *Plant and Soil*, 204(2), 235-241.

Krull, E. G., & Bray, S. S. (2005). Assessment of vegetation change and landscape variability by using stable carbon isotopes of soil organic matter. *Australian Journal of Botany*, 53(7), 651-661.

Krull, E. S., & Skjemstad, J. O. (2003). $\delta^{13}C$ and $\delta^{15}N$ profiles in 14 C-dated Oxisol and Vertisols as a function of soil chemistry and mineralogy. *Geoderma*, 112(1), 1-29.

Krull, E. S., Bestland, E. A., & Gates, W. P. (2002). Soil organic matter decomposition and turnover in a tropical ultisol: evidence from $\delta^{13}C$, $\delta^{15}N$ and geochemistry. *Radiocarbon*, 44(1), 93-112.

Kuzyakov Y, Domanski G. Carbon input by plants into the soil. Review. *Journal of Plant Nutrition and Soil Science*. 2000 Aug 1;163(4):421-31.

Kuzyakov, Y., Mitusov, A., & Schneckenberger, K. (2006). Effect of C 3–C 4 vegetation change on $\delta^{13}C$ and $\delta^{15}N$ values of soil organic matter fractions separated by thermal stability. *Plant and Soil*, *283*(1), 229-238.

Kuzyakov, Y. & Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: Concept & review. Soil Biology and Biochemistry, 83(C), pp.S29–S36.

Lane, C. S., Horn, S. P., & Mora, C. I. (2004). Stable carbon isotope ratios in lake and swamp sediments as a proxy for prehistoric forest clearance and crop cultivation in the Neotropics. Journal of Paleolimnology, 32(4), 375-381.

Lang, D. T., & Lang, M. D. T. (2016). Package 'RCurl'.

Lerch, T. Z., Nunan, N., Dignac, M. F., Chenu, C., & Mariotti, A. (2011). Variations in microbial isotopic fractionation during soil organic matter decomposition. *Biogeochemistry*, *106*(1), 5-21.

Li, Z., Wang, X., Zhang, R., Zhang, J., & Tian, C. (2011). Contrasting diurnal variations in soil organic carbon decomposition and root respiration due to a hysteresis effect with soil temperature in a Gossypium s.(cotton) plantation. *Plant and soil*, *343*(1-2), 347-355.

Lin, G., & Ehleringer, J. R. (1997). Carbon isotopic fractionation does not occur during dark respiration in C3 and C4 plants. *Plant Physiology*, 114(1), 391-394.

Liu, J., Wang, C., Peng, B., Xia, Z., Jiang, P., & Bai, E. (2016). Effect of nitrogen addition on the variations in the natural abundance of nitrogen isotopes of plant and soil components. *Plant and Soil*, 1-12.

Lloyd, J., & Taylor, J. A. (1994). On the temperature dependence of soil respiration. *Functional ecology*, 315-323.

Lu, H., Wu, N., Gu, Z., Guo, Z., Wang, L., Wu, H., Wang, G., Zhou, L., Han, J. and Liu , T. (2004). Distribution of carbon isotope composition of modern soils on the Qinghai-Tibetan Plateau. *Biogeochemistry*, *70*(2), 275-299.

Macdonald, B. C. T., Warneke, S., Maïson, E., McLachlan, G., & Farrell, M. (2015). Spatial decoupling of soil nitrogen cycling in an arid chenopod pattern ground. *Soil Research*, 53(1), 97-104.

Maechler, M., & Ringach, D. (2015). diptest: Hartigan's Dip Test Statistic for Unimodality—Corrected. *URL http://CRAN. R-project. org/package= diptest. R package version 0.75-7*.

Marin-Spiotta E, Smith AP, Atkinson EE, Chaopricha NT. Landscape Disturbance History and Belowground Carbon Dynamics. InAGU Fall Meeting Abstracts 2014 Dec (Vol. 1, p. L04).

Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., & Tardieux, P. (1981). Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. *Plant and soil*, *62*(3), 413-430.

Mary, B., Mariotti, A., & Morel, J. L. (1992). Use of $\delta^{13}C$ variations at natural abundance for studying the biodegradation of root mucilage, roots and glucose in soil. *Soil Biology and Biochemistry*, 24(10), 1065-1072.

Merlin, C., Masters, M., McAteer, S., & Coulson, A. (2003). Why is carbonic anhydrase essential to Escherichia coli?. *Journal of bacteriology*, 185(21), 6415-6424.

Mi, N. A., Wang, S., Liu, J., Yu, G., Zhang, W., & Jobbagy, E. (2008). Soil inorganic carbon storage pattern in China. *Global Change Biology*, 14(10), 2380-2387.

Miltner, A. et al., 2004. Assimilation of CO_2 by soil microorganisms and transformation into soil organic matter. *Organic Geochemistry*, 35(9), pp.1015–1024.

Miltner, A., Kopinke, F. D., Kindler, R., Selesi, D., Hartmann, A., & Kästner, M. (2005). Non-phototrophic CO_2 fixation by soil microorganisms. *Plant and Soil*, 269(1), 193-203.

Miltner, A., Richnow, H. H., Kopinke, F. D., & Kästner, M. (2004). Assimilation of CO_2 by soil microorganisms and transformation into soil organic matter. *Organic Geochemistry*, 35(9), 1015-1024.

Minasny, B., McBratney, A. B., & Salvador-Blanes, S. (2008). Quantitative models for pedogenesis—a review. *Geoderma*, 144(1), 140-157.

Mucina, L., & Rutherford, M. C. (2006). The vegetation of South Africa, Lesotho and Swaziland. *South African National Biodiversity Institute*.

Nair, V. D., Haile, S. G., Michel, G. A., & Nair, P. K. (2007). Environmental quality improvement of agricultural lands through silvopasture in southeastern United States. *Scientia Agricola*, 64(5), 513-519.

Novák, M., Buzek, F., Harrison, A. F., Přechová, E., Jačková, I., & Fottová, D. (2003). Similarity between C, N and S stable isotope profiles in European spruce forest soils: implications for the use of δ 34 S as a tracer. *Applied geochemistry*, 18(5), 765-779.

Nowak, M., Beulig, F., Von Fischer, J., Muhr, J., Küsel, K., & Trumbore, S. E. (2015). Autotrophic fixation of geogenic CO2 by microorganisms contributes to soil organic matter formation and alters isotope signatures in a wetland mofette. *Biogeosciences*, 12(3), 7169-7183.

Ometto, J. P., Ehleringer, J. R., Domingues, T. F., Berry, J. A., Ishida, F. Y., Mazzi, E., Higuchi, N., Flanagan, L.B., Nardoto, G.B. and Martinelli, L.A. (2006). The stable carbon and nitrogen isotopic composition of vegetation in tropical forests of the Amazon Basin, Brazil. Nitrogen Cycling in the Americas: *Natural and Anthropogenic Influences and Controls*, 251-274.

Parton W, Silver WL, Burke IC, et al (2007) Global-Scale Similarities in Nitrogen Release Patterns During Long-Term Decomposition. *Science* 315:361–364. doi: 10.1126/science.1134853

Pataki, D. E., Billings, S. A., Naumburg, E., & Goedhart, C. M. (2008). Water sources and nitrogen relations of grasses and shrubs in phreatophytic communities of the Great Basin Desert. *Journal of Arid Environments*, *72*(9), 1581-1593.

Perez, R. C., & Matin, A. B. D. U. L. (1982). Carbon dioxide assimilation by Thiobacillus novellus under nutrient-limited mixotrophic conditions. *Journal of bacteriology*, 150(1), 46-51.

Pessenda, L. C. R., Aravena, R., Melfi, A. J., Telles, E. C. C., Boulet, R., Valencia, E. P. E., & Tomazello, M. (1996). The use of carbon isotopes (13C, 14C) in soil to evaluate vegetation changes during the Holocene in central Brazil. *Radiocarbon*, 38(2), 191-201.

Pessenda, L. C. R., de Souza Ribeiro, A., Gouveia, S. E. M., Aravena, R., Boulet, R., & Bendassolli, J. A. (2004). Vegetation dynamics during the late Pleistocene in the Barreirinhas region, Maranhão State, northeastern Brazil, based on carbon isotopes in soil organic matter. *Quaternary Research*, 62(2), 183-193.

Ponsard S, Arditi R. What can stable isotopes (δ15n and δ13c) tell about the food web of soil macro-invertebrates?. *Ecology*. 2000 Mar 1;81(3):852-64.

Prentice, I. C. (1986). Vegetation responses to past climatic variation. *Vegetatio*, *67*(2), 131-141.

Price, P. B., & Sowers, T. (2004). Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proceedings of the National Academy of Sciences of the United States of America*, 101(13), 4631-4636.

Qian, J. H., Doran, J. W., & Walters, D. T. (1997). Maize plant contributions to root zone available carbon and microbial transformations of nitrogen. *Soil Biology and Biochemistry*, 29(9), 1451-1462.

Qiao, Y., Miao, S., Silva, L. C., & Horwath, W. R. (2014). Understory species regulate litter decomposition and accumulation of C and N in forest soils: A long-term dual-isotope experiment. *Forest Ecology and Management*, 329, 318-327.

Ramankutty N, Foley JA. Characterizing patterns of global land use: An analysis of global croplands data. Global Biogeochemical Cycles. 1998 Dec 1;12(4):667-85.
Ridgeway G, Southworth MH, RUnit S (2013) Package "gbm."

Rovira, P., & Vallejo, V. R. (2002). Labile and recalcitrant pools of carbon and nitrogen in organic matter decomposing at different depths in soil: an acid hydrolysis approach. *Geoderma*, 107(1), 109-141.

Rumpel, C., & Kögel-Knabner, I. (2011). Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. *Plant and soil*, 338(1-2), 143-158.

Saini, R., Kapoor, R., Kumar, R., Siddiqi, T. O., & Kumar, A. (2011). CO_2 utilizing microbes—A comprehensive review. *Biotechnology advances*, 29(6), 949-960.

Sanaiotti, T. M., Martinelli, L. A., Victoria, R. L., Trumbore, S. E., & Camargo, P. B. (2002). Past vegetation changes in Amazon savannas determined using carbon isotopes of soil organic matter. *Biotropica*, 34(1), 2-16.

Sanderman, J. & Amundson, R., (2009). A comparative study of dissolved organic carbon transport and stabilization in California forest and grassland soils. *Biogeochemistry*. 92, 45-59

Šantrůčková, H., Bird, M. I., & Lloyd, J. (2000a). Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. *Functional Ecology*, 14(1), 108-114.

Šantrůčková, H., Bird, M. I., Frouz, J., Sustr, V., & Tajovský, K. (2000b). Natural abundance of 13C in leaf litter as related to feeding activity of soil invertebrates and microbial mineralisation. *Soil Biology and Biochemistry*, 32(11), 1793-1797.

Scheu S, Falca M. The soil food web of two beech forests (Fagus sylvatica) of contrasting humus type: stable isotope analysis of a macro-and a mesofauna-dominated community. *Oecologia*. 2000 May 1;123(2):285-96.

Schimel, J. P., & Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, 85(3), 591-602.

Sharp, B. R., & Whittaker, R. J. (2003). The irreversible cattle-driven transformation of a seasonally flooded Australian savanna. *Journal of Biogeography*, 30(5), 783-802.

Shuster, W. D., Subler, S., & McCoy, E. L. (2001). Deep-burrowing earthworm additions changed the distribution of soil organic carbon in a chisel-tilled soil. *Soil Biology and Biochemistry*, 33(7), 983-996.

Sikora, F. J., & Moore, K. P. (2014). Soil test methods from the southeastern United States. S. *Coop. Ser. Bul*, (419).

Silfer, J. A., Engel, M. H., & Macko, S. A. (1992). Kinetic fractionation of stable carbon and nitrogen isotopes during peptide bond hydrolysis: experimental evidence and geochemical implications. *Chemical Geology: Isotope Geoscience Section*, *101*(3-4), 211-221.

Six, J., Feller, C., Denef, K., Ogle, S., de Moraes Sa, J. C., & Albrecht, A. (2002). Soil organic matter, biota and aggregation in temperate and tropical soils-effects of no-tillage. *Agronomie*, 22(7-8), 755-775.

Sollins, P., Kramer, M. G., Swanston, C., Lajtha, K., Filley, T., K Aufdenkampe, A.K., Wagai, R & Bowden, R. D. (2009). Sequential density fractionation across soils of contrasting mineralogy: evidence for both microbial-and mineral-controlled soil organic matter stabilization. *Biogeochemistry*, 96(1-3), 209-231.

Splechtna, B. E., Gratzer, G., & Black, B. A. (2005). Disturbance history of a European old-growth mixed-species forest–A spatial dendro-ecological analysis. *Journal of Vegetation Science*, 16(5), 511-522.

Spratt, E. R. (1915). The root-nodules of the Cycadaceae. *Annals of Botany*, 29(116), 619-626.

Stable carbon and nitrogen isotopes in a peat profile are influenced by early stage diagenesis and changes in atmospheric CO_2 and N deposition. W*ater, Air, & Soil Pollution*, 223(5), 2007-2022.

Stein, S., Selesi, D., Schilling, R., Pattis, I., Schmid, M., & Hartmann, A. (2005). Microbial activity and bacterial composition of H2-treated soils with net CO_2 fixation. *Soil Biology and Biochemistry*, 37(10), 1938-1945.

Steiner, H., Jonsson, B.H. & Lindskog, S., (1975). The catalytic mechanism of carbonic anhydrase. Hydrogen-isotope effects on the kinetic parameters of the human C isoenzyme. *European journal of biochemistry*, 59(1), 253–259.

Susfalk, R. B., Cheng, W. X., Johnson, D. W., Walker, R. F., Verburg, P., & Fu, S. (2002). Lateral diffusion and atmospheric CO2 mixing compromise estimates of rhizosphere respiration in a forest soil. *Canadian Journal of Forest Research*, 32(6), 1005-1015.

Swap, R. J., Aranibar, J. N., Dowty, P. R., Gilhooly, W. P., & Macko, S. A. (2004). Natural abundance of 13C and 15N in C3 and C4 vegetation of southern Africa: patterns and implications. *Global Change Biology*, *10*(3), 350-358.

Taybi, T., Nimmo, H. G., & Borland, A. M. (2004). Expression of phosphoenolpyruvate carboxylase and phosphoenolpyruvate carboxylase kinase genes. Implications for genotypic capacity and phenotypic plasticity in the expression of crassulacean acid metabolism. *Plant Physiology,* 135(1), 587-598.

Thuiller, W., Lavorel, S., Araújo, M. B., Sykes, M. T., & Prentice, I. C. (2005). Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America*, 102(23), 8245-8250.

Tian, H., Chen, G., Zhang, C., Melillo, J. M., & Hall, C. A. (2010). Pattern and variation of C: N: P ratios in China's soils: a synthesis of observational data. *Biogeochemistry*, 98(1-3), 139-151.

Tiwari, S. C., Tiwari, B. K., & Mishra, R. R. (1987). The influence of moisture regimes on the population and activity of soil microorganisms. *Plant and Soil*, 101(1), 133-136.

Troughton, J. H. (1979). $\delta^{13}C$ as an indicator of carboxylation reactions. In Photosynthesis II (pp. 140-149). Springer Berlin Heidelberg.

Trumbore, S. (2000). Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. *Ecological Applications*, 10(2), 399-411.

Trumbore, S. (2009). Radiocarbon and soil carbon dynamics. *Annual Review of Earth and Planetary Sciences*, 37, 47-66.

Tu, K., & Dawson, T. (2005). Partitioning ecosystem respiration using stable carbon isotope analyses of CO_2. Stable Isotopes and Biosphere–Atmosphere Interactions: *Processes and Biological Controls*, 125-148.

Valentine, K. W. G., & Dalrymple, J. B. (1976). Quaternary buried paleosols: a critical review. *Quaternary Research*, 6(2), 209-222.

Van Der Hammen, T., & Hooghiemstra, H. (2000). Neogene and Quaternary history of vegetation, climate, and plant diversity in Amazonia. *Quaternary Science Reviews*, 19(8), 725-742.

Wallander, H., Nilsson, L. O., Hagerberg, D., & Rosengren, U. (2003). Direct estimates of C: N ratios of ectomycorrhizal mycelia collected from Norway spruce forest soils. *Soil Biology and Biochemistry*, 35(7), 997-999.

Wang, G., Jia, Y., & Li, W. (2015). Effects of environmental and biotic factors on carbon isotopic fractionation during decomposition of soil organic matter. *Scientific reports*, 5.

Wentworth, C. K. (1933). Fundamental limits to the sizes of clastic grains. *Science*, 77, 633-634.

Werth, M., & Kuzyakov, Y. (2010). 13C fractionation at the root–microorganisms–soil interface: a review and outlook for partitioning studies. *Soil Biology and Biochemistry*, 42(9), 1372-1384.

West, A. G., Bond, W. J., & Midgley, J. J. (2000). Soil carbon isotopes reveal ancient grassland under forest. *S. Afr. J. Sci*., 96, 253.

Whitlock, C., Shafer, S. L., & Marlon, J. (2003). The role of climate and vegetation change in shaping past and future fire regimes in the northwestern US and the implications for ecosystem management. *Forest ecology and management*, 178(1), 5-21.

Wickham, H. (2009). ggplot2: elegant graphics for data analysis. Springer Science & Business Media.

Wilson, A. T., & Grinsted, M. J. (1977). 12C/13C in cellulose and lignin as palaeothermometers. *Nature*, 265(5590), 133-135.

Wingate, L. (2008). Weighty issues in respiratory metabolism: intriguing carbon isotope signals from roots and leaves. *New Phytologist*, 177(2), 285-287.

Wu, X., Ge, T., Wang, W., Yuan, H., Wegner, C.E., Zhu, Z., Whiteley, A.S. and Wu, J., (2015). Cropping systems modulate the rate and magnitude of soil microbial autotrophic CO2 fixation in soil. *Frontiers in microbiology*, 6.

Wu, X., Ge, T., Yuan, H., Li, B., Zhu, H., Zhou, P., Sui, F., O'Donnell, A.G. and Wu, J., (2014). Changes in bacterial CO2 fixation with depth in agricultural soils. *Applied microbiology and biotechnology*, 98(5), 2309-2319.

Wynn, J. G., Harden, J. W., & Fries, T. L. (2006). Stable carbon isotope depth profiles and soil organic carbon dynamics in the lower Mississippi Basin. *Geoderma*, *131*(1), 89-109.

Wynn, J. G., Bird, M. I., & Wong, V. N. (2005). Rayleigh distillation and the depth profile of 13 C/12 C ratios of soil organic carbon from soils of disparate texture in Iron Range National Park, Far North Queensland, Australia. *Geochimica et cosmochimica acta*, 69(8), 1961-1973.

Wynn, J. G., Harden, J. W., & Fries, T. L. (2006). Stable carbon isotope depth profiles and soil organic carbon dynamics in the lower Mississippi Basin. *Geoderma*, 131(1), 89-109.

Wynn, J. G. (2007). Carbon isotope fractionation during decomposition of organic matter in soils and paleosols: implications for paleoecological interpretations of paleosols. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *251*(3), 437-448.

Yin, Y. F., & Cai, Z. C. (2007). Organic carbon decomposition rate in different soil types. *Ying yong sheng tai xue bao= The journal of applied ecology*, *18*(10), 2251-2255.

Zech, M., Leiber, K., Zech, W., Poetsch, T., & Hemp, A. (2011). Late Quaternary soil genesis and vegetation history on the northern slopes of Mt. Kilimanjaro, East Africa. *Quaternary international*, 243(2), 327-336.

Zeller, B., Brechet, C., Maurice, J. P., & Le Tacon, F. (2007). $\delta^{13}C$ and $\delta^{15}N$ isotopic fractionation in trees, soils and fungi in a natural forest stand and a Norway spruce plantation. *Annals of Forest Science*, 64(4), 419-429.

Zhang, D., Hui, D., Luo, Y., & Zhou, G. (2008). Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of Plant Ecology*, *1*(2), 85-93.

Zier, J. L., & Baker, W. L. (2006). A century of vegetation change in the San Juan Mountains, Colorado: an analysis using repeat photography. *Forest Ecology and Management*, 228(1), 251-262.