Geoderma 335 (2019) 170–178

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Soil microbial anaplerotic CO_2 fixation in temperate soils

Jacques A. Nel*, Michael D. Cramer

Department of Biological Sciences, University of Cape Town, Private Bag X1, Rondebosch 7701, South Africa

ARTICLE INFO

Handling Editor: I. Kögel-Knabner

Keywords:
$\delta^{13}C$
$\delta^{15}N$
Anaplerotic fixation
Soil organic matter
Soil microbes

ABSTRACT

In soils both $\delta^{13}C$ and $\delta^{15}N$ values of soil organic matter (SOM) tend to increase with soil depth. This change in soil C and N isotopic composition is at least partially linked to soil microbial activity that has been suggested to drive fractionation during decomposition, preferential microbial decomposition of components of soil organic matter and mixing of diverse sources of C. Soil microbes are capable of fixing N_2 and CO_2 from the soil atmosphere. Apart from surface photosynthetic CO_2 fixation and chemoautotrophic 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 may 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). We did not detect significant N_2 fixation in any treatment. Significant soil microbial 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, indicating a possible 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. Diffusion of bulk atmospheric CO_2 ($\delta^{13}C$ ca. −10‰) into the soil atmosphere ($\delta^{13}C \ll -10‰$) drives soil atmospheric CO_2 $\delta^{13}C$ values up towards those of the bulk atmosphere. Anaplerotic CO_2 fixation in this CO_2 may contribute to determining soil $\delta^{13}C$ values.

1. Introduction

Both $\delta^{13}C$ and $\delta^{15}N$ values of soil organic matter (SOM) tend to increase with depth in soil in tropical, temperate and boreal systems (Hobbie and 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 and 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 C and 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 and Swank, 1998; Schimel and 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 and Swank, 1998; Chen et al., 2013). Microbial mineralization involves biochemical reactions that are sensitive to activation energies, substrate supply and moisture. This mineralization leads to isotopic fractionation with retention of heavier isotopes, i.e. ^{13}C and ^{15}N relative to ^{12}C and ^{14}N, respectively, with the limit that no fractionation can occur under substrate-limiting conditions where reactants are entirely converted into products (Cerling et al., 1991). This fractionation of SOM is most evident from ^{13}C enrichment through soil depth profiles and has been attributed to four explanations: 1) The influence of historical changes in atmospheric CO_2 $\delta^{13}C$ values driven by fossil fuel use, 2) Microbial respiratory fractionation during decomposition, 3) Preferential microbial decomposition of less recalcitrant fractions that have lower $\delta^{13}C$ values and 4) incorporation of a proportion of atmospheric CO_2 into microbial biomass (Ehleringer et al., 2000). Indeed, it is likely that some combination of these processes contribute to changes in SOM $\delta^{13}C$ with depth (Boström et al., 2007). The role of microbial respiratory

* Corresponding author.
E-mail address: jacques.a.nel@gmail.com (J.A. Nel).

https://doi.org/10.1016/j.geoderma.2018.08.014
Received 13 March 2018; Received in revised form 6 August 2018; Accepted 7 August 2018
Available online 23 August 2018

fractionation has received much attention with the suggestion that this leads to the fractionation of both C and N as lighter isotopes are progressively used up as mineralization progresses, leaving SOM ^{13}C- and ^{15}N-enriched (Price and 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 (Lin and Ehleringer, 1997; Ekblad and Högberg, 2000; Ekblad and Nordgren, 2002; Boström et al., 2007; Breecker et al., 2015; Hall et al., 2017), while others have demonstrated a significant fractionation of respired CO_2 during decomposition (Fernandez et al., 2003; Šantrůčková et al., 2000a, 2000b). Amongst microbes, however, some are able to fix CO_2 photosynthetically, chemoautotrophically and anaplerotically and some also fix N_2. These processes incorporate C and N into biomass, possibly resulting in microbes exhibiting $\delta^{13}C$ and $\delta^{15}N$ values, respectively, closer to those of the source CO_2 and N_2.

Soil is generally a net source of CO_2, although some microorganisms photosynthetically fix CO_2 in surface soils, and this can 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) that is apparently ubiquitous in living organisms. 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 and 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 few studies have addressed its relevance for soil microorganisms (Šantrůčková et al., 2018). The potential role of anaplerotic CO_2 fixation in influencing $\delta^{13}C$ values of SOM has, however, been suggested (Ehleringer et al., 2000; Boström et al., 2007), and there is empirical support for the role of anaplerotic CO_2 fixation in SOM ^{13}C enrichment with depth in arctic soils (Šantrůčková et al., 2018). The influence of anaplerotic CO_2 fixation on SOM $\delta^{13}C$ is likely to vary with depth because the $\delta^{13}C$ and $\delta^{15}N$ values of CO_2 and N_2 in the soil are not constant, being influenced by the degree of mixing between soil and bulk atmospheric reservoirs of these gasses (Susfalk et al., 2002; Clough et al., 2003) and by diffusion of CO_2 through the soil profile (Cerling et al., 1991). For example, shallow soils have a larger component of atmospheric gasses and a steep gradient of soil CO_2 $\delta^{13}C$ values (Cerling et al., 1991), whereas diffusional limitations result in dilution of atmospheric CO_2 deeper in the soil (Susfalk et al., 2002). Both waterlogging (Greenway et al., 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, 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, although the respiration rate of some soils has been shown to continue increasing up to 40 °C (Lloyd and 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 common in soils, not only in the arctic (e.g. Šantrůčková et al., 2018), 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 C and N are less available may exhibit higher rates of N_2 fixation and anaplerotic CO_2 fixation compared to soils which are nutrient rich. We determined the effect of soil microbial $^{15}N_2$- and anaplerotic $^{13}CO_2$-fixation on soil $\delta^{13}C$ and $\delta^{15}N$ values under varying soil conditions in order to ascertain whether these are incorporated into soils. 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.

2. Materials and methods

In order to determine the effects of soil conditions on both CO_2 and N_2 fixation, $^{13}CO_2$ and $^{15}N_2$ were supplied to soils sampled from a variety of edaphic circumstances. The effects of microbial activity, soil moisture and temperature 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. We used sterilized soils and unenriched soils to provide controls for microbial fixation of CO_2 and N_2.

2.1. 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 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.

2.2. Soil variability

Most of the soils were collected from within the Cape Floristic Region, but in diverse settings representing both open-canopy nutrient poor Fynbos vegetation on shale-derived soils (Table 1; soils 2, 3) and closed canopy Afrotemperate forests both on relatively nutrient-rich granitic (soil 4) and nutrient-poor sandstone-derived soils (soils 5, 6, 10). Within this region we also collected soil from a shale-derived Renosterveld site (soil 1). Further afield, samples were taken from shale-derived Karoo soils (soils 7, 8) as well as grassland sites (soil 9). 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% (Fig. A.1).

2.3. 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