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Is the rate of mineralization of soil organic carbon under microbiological control?

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ABSTRACT

A theory called the Regulatory Gate Hypothesis was previously proposed to considers that the rate limiting step in soil organic carbon (SOC) mineralization is independent of the size, community stricture or specific activity (mg CO_2-C evolved g^{-1} biomass C) of the soil microbial biomass. Here we report new experiments to test this hypothesis. In the first experiment, six different soils were perfused with $CHCl_3$-saturated water to model SOC release and to stop microbial activity. Apart from one highly organic soil, they all released SOC at low and roughly constant rates, over sixty three days. In the second experiment, when the freeze-dried perfusates were returned to the parent soils, their % mineralization ranged from 17 to 35% over ten days, in contrast to bulk SOC (range 0.46–0.77%). In the third experiment, two soils were given three consecutive fumigations, each followed by 10 days aerobic incubation. The microbial biomass was decreased by > 90%, yet SOC mineralization proceeded at the same rate as in nonfumigated soil. In the fourth experiment, the six soils were subjected to various perturbations, including non-perturbed controls, fumigation-incubation, air-drying rewetting, freeze-thaw (−20 °C and −80 °C) and sieving < 0.3 mm. After an initial flush due to the perturbations, the rates of mineralization became roughly equal in nearly all soil treatments and comparable to the control, despite significant declines in biomass. This shows that basal respiration was little affected by the perturbations. In Experiment five the effects of the perturbations on the microbial communities in the different soils and perturbations were determined. The bacterial community was significantly modified by both fumigation and air drying-rewetting, due mainly to increased Firmiculites and decreased Proteobacteria populations. Our findings suggest that mineralization of SOC is a two-stage process: firstly, non-bioavailable forms are converted abiologically to bioavailable forms (termed the Regulatory Gate), which, only then, undergo second process, biological mineralization. This finding has serious implications for theories of e.g. SOC dynamics, effects of global warming and soil nutrient cycling.

1. Introduction

Stabilized soil organic carbon (C) comprises around 1500 Pg C, twice that in the atmosphere (Lal, 2004). It is therefore vital that we have a correct understanding of the key processes involved in the dynamics of this huge C pool if we are to better manage it. It is remarkable that no one has yet satisfactorily explained a particular characteristic of soil organic C mineralization. Thus, even if > 90% of the soil microorganisms are destroyed by $CHCl_3$ fumigation, following fumigant removal and the initial flush of mineralization of fumigant-killed biomass C, soil organic C mineralization continues at the same rate (defined as µg CO_2-C evolved g^{-1} soil day^{-1}) as in the nonfumigated soil (Jenkinson and Powlson, 1976; Wu et al., 1996; Kemmitt et al., 2008). This phenomenon has been known for nearly 60 years but has never been satisfactorily explained, even though $CHCl_3$ fumigation has formed the basis of the widely used Fumigation Incubation method for measuring soil microbial biomass C over this period (Jenkinson and Powlson, 1976).

The long held belief that SOC consists of large, structurally nonspecific, moieties that are largely resistant to microbial attack (loosely termed humified material), is currently being strongly

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challenged. Instead, an alternative model is emerging that considers the stability of SOC to mainly depend upon its interactions with the biotic and abiotic environment e.g. chemical interactions, reactive mineral surfaces, climate, water availability, pH and presence of microbial degraders (Schmidt et al., 2011; Dungait et al., 2012; Lehmann and Kleber, 2015). However, this debate still does not yet reveal how a small, $CHCl_3$ depressed, species poor, microbial population can mineralize the same amount of highly biologically resistant SOC to CO_2-C as the large, intact population in non-fumigated soil. This suggests that the SOC-derived substrate is being made available to these two, very different, populations in the same way, rate, time, and in the same chemical forms, for weeks or even months, without any fresh substrate inputs (e.g. Joergensen et al., 1990; Kemmitt et al., 2008). The question is, how can this be?

Kemmitt et al. (2008) proposed a new theory to explain this phenomenon. They considered that soil organic C had to first undergo an abiotic process, or processes, before it could be mineralized by the microbial biomass. They termed this the Regulatory Gate Hypothesis (SI Fig. 1). ***K1*** is the abiological transformation of non-bioavailable soil organic C. ***K2*** is the biological mineralization of (now) bioavailable soil organic C. Arrows indicate that the soil microbial biomass may create both non-biologically available and biologically available soil organic C but is not able to directly influence the rate of ***K1***. The precise nature of ***K1*** is not known. It may include processes such as desorption, chemical oxidation, free radical activity, cleavage of phenolic rings (Majchner et al., 2000) or stabilized exocellular enzymes. Probably, several mechanisms operate simultaneously.

Kuzyakov et al. (2009) suggested that enzymes released from the $CHCl_3$-induced lysis of microbial cells remained active and could mineralize soil organic C for several months. However, it seems more likely that exocellular non-stabilized enzymes would be utilized as substrate and have a short half-life in soil (Burns, 1982; Nannipieri, 2003). The Regulatory Gate Hypothesis does not necessarily conflict with the view of Kuzyakov et al. (2009) that exoenzymes are involved, as the water soluble C could, presumably, diffuse to them along a concentration gradient. However, it is difficult to reconcile the random activity of exoenzymes, either stabilized in bulk soil organic matter, or freshly released from microbial cells, after, for example, $CHCl_3$ fumigation (Brookes et al., 1982) with the highly regulated metabolic processes involved in (say) the mineralization of a tertiary protein to CO_2. This also agrees with the view of Paterson (2009) that although it might be possible for high rates of soil organic matter mineralization to be maintained in fumigated soil by the activity of stabilized enzymes, this would be "a striking and, in my view, *sic*, an improbable uncoupling of soil biological activities and functions".

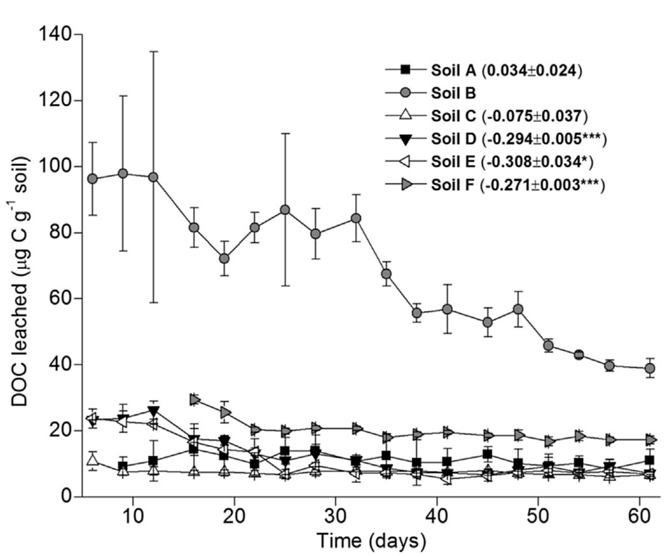

Fig. 1. Total soil organic C leached from six soils (A–F) over 63 days. Errors are standard errors of the mean. Bars that do not share the same letters above them are significantly different ($p < 0.05$).

It is also theoretically possible that the small recolonizing microbial population that survives $CHCl_3$ fumigation has the same community structure, comprising the same population of organic matter mineralizing organisms, as that in the intact non-fumigated soil. We also discount this for two reasons. Firstly, adenosine triphosphate (ATP) concentrations in fumigated soil before $CHCl_3$ removal are very small (Kemmitt et al., 2008) so the recolonizing population must develop after, rather than survive, fumigation. Secondly, it implies that most of the soil microbial biomass is redundant, at least in terms of soil organic C mineralization, in non-fumigated soil. The Regulatory Gate Hypothesis offers an explanation why the mineralization of soil organic C proceeds at the same rate in both nonfumigated and fumigated soil, even if the microbial population size has been decimated, and specific activity and diversity drastically changed. If correct, both the large, intact population and the damaged small population are mineralizing precisely the same small, but constant, supply of soil organic C, which has been transformed from non-available soil organic C, by processes that we do not yet really understand, to biologically available substrates, and which has been brought to both of them via diffusion in soil solution. We believe the alternative possibility, that the soil organic C is being mainly mineralized directly by the microbial population, without firstly undergoing an abiotic transformation from non-available to available, is unlikely in the light of the above evidence. Curtin et al. (2012) reviewed data which agreed with this hypothesis, and similarly considered that chemical factors must be controlling soil organic C desorption rather than biotic ones. In particular, chemical factors causing decreased soil anion absorption capacity can increase soluble organic C concentrations. For example, increasing soil pH can increase desorption of SOC, which can increase mineralization. Similarly, Dungait et al. (2012) cited work which suggested that there must be a transport mechanism which enabled substrate to be transported to the microbial biomass. This suggests that the initial step in the mineralization rate of soil organic C is not under microbial control, i.e. abiotic processes convert non-biologically available to biologically available soil organic C, which then undergoes mineralization. If so, the question is; how could abiotic mechanisms operate?

A main aim of this work was to investigate the phenomenon that if a soil is given a 24 h $CHCl_3$ fumigation, followed by its removal and then an anaerobic incubation, then the rate of SOC mineralization in the previously fumigated soil is very similar to that in the corresponding nonfumigated soil. This observation forms the basis of the Regulatory Gate Hypothesis. This is despite most (around 90% or more) of the microbial population in the fumigated soil being killed by the fumigant. A second aim was to confirm the findings apparently non-biologically available SOC could be mobilized independently of the soil microbial biomass and subsequently mineralized. This was done by slowly perfusing $CHCl_3$ saturated water, to inhibit microbial activity, through soil columns and measuring the soluble C in the perfusates at intervals up to 60 days. The perfusates were then freeze dried, added back to the parent soils and their mineralization measured. Other experiments were done to investigate the effects of air-drying-rewetting, freeze-thaw (−20 and −80 °C) and sieving (<0.3 mm) on basal soil organic matter mineralization and soil microbial community structure.