1 Modeling coupled enzymatic and solute transport controls on decomposition in drying soils

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Abstract

Mechanistic descriptions of microbial processes are difficult to embed in ecosystem models because they require complex mathematical formulations. The interactions between microbes, soil carbon (C), and water availability are particularly complex, as they involve coupled physical (advection and diffusion in unsaturated media) and biochemical processes (enzymatic reactions, C uptake by microbes). Here we propose an approximated equation based on a quasi-equilibrium assumption that describes microbial uptake of soil C as a function of soil moisture and organic matter content during soil drying. The equation predicts that uptake depends on two terms, one dependent on soil organic C concentration and enzyme availability (analogous to a Michaelis-Menten equation) and one dependent on soil moisture via its effects on enzyme and solute mass transfer, and microbial uptake kinetics. Assuming that uptake is proportional to microbial respiration, model results are compared to measured respiration-water potential curves. Using independently estimated parameter values (except for the calibrated microbial uptake efficiency), the theoretical model captures well the respiration decline during drying and provides an explanation of respiration pulses at rewetting. Thus, this simple formulation could be employed in ecosystem models as an alternative to empirical respiration-moisture response functions.

Keywords

Soil water potential; Microbial respiration; Extra-cellular enzymes; Process-based model; Equilibrium approximation

1. Introduction

Recent soil biogeochemical models employ nonlinear kinetic equations to describe carbon (C) mineralization, accounting for the feedback between microbial biomass, which produces extra-cellular enzymes, and soil organic matter, which is decomposed to soluble compounds by such enzymes (Schimel and Weintraub, 2003; Allison et al., 2010; Sinsabaugh and Shah, 2012; Todd-Brown et al., 2012). In these equations, however, soil moisture effects are included *a posteriori* through rate modifiers, thus neglecting possible interactions between reaction and transport processes. Understanding and capturing these interactions is a key step towards predicting biogeochemical responses to hydro-climatic changes. Interactions arise because extracellular enzymes must first reach the complex organic compounds and then react and degrade these compounds. Enzymes and substrates in unsaturated soil may move via diffusion or mass flow, with molecular diffusion becoming the dominant mode of transport as soils dry (Jury et al., 1983) and at the smallest spatial scales (Figure 1). Accordingly, for microbes to decompose a complex substrate three steps are required before uptake and metabolism can occur: i) transport of enzymes to the site of decomposition, ii) enzyme-mediated reaction, and iii) transport of products back to the cells (Vetter et al., 1998).

When diffusion dominates, the transport of enzymes and soluble substrate between microbial cells and organic matter compounds can be described by Fick's law as the product of diffusivity and the concentration gradient. Diffusion is limited by the amount of water-filled pores, resulting in a power-law relationship between solute diffusivity and volumetric soil moisture (Moldrup et al., 2001; Hamamoto et al., 2010). Hence, soil moisture mediates steps i) and iii) of the decomposition process, in addition to imposing physiological stress on decomposing microbes (Harris, 1981; Schimel et al., 2007; Manzoni et al., 2014). Natural soils,

however, are highly heterogeneous, and the occurrence of ‘immobile zones’ may hinder Fickian diffusion by trapping solutes as they are transported towards the microbes. While the concept of immobile zones has been employed in groundwater and vadose zone solute transport models (Griffioen et al., 1998; Padilla et al., 1999; Kätterer et al., 2001; Toride et al., 2003), only recently it has been applied in the context of soil biogeochemical models (Zhang et al., 2014). Moreover, as solute concentration in the liquid phase increases in dry soils, adsorption is favored compared to desorption, and bioavailable C may be removed from the soil solution. These transport processes and physical protection mechanisms limit the availability of enzymes at the decomposition site and of the soluble substrates that can be taken up by microbes, thus constraining the overall rate at which organic matter is transformed into new biomass.

In contrast to the transport processes, enzyme-mediated reactions (step ii) are weakly dependent on soil moisture, but they are controlled by the availability of substrates and enzymes. The rate of enzymatic reactions can be described by Michaelis-Menten kinetics, in which reaction rates saturate at high substrate concentration (Michaelis and Menten, 1913; Briggs and Haldane, 1925; Tang and Riley, 2013). The same functional dependence is often used in soil C models, on the ground that the complex, enzyme-catalyzed reactions in soils can be approximated by homogeneous reactions (Schimel and Weintraub, 2003; Davidson et al., 2012; Tang and Riley, 2013; Wang and Post, 2013; Zhang et al., 2014). The original Michaelis-Menten formalism, however, neglects the transport constraints imposed by soil moisture and no equation currently accounts for enzyme and substrate transport as well as reaction constraints in a consistent way, leaving soil moisture effects as empirical rate or C availability modifiers (Bauer et al., 2008; Davidson et al., 2012).

Macroscopic equations describing decomposition in a mechanistic way should account for these processes, albeit in a simplified way so that they can be applied in ecosystem models. The goal of this contribution is to develop an equation linking C uptake by microbes and subsequent respiration to substrate availability and soil moisture, while also accounting for enzymatic reactions and transport limitations at the daily time scale typical of most biogeochemical models. Our aim is to derive an expression where the overall effect of soil moisture on microbial respiration is not imposed *a posteriori*, but emerges from the coupling of reaction and transport processes.

2. Theory

2.1. Model rationale and main assumptions

We propose a theoretical model of carbon cycling during soil drying. As shown in Figure 1, several conceptual C pools are considered based on their biogeochemical properties and spatial location. C pools located in the bulk soil and pools near the microbial cells are distinguished to provide a simplified, lumped description of the spatial organization in the soil micro-environment. We further separate bio-available C pools in the soil solution from pools of C trapped in hydraulically-disconnected soil patches or sorbed. A sequence of physical and reaction processes linking these pools is considered: enzyme production and transport to the reaction site, reactions yielding bio-available C and consuming stable organic matter, transport of C to the microbial cells and uptake, and removal of bioavailable C due to trapping in the unavailable pools. Moreover, we focus on C pools that are degraded by extracellular enzymes and neglect labile compounds that become bioavailable as soon as they are incorporated in the soil.

In principle, a model based on mass balance equations could track the fate of C in all these pools and thus predict C fluxes among them, providing a process-based representation of C cycling in variably saturated conditions. However, most ecosystem-scale biogeochemical models lack this level of detail in their pool structure and thus require kinetic laws that account for detailed processes and yet only depend on a few modeled pools (i.e., ‘macroscopic’ kinetic laws). Our main goal here is to derive a ‘macroscopic’ kinetic law describing the flux of C from an organic matter pool to a microbial pool. We aim at collapsing the complexity illustrated in Figure 1B (top C flow scheme) into a simple expression that only depends on pools compatible with ecosystem models (Figure 1B, bottom). To derive this equation, we adopt the method of time scale separation (Holmes, 2009). The idea behind this technique is that pools turning over much faster than the others can be assumed in ‘quasi steady state’; in other words, these pools equilibrate rapidly due to the large fluxes they exchange and can thus be considered in equilibrium at the daily time scale. The mass balance equations for these ‘fast’ pools can then be solved analytically at steady state, immediately providing a value for the ‘fast’ C pools as a function of the slower ones. This is precisely what we need to collapse small, ‘fast’ pools into kinetic laws that only depend on the ‘slow’ C pools described by ecosystem models.

Three separate time scales characterize C cycling in drying soils: a fast scale for the enzymatic reactions at which the total enzyme pool is approximately constant (sub-daily time scale), an intermediate one at which the enzyme and product pools are allowed to vary, but can be assumed in equilibrium by exchanging mass through biogeochemical and transport processes (daily time scale), and a slower time scale at which the substrate and microbial biomass pools vary (longer than daily). By considering the C compartments that change rapidly during a soil dry-down at quasi-steady state, an analytical expression that links microbial activity to soil

moisture and soil organic C valid at the daily time scale is thus obtained. Section 2.2 presents the mass balances for each pool, Section 2.3 defines the C fluxes and their relations to soil moisture, and Section 2.4 briefly presents the published soil respiration data we used. Time scales are indicated in the following by the symbol τ ; all biogeochemical pools and fluxes are defined in Table 1 and Figure 1, and parameter values are reported in Table 2.

2.2. Mass balance equations

We conceptualized soil C cycling as a set of five interconnected compartments: bio-unavailable organic matter (C_{OM}), bio-available or soluble C in the bulk soil (subscript S) and around the microbial cells (subscript $S,0$), enzyme C (subscript E), and microbial biomass C (C_B). The C_{OM} and C_B compartments are each described as single pools. In contrast, the substrate and enzyme compartments are further partitioned into an available phase (superscript a) and an un-available phase (superscript u). The available phase includes C dissolved (or readily soluble) in pore-water *connected* to microbial cells (the mobile water phase), while the unavailable phase includes C dissolved in pore-water *disconnected* to microbial cells (the immobile water phase) and C sorbed to soil particles. These variables are expressed as C-mass per unit volume of soil (gC m^{-3} soil). The C_E^a , C_S^a , and $C_{S,0}^a$ pools are converted to concentrations in liquid phase when necessary (C-mass per unit volume of soil water, gC m^{-3} water). The conversion is done by assuming that a fraction f of the total soil water θ resides in the mobile water phase (m^3 water m^{-3} soil). Thus, the concentrations in liquid phase are given as $C_i^a / (f\theta)$ and $C_i^u / [(1-f)\theta]$ (for $i=S$ or $i=E$). These different definitions are required to express diffusion-driven mass transfer as a function of gradients in the liquid phase only, whereas mass balance equations are written for total soil C (i.e., the control volume encompasses the whole soil volume).

Because both C_{OM} and C_B are approximately constant at the daily time scale, we can focus on the mass balance equations for the remaining compartments, expressed as

$$\frac{dC_E^a}{dt} = F_E - L_E - \phi_E - D_E,$$

$$\frac{dC_E^u}{dt} = \phi_E,$$

$$\frac{dC_S^a}{dt} = D_{OM} - F_S - L_S - \phi_S, \quad (1)$$

$$\frac{dC_S^u}{dt} = \phi_S,$$

$$\frac{dC_{S,0}^a}{dt} = F_S - U,$$

where mass transfers are indicated by F , decomposition by D (either de-polymerization, D_{OM} , or enzyme decay, D_E), leaching by L , and microbial uptake by U ; where ambiguities may arise, subscripts are added to indicate which compartment each flux refers to. Enzymes (C_E^a) are produced by the microbial biomass (production is modeled implicitly as a set enzyme concentration around the cell, $C_{E,0}$, see Equation (5)), and transported to the site of decomposition by diffusion (F_E). Available C, C_S^a , is also transported by diffusion to the microbial cells (F_S). Both enzymes C and available C may be transferred from the mobile phase to unavailable C pools (respectively ϕ_E and ϕ_S) or lost through leaching (L_E and L_S), and enzymes are deactivated at a rate D_E . Deactivated enzymes are transferred to the C_{OM} pool. The unavailable pools (C_E^u and C_S^u) only exchange C with the available pools. Hence, in this conceptual scheme, de-polymerization, transport to the microbes, and C uptake simultaneously limit the C mineralization rate. Environmental conditions and the value of the kinetic constants (Section 2.3) determine the relative importance of each of these processes.

To obtain an analytical expression for C supply during soil drying, we focus on a single period without rain. Due to the reduction in soil moisture, the concentrations of solutes and enzymes in the available phase increase partly because the solvent content decreases and partly because the total pool size increases (Williams and Xia, 2009; Kakumanu et al., 2013; Guo et al., 2014; Warren, 2014; Kaiser et al., 2015). In turn, C accumulation in solution favors mass transfer towards the unavailable pools (immobile or sorbed phases). Hence, we can approximate the phase exchanges as a net loss from the available phase (i.e., ϕ_E and ϕ_S are positive when soil dries). The release of C from the unavailable pools would instead occur during re-wetting or when the soil remains moist for prolonged periods. During the drying period, we suggest that the dynamics of the available substrate and enzyme C can be effectively approximated as quasi-stationary, whereas C accumulates in the unavailable pools.

In order to track the temporal changes in the mass transfer rates and water volume available for the dissolved phase, we modeled soil moisture dynamics with a simplified soil water balance equation,

$$\frac{d\theta}{dt} = -\varepsilon_{max} \min\left(\frac{\theta}{\theta_{fc}}, 1\right) - l, \quad (2)$$

where θ is the volumetric soil moisture, ε_{max} is the maximum evaporation rate per unit soil volume (d^{-1}), θ_{fc} is the soil moisture at field capacity, and l is the rate of deep percolation per unit of soil volume (d^{-1}). In the results presented below, we focus on conditions where drying is induced by evaporation alone (e.g., in controlled laboratory conditions), which motivated the choice of the simple piece-wise linear evaporation function. The soil moisture dependence of l is described by $l = k_{sat}/Z (\theta/\eta)^{2b+3}$, where k_{sat} is the hydraulic conductivity in saturated conditions, Z the soil depth, and b is the exponent of the water retention curve (Campbell, 1974).

Normalizing the hydraulic conductivity (m d^{-1}) by soil depth is necessary to have consistent units in all terms in Equation (2). The water volume is further partitioned into a mobile (hydraulically-connected) phase that is accessible to microbes and an immobile (disconnected) phase. Moisture in the mobile zone is for simplicity assumed to be a constant fraction f of the total moisture (Griffioen et al., 1998). Once initial conditions are prescribed and the fluxes are defined, Equations (1) and (2) fully describe the dynamics of the C pools and soil moisture during drying.

2.3. Definition of the C fluxes

2.3.1. Biogeochemical reactions

The kinetic rate for enzymatic reactions producing bio-available C from stable organic compounds can be described by a saturating function of C_{OM} (Michaelis and Menten, 1913),

$$D_{OM} = \frac{V_{max} C_E^a C_{OM}}{k_m + C_{OM}} = \nu C_E^a, \quad (3)$$

where V_{max} is the kinetic constant for the reaction (d^{-1}) and k_m is the half saturation constant (gC m^{-3}). Following the derivation of Briggs and Haldane (1925), Equation (3) is obtained when the enzymatic reaction is much faster than the turnover rate of enzymes and reaction products – an assumption justified at the daily scale. Because C_{OM} can be approximated as constant at the daily time scale, kinetic constants and C_{OM} can be grouped in a single parameter, ν , which summarizes availability and decomposability of organic C. Clearly, C_{OM} and thus ν may change at longer time scales, as represented in ecosystem models. However, we here look at the daily-scale moisture effect, and we can ignore the specific values of V_{max} and k_m (and thus ν) by normalizing decomposition rates to optimal moisture conditions. Moreover, in Equation (3), only the available enzyme fraction contributes to the reaction, whereas the enzyme fraction sorbed to the mineral matrix or trapped into immobile zones is inactive.

Different models of microbial uptake U have been proposed, ranging from a constant rate independent of substrate concentration (Merchuk and Asenjo, 1995) to Michaelis-Menten kinetics (Bosma et al., 1997; van Hees et al., 2005a; Ågren and Wetterstedt, 2007; Hobbie and Hobbie, 2012). Because substrates are unlikely to be at saturating concentrations around microbial cells, uptake rate can be described by a linear rather than a saturating function of substrate concentration in the dissolved phase,

$$U = u \frac{C_{S,0}^a}{f\theta}, \quad (4)$$

where u is the uptake kinetic constant, with units of m^3 of water m^{-3} of soil d^{-1} – indicated for conciseness as d^{-1} . The value of u depends on the activity state of the microbial biomass, and thus probably varies as soil moisture decreases. Because the value of u is not known *a priori*, we first carried out a sensitivity analysis and then estimated how u varies as a function of water potential, using a simple inversion approach (Section 2.4). Enzyme deactivation is assumed to follow first order kinetics as $D_E = k_E C_E^a$ (Schimel and Weintraub, 2003).

2.3.2. Mass transfer by diffusion and leaching

Diffusion of enzymes and soluble compounds was modeled based on mass transfer equations,

$$F_E = h_E \frac{C_{E,0} - C_E^a}{f\theta}, \quad (5)$$

$$F_S = h_S \frac{C_S^a - C_{S,0}^a}{f\theta},$$

where the subscript 0 indicates concentrations near the microbial cells and the transfer coefficients for enzymes and soluble substrates are denoted by h_E and h_S , respectively. Note that

the concentration gradients driving these fluxes develop in the dissolved phase; therefore, the C pools are converted to units of mass per unit soil water volume. The transfer coefficients (with units of m^3 of water m^{-3} of soil d^{-1} – indicated as d^{-1}) need to incorporate information on the spatial structure of the soil system, and specifically the distance between substrates and microbes. For simplicity, we assume that microbial cells or colonies are located on a three-dimensional square lattice (Figure 1A), so that the mass transfer coefficients are expressed as (Manzoni et al., 2014),

$$\begin{aligned} h_E(\theta) &= \frac{\nu D_E(\theta)}{\delta^2} \\ h_S(\theta) &= \frac{\nu D_S(\theta)}{\delta^2} \end{aligned} \quad (6)$$

where D_E and D_S are the diffusivities of enzyme C and bio-available C (a function of volumetric soil water content, θ), and δ is the characteristic distance between microbial cells or colonies and their C substrate. The units of the diffusivities are m^3 of water m^{-1} of soil d^{-1} ; i.e., in more compact notation, $\text{m}^2 \text{d}^{-1}$ (adopting the unit convention in Hamamoto et al., 2010). Hydrodynamic dispersion is neglected, as we focus on unsaturated conditions with slow water movement driven mainly by evapotranspiration (Jury et al., 1983; Sato et al., 2003). This assumption is conservative, since dispersion would contribute to the effective diffusivity and thus to the net transport of solutes towards the microbes.

To derive Equation (6), the transfer coefficient was approximated as the ratio of Fickian diffusivity and the distance δ , multiplied by the surface around a microbial cell crossed by the substrate or enzyme flux, and divided by the volume of soil enveloped by this surface, to obtain fluxes per unit soil volume (Cussler, 1995; Bosma et al., 1997) (Figure 1A). In turn, the surface-

to-volume ratio is approximated by ν/δ (with $\nu=6$) assuming that microbes are located at the vertices of a cubic lattice of side δ and that the same length scale characterizes the microbe-substrate distance (Manzoni et al., 2014). The value of δ depends on the microbial cell distribution in the soil with respect to the C sources (Section 2.3.3). It should be noted that microbial cells often form clusters around nutrient- or C-rich hotspots (Nunan et al., 2002; Eickhorst and Tippkoetter, 2008; Raynaud and Nunan, 2014); in that case the single value assumed here for the distance δ should be regarded as the mean value of the actual distribution. Because estimates of δ are uncertain, sensitivity analyses were performed on this parameter.

Solute diffusivities were modeled as a function of volumetric water content as (Hamamoto et al., 2010),

$$\frac{D_i(\theta)}{D_{i,0}} = (\eta - \theta_t)^{m_1} \left(\frac{\theta - \theta_t}{\eta - \theta_t} \right)^{m_2}, \quad (7)$$

where θ_t is the percolation threshold for solute diffusion (here computed as the soil moisture at $\psi_t = -15$ MPa), m_1 and m_2 are two empirical exponents (1.5 and 2, respectively), η is the soil porosity, and $D_{i,0}$ is the diffusivity of either enzymes ($i=E$) or substrate ($i=S$) in pure water (Table 2).

The rate of C transfer from the available to the unavailable pools is described as a mass transport in soil solution, with a first order transfer coefficient denoted by α_i ($i=S$ or $i=E$),

$$\phi_E = \alpha_E \left[\frac{C_E^a}{f\theta} - \frac{C_E^u}{(1-f)\theta} \right] \approx \alpha_E \frac{C_E^a}{f\theta}, \quad (8)$$

$$\phi_S = \alpha_S \left[\frac{C_S^a}{f\theta} - \frac{C_S^u}{(1-f)\theta} \right] \approx \alpha_S \frac{C_S^a}{f\theta},$$

where the concentration in the available and un-available pools are expressed as C-mass per unit volume of water in the corresponding phases. The approximations on the right hand side only hold during soil drying, when the concentrations of the available pools increase sufficiently fast that the concentrations in the un-available pools can be neglected (i.e., C_E^u and C_S^u are temporarily net C sinks). During re-wetting or in moist conditions, the un-available pools would not act merely as receivers, but would also supply C to the available pools. The mass transfer coefficients α_i can be interpreted as first-order rates of adsorption (Wang et al., 2013) or transfer to the immobile zone (Griffioen et al., 1998) (with the same units of h_i). Lacking specific information on the values of α_i , we assumed them to be equal ($\alpha_E = \alpha_S = \alpha$), and performed a sensitivity analysis.

Leaching of enzymes and dissolved substrate (respectively denoted by L_E and L_S) becomes increasingly important as the percolation of soil water increases at high soil moisture, as long as the soil is free to drain. These fluxes are modeled as the product of enzyme or substrate concentration in pore water and the corresponding leakage rate,

$$\begin{aligned} L_E &= l \frac{C_E^a}{f\theta}, \\ L_S &= l \frac{C_S^a}{f\theta}. \end{aligned} \tag{9}$$

In turn, the leakage rate is the volume of water percolating per unit volume of soil per unit time (which depends on soil moisture, see Equation (2)).

2.3.3. Model parameters, simulations, and analytical solutions

The model requires estimates for several parameters (listed with their literature source in Table 2). Some of these parameters, however, are poorly quantified, requiring sensitivity analyses over a reasonable range of values. In particular, we consider the following ranges:

- Characteristic distance between substrates and microbes, δ : between 10^{-5} and 10^{-3} m (Manzoni and Katul, 2014). Soils that have been disturbed (e.g., through sieving) likely have a more homogeneous distribution of microbial cells, resulting in low $\delta \sim 10^{-5}$ to 10^{-4} m. Soils in a natural, undisturbed state are probably characterized by larger distance between microbes and C sources, since at least a fraction of the microbial colonies might have remained isolated from richer soil patches (e.g., around an extending root). In a conceptually similar, lumped model applied to contaminated soil, δ was approximated as 10^{-4} m (Bosma et al., 1997), consistent with our estimate.
- Rate constant of sorption and transport to immobile zones, α : between 10^{-2} and 10 d^{-1} to span a reasonably large range of time scales; we selected $\alpha=1$ d^{-1} as a default value, in agreement with estimates of mass transfer coefficients between mobile and immobile zones at low soil moisture (Padilla et al., 1999; Toride et al., 2003) and a modeling study assuming rapid equilibration between these zones (Zhang et al., 2014). If C removal from the bioavailable pool is interpreted as due to adsorption, α would be lower, in the order of 0.1 d^{-1} (Wang et al., 2013). Some extra-cellular enzymes sorbed to mineral particles can still be chemically active (Yan et al., 2010), suggesting that the distinction between C_E^a and C_E^u might not be as strong as we assumed. To test the role of the inactive enzyme pool, we set $\alpha=0$ for the enzyme pool, i.e., $\phi_E=0$, in a separate analysis.

- Rate constant for microbial uptake, u : between 0.1 and 10 d⁻¹, based on the measured uptake rate of low molecular weight compounds at low concentration (typically >1 d⁻¹, see Hobbie and Hobbie (2012)). The 0.1 to 10 d⁻¹ range is used in a sensitivity analysis where u is kept constant with respect to soil moisture; however, model-data comparisons suggest that this assumption is not reasonable, so that a function $u(\psi)$ must be estimated. To this purpose, u was estimated from inversion of Equation (11) for each dataset (see details in Section 2.4).
- Rate constant for enzyme turnover, k_E : between 0.01 and 1 d⁻¹ (Allison, 2006; Becerra et al., 2014). We selected $k_E=0.1$ d⁻¹ as default value, and verified that changes in k_E have limited impact on the C uptake-moisture relation.

Unless otherwise stated, baseline parameter values were: $\alpha=1$ d⁻¹, $\delta=10^{-4}$ m, $k_E=0.1$ d⁻¹, $C_{E,0}=0.1$ gC m⁻³, and soil textural properties typical of a sandy loam.

Equations (1) and (2) are solved together to describe the temporal changes in all the soil soluble pools during a dry period, when organic matter and microbial biomass remain relatively stable. The numerical solution has then been compared to the analytical solution of Equations (1) at steady state, under the assumption that the ‘fast’ pools equilibrate rapidly with the incoming and outgoing fluxes. The steady state solutions are obtained by setting to zero the time derivatives in the mass balance equations for C_E^a , C_S^a , and $C_{S,0}^a$ (Equation (1)), and solving the resulting system of algebraic equations.

2.4. Soil respiration data and data analysis

Model results are compared to measured soil respiration vs. soil water potential curves. Respiration data were collected from published laboratory studies, using soils spanning a range

of textures (5% to 80% sand) and origin (from mesic to semiarid climates). Soils were incubated either at constant moisture levels (75% of datasets) or throughout one or multiple drying periods (25% of datasets), recording respiration rates. In the former experimental setup, respiration rate was measured several days or weeks after equilibration at the required moisture level (except for one experiment in which respiration was measured after a day). In the latter experimental setup, the drying period lasted 2-9 weeks, similar to a natural dry-down. Therefore, in both cases, the experimental conditions were consistent with our model assumptions. Only datasets in which respiration rates decreased to less than 20% of the maximum rate were retained (50% for intact soil cores, due to limited availability), resulting in 32 datasets in total. To compare different datasets, respiration rates were normalized to the values achieved at field capacity. Details on the respiration data can be found in Manzoni et al. (2014).

The respiration data were used as proxies of the microbial C uptake rate, based on the assumption that the microbial C-use efficiency is relatively stable. The same respiration datasets were used to infer changes of the uptake rate constant u during drying. For each experiment, we equated the uptake rate model at steady state (Equation (11) in the Results section) to a nonlinear least-square regression of the respiration data, yielding an equation where u is the only unknown. Inverting analytically this equation, an estimate of u was obtained for all respiration datasets and water potential levels.

In all figures, water potential (ψ) is used instead of volumetric soil moisture (θ) to compare different datasets and express water content in terms of its actual availability to the microbes. In most datasets used, water potential values were already reported. Where water potential was not reported and in all model results, water potential is obtained from soil moisture using a standard water retention curve in the form $\psi = \psi_{sat}(\theta/\eta)^{-b}$, where ψ_{sat} is the water

potential at saturation, η is the soil porosity, and b is the exponent of the water retention curve (Campbell, 1974). The values of these parameters were changed with soil texture following Rodriguez-Iturbe and Porporato (2004).

3. Results

Conditions under which the dissolved bio-available C and enzyme C compartments equilibrate rapidly compared to changes in soil moisture are evaluated first (Section 3.1). Second, the steady state solutions of the mass balance equations (1) are derived (Section 3.2). These solutions are finally compared to observed rates of heterotrophic respiration as a function of water availability (Section 3.3).

3.1. Validity of the equilibrium approximation

The bulk soil organic matter and the microbial pool change at time scales much longer than daily (respectively ≈ 5 -100 years and ≈ 1 year) (Wardle, 1998; Barré et al., 2010), despite large variations in the fluxes leaving (D_{OM}) and entering (U) these pools as soil moisture declines. Thus, these pools can be regarded as constant in the following analyses. The dissolved bio-available and enzyme C pools are instead more dynamic and the time scales at which they vary depend on soil moisture. These timescales are estimated as turnover times, i.e., ratios of pool size (per unit soil volume) over outflow. At high soil moisture, rapid leaching and diffusion decrease the turnover time, whereas in dry conditions slow biochemical reactions (enzyme deactivation) prevail and hence the turnover times increase. Using Equations (1), and assuming that the uptake kinetic constant u is sufficiently high to maintain $C_{S,0}$ near zero, the turnover time for the enzyme pool is given by $\tau_E = f\theta(l + \alpha + f\theta k_E)^{-1}$ and for bioavailable C is obtained as $\tau_S = f\theta(l + \alpha + h_S)^{-1}$. Because both leaching and mass transfer depend on soil moisture in a

nonlinear way with an exponent β larger than one, then $\tau \propto \theta \times \theta^{-\beta} = \theta^{1-\beta}$, i.e., these time scales lengthen as soil becomes drier (Figure 2). In comparison, the soil moisture time scale when $\theta < \theta_{fc}$ is approximately 4 days, with parameters in Equation (2) suitable for laboratory conditions. In natural setting, a typical soil moisture time scale would be in the order of weeks (Katul et al., 2007). Hence, the quasi-equilibrium approximation for the pools of dissolved enzyme C and bio-available C is justified. Also the soluble C around the microbial cells turns over relatively fast, as long as the uptake kinetic constant in dry conditions remains in the order of 1 d^{-1} (this is the case, as explained in Section 3.3). To check the performance of the equilibrium approximation, it was compared to the numerical solution, resulting in nearly identical trajectories (not shown).

This equilibrium approximation does not hold after rainfall or during wet periods when soil moisture is high; under these conditions, release of dissolved C from the unavailable phases plays an important role, and transport is not inhibited. Also, as shown in Figure 2A (light gray lines), the time scales of bioavailable and enzyme C approach one day at low soil moisture if the average distance between microbes and substrates is large (in the shown example $\delta=10^{-3} \text{ m}$) and the mass transfer coefficient to the sorbed or immobile phase is low (α much lower than 1 d^{-1}). These conditions represent the limit of applicability of the equilibrium approximation.

3.2. Equilibrium solution

Steady state solutions for dissolved enzyme C and bio-available C in the bulk soil and around the microbial cells are obtained analytically by setting the time derivatives in Equation (1) to zero and solving for C_E^a , C_S^a , and $C_{S,0}^a$,

$$C_E^{a*} = \frac{C_{E,0} h_E}{h_E + l_E + f\theta k_E + \alpha},$$

$$C_S^{a*} = \frac{v C_E^{a*} f \theta (h_S + u)}{u(l_S + \alpha) + h_S(l_S + \alpha + u)}, \quad (10)$$

$$C_{S,0}^{a*} = \frac{v C_E^{a*} f \theta h_S}{u(l_S + \alpha) + h_S(l_S + \alpha + u)},$$

386 where equilibrium conditions are indicated by an asterisk. Equations (10) are in turn substituted
387 in Equations (3), (4), and (5) to obtain the equilibrium fluxes – specifically the microbial uptake,

$$U^* = v C_{E,0} \kappa(\theta)$$

$$\kappa(\theta) = \frac{u h_E h_S}{(h_E + l_E + \alpha + f\theta k_E) [u(l_S + \alpha) + h_S(l_S + \alpha + u)]}. \quad (11)$$

388 The function U^* has been written in Equation (11) by separating a reaction term $v C_{E,0}$ from a
389 moisture-dependent rate modifier $\kappa(\theta)$, which tends to one when C is not lost from the
390 available pool and enzyme de-activation is slow compared to the transport and uptake processes.
391 It is important to emphasize four aspects regarding Equation (11):

- 392 1) U^* describes microbial uptake when the *available* C pools are in equilibrium. Therefore,
393 U^* is generally lower than the actual de-polymerization rate D_{OM} (Equation (3)) because
394 part of the C from de-polymerization is temporarily stored in the *unavailable* (sorbed or
395 immobile) C pools. In other words, the soil system is far from being at steady state, but
396 some compartments (C_E^a , C_S^a , and $C_{S,0}^a$) equilibrate rapidly and reach a quasi-equilibrium.
- 397 2) The reaction term $v C_{E,0}$ retains the form of a Michaelis-Menten function (Equation (3)),
398 where the maximum reaction velocity and the half saturation constant are independent of

soil moisture. Had we assumed that de-activated enzymes feed the soluble C pools, D_{OM} would include an additional term dependent on k_E .

- 3) Microbial biomass plays a role in setting the concentration of enzymes outside the microbial cells ($C_{E,0}$), which in turn controls the reaction term and thus also the maximum uptake rate. Also the uptake kinetic constant u affects U^* , but only through interactions with the transport kinetics in the coefficient $\kappa(\theta)$.
- 4) The predicted U^* can be linked to heterotrophic respiration, which is often measured. In fact, uptake is proportional to respiration, with a proportionality constant given by one minus the microbial C use efficiency. Hence, if C use efficiency is weakly dependent on soil moisture, patterns in normalized U^* and respiration should be comparable.

Around the soil field capacity ($\theta = \theta_{fc}$), all microbes are active ($u \gg 1 \text{ d}^{-1}$) and leaching can be neglected, allowing us to define a maximum uptake rate to normalize U^* . This normalization allows us comparing Equation (11) to different respiration datasets and focus on the scaling between respiration and water availability without confounding factors such as temperature and organic matter content. In Table 3 the coefficient $\kappa(\theta)$ from Equation (11) is further simplified by considering the limiting cases of negligible leaching, mass transfer to sorbed/immobile phases, or transport by diffusion, as well as the case of limiting uptake kinetics (i.e., low physiological activity).

Figure 3 shows the effect of different mass transfer rates to the unavailable pools, uptake kinetics, microbe-substrate distances, and soil texture on the normalized uptake. Larger mass transfer to the unavailable pools lowers the available C, thereby reducing microbial uptake (Figure 3A). In contrast, slow mass transfer allows larger fluxes towards the microbes and

sustained uptake even in relatively dry conditions, provided that u remains larger than zero at low moisture levels. This sustained uptake is due to the accumulation of bio-available C that in turn increases the uptake flux to the point of partly compensating the reduction of u at low moisture. Increasing the uptake rate u or decreasing the microbe-substrate distance δ improves microbial activity at any moisture level (Figure 3B, D). Fine-textured soils, due to the larger diffusivity at a given volumetric moisture level, also improve microbial activity, as shown in Figure 3C. Altering the turnover rate of enzymes has only limited effects on U^* (not shown).

The scenario in which enzymes remain active regardless of soil moisture content, whereas bio-available C is lost to unavailable pools, was also considered (not shown for conciseness). Because more enzymes are active compared to the initial scenario, decomposition of organic matter may proceed faster at any given soil water potential. As a consequence, the uptake-water potential curves shift rightwards and become steeper around the water potential values where substrate transport and microbial physiology become limiting.

3.3. Moisture effects on the microbial uptake rate constant

In Figure 4 the predicted declines in microbial uptake are compared to measured respiration declines from incubation experiments (Section 2.4). We note that both modeled and measured respiration rates are normalized to allow comparing widely different experimental setups and that modeled curves are parameterized for a soil of intermediate texture (sandy loam) for clarity of representation. As emphasized above, this comparison is based on the assumption that uptake and respiration are proportional, i.e., the carbon use efficiency varies little along moisture gradients. If U^* is solely controlled by abiotic factors, microbial respiration is overestimated at intermediate water potential levels (thin lines in Figure 4). The differences

between modeled and measured respiration rates can thus be attributed to reduced microbial uptake efficiency (i.e., low u) as moisture decreases.

Because we had no *a priori* knowledge of the dependence of the uptake rate constant u on soil water potential, we adopted an inversion approach and estimated the function $u(\psi)$ from Equation (11) and the measured respiration-moisture curves. As expected, the estimation procedure predicts very large u values under moist conditions ($u \gg 1 \text{ d}^{-1}$), subsequently declining as ψ becomes more negative (solid curve in Figure 5). Around $\psi = -8 \text{ MPa}$ the relation breaks down as the predicted respiration nears the measured values even assuming unlimited uptake capacity (below $\psi = -8 \text{ MPa}$ numerical instabilities make this approach unreliable). Using the u values at $\psi > -8 \text{ MPa}$, a predictive equation for u is obtained by nonlinear least-square fitting (dashed curve in Figure 5),

$$u(\psi) = 0.60 + 0.28|\psi|^{-1.23}. \quad (12)$$

Equation (12) predicts that the uptake rate decreases as soil water potential becomes more negative. This decrease can be caused by declining number of active microbial cells, or decreasing uptake rate due to downregulation under water stress. This approach cannot distinguish between the contributions of these different physiological responses, because it lumps all microbial physiological limitations into a single equation. Accounting for these physiological limitations provides a better fit of U^* to the respiration data compared to the case of unlimited uptake efficiency. This procedure does not represent a proper validation, since the same dataset was used to estimate u . However, the dependence of u on ψ follows our general expectations and its rate of decline is consistent with the increasing number of inactive species during drying (dotted curve in Figure 5) (Lennon et al., 2012).

Overall, using default parameters and only calibrating u , the theoretical predictions are in line with respiration measurements during drying in sieved soils ($R^2 = 0.76$). The effect of soil type is not large as long as soils of intermediate texture are considered (Figure 3C), such as the ones in these experiments, leading to a similar pattern of respiration decline in all datasets. Respiration declines are faster in soil samples that were maintained intact, possibly due to their less negative diffusion threshold ($\psi_t = -1$ MPa; see Table 2) and larger distances δ . This faster respiration decline is captured by Equation (11) when the distance δ is increased to $10^{-3.5}$ m to simulate a heterogeneous soil structure, in which substrates have not been artificially moved near the microbes by soil mixing and sieving (compare dashed and solid curves; $R^2 = 0.61$).

3.4. Effect of interacting abiotic and biotic factors on C uptake rate

Having established a relation between the uptake constant and soil water potential, it is now possible to identify how different biotic (i.e., captured by $u(\psi)$) and abiotic factors (mass transfer coefficients) contribute to the overall microbial uptake rate U^* . To isolate different factors, we sequentially removed contributing processes from the general Equation (11) (Figure 6). As also shown in Figures 4 and 5, microbial physiology becomes limiting at moderate water potential levels (near -0.2 MPa), while transport constraints gain importance around -2 MPa. The combined effects of reduced solute diffusion and increased transfer to the unavailable C pool inhibits U^* towards the diffusion threshold (-15 MPa). In even drier conditions, enzyme transport and decay are predicted to be the primary limiting factors.

4. Discussion

4.1. Comparison with previous approaches

Some components of the proposed model (Figure 1) are related to previous works that derived uptake equations by combining reaction and transport processes. Some models considered diffusion limitations and uptake kinetics, but neglected enzyme dynamics (Merchuk and Asenjo, 1995; Bosma et al., 1997; van Hees et al., 2005b; Ågren and Wetterstedt, 2007; Liu, 2007); others considered both enzyme and substrate dynamics, but did not consider diffusion separately from the biochemical reactions (Lawrence et al., 2009; Zhang et al., 2014). All these processes have been considered by Tang and Riley (2013), but enzyme availability was set independent of transport properties. Manzoni et al. (2014) considered diffusion using a similar lumped approach, but microbial uptake was assumed to be equal to the diffusion rate, possibly leading to overestimated respiration pulses after rewetting. Recent individual-based models account for the main mechanisms considered here and are spatially explicit (Allison, 2005; Ginovart et al., 2005; Resat et al., 2012; Kaiser et al., 2014). These models allow exploring spatially structured interactions among organisms but are difficult to scale up and use in ecosystem-level models. Here we propose an approximated, process-based framework that can be used in ecosystem models to capture the dependence of heterotrophic respiration on soil moisture during drying.

The microbial uptake flux obtained under equilibrium conditions retains a basic Michaelis-Menten dependence on soil C (through the reaction term $vC_{E,0}$ in Equation (11)). However, moisture availability only affects the maximum uptake rate, and not the half-saturation, as assumed in a recent model (Davidson et al., 2012). In other words, in our

derivation, moisture effects can be isolated from the reaction terms and thus soil moisture does not interact with C_{OM} in the Michaelis-Menten function. This lack of interaction with C_{OM} is due to the fact that soil moisture limits the fluxes among pools, and not the pool sizes themselves. Thus, changing moisture will cause a re-equilibration of the C pools that depend on how the different inputs to and outputs from each pool vary. For example, the bio-available C concentration cannot be calculated multiplying soil organic C by a moisture-dependent coefficient (Davidson et al., 2012), because the size of such a pool depends on how the various inputs and outputs equilibrate. In moist conditions, bio-available C concentrations will decrease due to larger outputs (leaching and microbial uptake) compared to the inputs, but using a moisture-dependent coefficient would instead predict increased bio-available C concentration. Thus, our results suggest that moisture effects can be captured by the moisture multiplier $\kappa(\theta)$ – as often assumed on a purely empirical basis in biogeochemical models (Rodrigo et al., 1997; Bauer et al., 2008). However, the steady-state enzyme concentration is moisture-dependent, so soil moisture effects would also appear in the denominator of the reaction term in models including enzymatic saturation (Tang and Riley, 2013). We emphasize that, different from previous models, this expression has been derived without *a priori* knowledge on the form of the multiplier $\kappa(\theta)$.

Different factors simultaneously affect the shape of the multiplier $\kappa(\theta)$, including physical losses, transport of solutes, and uptake kinetics, which limit C fluxes at different times during a dry period (Figure 6). Leaching depletes soluble C in moist conditions; as soils dry, microbial uptake is reduced faster than diffusivity, thus becoming limiting at intermediate soil moisture; in dry conditions the transport of soluble C to microbes is the most limiting factor. The

equations for $\kappa(\theta)$ predict that these factors cause saturating dependencies of uptake rate on any mass transfer or kinetic constant (Table 3); i.e., when one process is faster than the others, it loses importance. This mathematical form allows smooth transitions among the different limitations as moisture varies (as in the conceptually similar model by Ågren and Wetterstedt, 2007). The disadvantage of explicitly representing these different physical and biological processes is the increased number of parameters compared to simpler empirical approaches. Some of these parameters could be difficult to estimate from respiration-moisture curves due to equifinality, because they change the shape of the respiration curve in similar ways. For example, increasing either δ or α lowers the C fluxes (Figure 3). Despite this problem, having a process-based equation is advantageous as it allows attributing changes in respiration to specific processes once parameters are estimated from independent datasets.

4.2. Simplifying assumptions

The approximated equation for microbial uptake (Equation (11)) is based on the assumption that the dissolved enzyme C and bio-available C pools turn over quickly compared to soil moisture (Figure 2). Evidence suggests that turnover times of amino-acids in soils is at most a few hours (Jones et al., 2009), although they are often taken up immediately upon addition (Hobbie and Hobbie, 2012). Similarly, simple sugars are taken up rapidly in surface layers (turnover times of 4-20 hours), but not in deeper layers where microbial biomass is scarce (van Hees et al., 2008). In deep layers, the distance δ would be higher, and in such conditions our model predicts a lengthening of the turnover times (Figure 2A). Thus, equilibrium can only be achieved when mass transfer is rapid; i.e., as long as the microbe-substrate distance δ is small (lower than 10^{-3} m). The δ can be estimated based on microbial biomass concentrations and assuming that microbial cells or colonies are homogeneously distributed, yielding values in the

order of 10^{-5} - 10^{-4} m (Manzoni et al., 2014). Geo-statistical analyses of intact soil cores identified a characteristic spatial scale for microbial biomass distribution in the same range (Nunan et al., 2002; Raynaud and Nunan, 2014). Therefore, the quasi-equilibrium approximation is supported.

In the comparison between measured respiration and C uptake, the ratio of these fluxes (i.e., one minus microbial C use efficiency) was assumed independent of soil moisture. That is, we assumed a one-to-one relation between C uptake and C usage for respiration. Any change in C use efficiency with soil moisture would alter the relation between C uptake and respiration rate, thus shifting the respiration-moisture curve from the modeled C uptake-moisture curve. Unfortunately, there is little evidence to quantify shifts in C use efficiency along moisture gradients. On one hand, some evidence shows increased C use efficiency under dry conditions, possibly because of accumulation of C-based osmolytes (Herron et al., 2009); on the other hand, water stress may decrease the C use efficiency (Tiemann and Billings, 2011). We suggest that these contrasting responses may compensate each other, thereby stabilizing the C use efficiency and justifying our approach. This approximation will require a further evaluation as evidence of trends in C use efficiency become available.

So far, we have considered stable temperature conditions during soil drying. However, temperature and moisture may co-vary and temperature affects all reaction and transport rates (Ågren and Wetterstedt, 2007; Davidson et al., 2012), thereby potentially affecting our results. Therefore, we tested whether temperature can alter the shape of the uptake-water potential curves by including temperature effects in all model parameters (Supplementary Online Materials). Increasing temperature slightly enhances the normalized uptake for a given water potential, but the change is minor compared to the effects of soil moisture and parameter uncertainties.

4.3. Relation between microbial activity and water potential

Equation (11) predicts a sigmoidal decline of microbial uptake as a function of water potential on a logarithmic scale if uptake kinetics are assumed not to limit the process (Figure 3). An approximately linear decline more consistent with observations (Orchard and Cook, 1983; Rodrigo et al., 1997) is obtained only when accounting for reduced uptake kinetics in dry conditions (Figure 4). The curves decline more rapidly in intact soil cores than in disturbed (sieved) soils – a difference that might be explained by larger microbe-substrate distances in intact soils (Figure 4). Data from intact cores are from different soils, but from a single source (Petersen et al., 2008), so conclusions based on differences between sieved and intact samples should be interpreted with caution. The decline in uptake efficiency that minimizes model errors mirrors the increase in the number of inactive species from an independent study (Lennon et al., 2012), lending some support to the proposed approach.

Overall, our results suggest that in moist conditions (between the soil field capacity and approximately -0.5 MPa) the reduction of heterotrophic respiration can be attributed to declining microbial uptake efficiency (Figures 4 and 6). Because soil fauna is more sensitive to reduced moisture than microbes (Freckman, 1986), part of this early decline could also be attributed to reduced grazer activity rather than changes in microbial uptake *per se*. At intermediate water potential levels (between -0.5 and -2 MPa) diffusion limitations become as or more important than reduced uptake efficiency (Figure 6). In a study on nitrifiers, both desiccation stress and substrate availability were shown to play a role in this range of water potentials, supporting our findings (Stark and Firestone, 1995). In dry soils (below -2 MPa) soluble C becomes unavailable to microbes due to reduced diffusivity and loss of C to unavailable zones, and we suggest that the drought-tolerant organisms that remain active under these conditions are primarily C-limited.

4.4. Interpretation of Birch effect and applicability in moist conditions

The proposed model takes into account soluble C that is trapped in unavailable compartments during the drying phase. This C can be released upon rewetting, providing substrates that could fuel a pulse of microbial activity – the so-called Birch effect (Birch, 1958). These pulses have been attributed to several mechanisms, including increased microbial mortality and increased substrate accessibility (Blazewicz et al., 2014; Kaiser et al., 2015). Previous models have considered the accumulation of soluble C due to reduced uptake (Lawrence et al., 2009; Manzoni et al., 2014; Oikawa et al., 2014), but only recently have unavailable pools been implemented and dynamically linked to the other compartments, allowing improved predictions of respiration pulses (Zhang et al., 2014). The assumptions of the present model hold only in the drying phase, but it is still possible to use the model to calculate how much C can be released upon rewetting, without however specifying the precise timing of release.

The C accumulated in the unavailable pools was calculated by integrating the mass transfer rates to the unavailable pools. The unavailable C increases with the duration of the dry period and with decreasing water potential, following a sigmoidal shape with an inflection point at -1 MPa, where the accumulation rate is fastest (curves in Figure 7). Experimental approaches have shown accumulation of soluble organic C when the soil water potential decreases below -1 MPa (Williams and Xia, 2009; Kakumanu et al., 2013; Guo et al., 2014; Warren, 2014; Kaiser et al., 2015), consistent with the trends predicted here. Moreover, observed respiration pulses following dry periods of different duration show the same pattern (Xu et al., 2004; Fischer, 2009; Lado-Monserrat et al., 2014) and are well correlated to the depletion of soluble C (Guo et al., 2014). To test model predictions, we compared the size of the unavailable C pool at a water

potential ψ , to measured respiration pulses after rewetting of soils dried to the same ψ (data from Fischer, 2009). Predicted respiration pulses agree well with measurements from soils drier than $\psi = -0.1$ MPa, but underestimate the pulse sizes from soils that were still relatively moist (respiration pulses and accumulated C were both normalized to their values at the inflection point $\psi = -1$ MPa for comparison). Hence, this analysis supports the hypothesis that C that is unavailable in dry soils may fuel respiration pulses upon rewetting (Xiang et al., 2008; Schimel et al., 2011), but it does not exclude other explanations (Blazewicz et al., 2014).

To implement the proposed approach in ecosystem models, Equation (11) and the other fluxes at steady state (or similar formulations derived following the general scheme illustrated here) can be used instead of an empirical rate modifier. During dry periods, C lost to unavailable pools can be calculated and the cumulative value stored in an additional variable. Upon rewetting, such pools can be rapidly transferred back to the dissolved available pool, and then used by microbes in ‘competition’ with leaching (Warren, 2014), providing a simple representation of respiration pulses. However, this implementation approach would neglect several processes occurring at rewetting (e.g., delayed release of C from the unavailable pools and dynamics of microbial re-activation), and the advantage of simplicity would need to be balanced against the disadvantage of a highly idealized system.

4.5. Conclusions

An analytical expression linking microbial C uptake to moisture availability is obtained by assuming that enzyme and bio-available substrate C pools equilibrate rapidly to changing soil moisture. This equilibration occurs because the time scales of some biogeochemical and mass transfer processes are much faster than those characteristic of microbial biomass and soil organic C. Within the range of conditions where the equilibrium assumption holds, the obtained relation

641 i) provides a process-based alternative to currently employed empirical functions, ii) links
642 biogeochemical and physical processes in a single expression, and iii) is relatively simple and
643 can therefore be implemented in ecosystem and Earth system models with minor changes to their
644 model structure.

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Figure captions

Figure 1. A) Geometry of the substrate-microbe system. B) Illustration of the modeled C-cycle processes: diffusion (dashed blue arrows), leaching (dotted blue arrows), reaction (solid black arrows), and mass transfer to unavailable pools (dot-dashed green arrows). When transport and reaction time scales (τ_C) are shorter than organic matter (C_{OM}), microbial (C_B), and soil moisture (θ) time scales, an ‘effective’ steady state model for the rate of C uptake by microbes (U^*) can be obtained as a function of C_{OM} , C_B and θ (bottom scheme). The orange dashed outline includes processes that are lumped into the effective kinetic parameters at the daily time scale. Superscripts a and u respectively indicate bio-available and unavailable pools; complete symbol definitions are reported in Table 1.

Figure 2. Turnover times of A) available C (τ_{C_S}) and B) enzyme C (τ_{C_E}), as a function of soil water potential (ψ). Lighter gray shades refer to smaller mobile-immobile zone exchange rate (α); solid-to-dotted curves indicate increasing distance δ between microbes and substrates ($\delta=10^{-5}$, 10^{-4} , and 10^{-3} m). Note that τ_{C_E} is independent of δ . The dot-dashed thin line indicates a time scale of 1 day; the farther the curves are from this line the safer the assumption of steady state. A loamy soil of depth $Z = 0.1$ m is assumed.

Figure 3. Relations between C uptake (normalized by the rate at field capacity with unlimited microbial uptake) and soil water potential (ψ) as a function of A) mobile-immobile phase exchange rate (α), B) microbe-substrate distance (δ), C) soil texture, and D) uptake rate constant (u), here assumed invariant with ψ . Baseline parameters are $\alpha=1 \text{ d}^{-1}$, $\delta=10^{-4} \text{ m}$, $k_E=0.1 \text{ d}^{-1}$, and loamy texture.

Figure 4. Comparison of observed normalized respiration rate (data) and normalized C supply rate (model curves) as a function of soil water potential (ψ). Theoretical curves refer to different microbe-substrate distances (δ) and uptake kinetic constant (u). Larger values of δ indicate higher heterogeneity, as expected in intact soil cores (black open squares) compared to sieved samples (gray filled circles). Respiration data are from published sources (Section 2.4); the dependence of u on ψ is estimated from respiration data (thick dashed curve in Figure 5); $\alpha=1 \text{ d}^{-1}$, $k_E=0.1 \text{ d}^{-1}$ and sandy loam texture were assumed.

Figure 5. Estimated uptake rate u as a function of soil water potential ψ , compared to the fraction of inactive species from an independent study (Lennon et al., 2012) (open squares; values multiplied by 10 for visual comparison). For each respiration dataset, u was estimated from inversion of Equation (11), resulting in a distribution of u values at each ψ level; the shaded area envelops u values between the first and third quartiles of this distribution. The thick dashed line is the relation approximating the mean estimated $u(\psi)$.

859 Figure 6. Relative contributions of different abiotic and biotic factors to the decline in C uptake
 860 rate during soil drying. Contributions are isolated by sequentially removing limiting factors in
 861 $\kappa(\theta)$ (Equation (11)): the role of microbial physiology is identified by assuming $u \rightarrow \infty$ in the
 862 full model, and substrate transport is isolated by letting $h_s \rightarrow \infty$ and $\alpha \rightarrow 0$, resulting in an
 863 equation that only depends on enzyme properties (h_E and k_E). Curves are obtained using
 864 baseline parameters and Equation (12) for $u(\psi)$.

865

866 Figure 7. Effect of soil water potential at the end of the experimental drying (ψ) on the
 867 respiration pulse at rewetting. Data points are measured respiration pulses in three soils,
 868 normalized by the pulse size after drying to $\psi_{ref} \approx -1$ MPa (data from Fischer, 2009). Curves
 869 represent modeled soluble C in unavailable pools at the end of the dry period (as a proxy of C
 870 available for a respiration pulse), also normalized with respect to the value attained at $\psi_{ref} =$
 871 -1 MPa. Solid curve: $\alpha = 1 \text{ d}^{-1}$, dashed curve: $\alpha = 0.1 \text{ d}^{-1}$; baseline parameters are as in Figure 3
 872 and u is computed using Equation (12).

Table 1. List of state variables and definition of C and water fluxes (see also Figure 1). Fixed parameters are listed in Table 2.

Symbol	Description	Units
C_B	Microbial biomass	gC m^{-3}
C_E^a	Active enzyme	gC m^{-3}
C_E^u	Unavailable enzyme	gC m^{-3}
C_{OM}	Organic matter	gC m^{-3}
C_S^a	Bio-available substrate	gC m^{-3}
C_S^u	Unavailable substrate	gC m^{-3}
$C_{S,0}^a$	Bio-available substrate near the microbial cells	gC m^{-3}
D_E	Enzyme deactivation rate ($D_E = k_E C_E$)	$\text{gC m}^{-3} \text{d}^{-1}$
D_{OM}	Organic matter decomposition rate (Equation (3))	$\text{gC m}^{-3} \text{d}^{-1}$
F_E	Enzyme diffusion from microbes to bulk soil (Equation (5))	$\text{gC m}^{-3} \text{d}^{-1}$
F_S	Substrate diffusion from bulk soil to microbes (Equation (5))	$\text{gC m}^{-3} \text{d}^{-1}$
h_E	Mass transfer coefficient for enzyme diffusion (Equation (6))	d^{-1}
h_S	Mass transfer coefficient for substrate diffusion (Equation (6))	d^{-1}
l_E	Leakage coefficient for enzymes ($l_E = l$)	d^{-1}
l_S	Leakage coefficient for substrates ($l_S = l$)	d^{-1}
l	Deep percolation rate per unit soil volume ($L = k_{sat}/Z (\theta/\eta)^{2b+3}$)	d^{-1}
L_E	Enzyme loss through leaching (Equation (9))	$\text{gC m}^{-3} \text{d}^{-1}$
L_S	Substrate loss through leaching (Equation (9))	$\text{gC m}^{-3} \text{d}^{-1}$
U	C uptake by microbes (Equation (4))	$\text{gC m}^{-3} \text{d}^{-1}$
ϕ_E	Rate of enzyme transfer to the unavailable pool C_E^u (Equation (8))	$\text{gC m}^{-3} \text{d}^{-1}$
ϕ_S	Rate of substrate transfer to the unavailable pool C_S^u (Equation (8))	$\text{gC m}^{-3} \text{d}^{-1}$

θ	Soil moisture (Equation (2))	$\text{m}^3 \text{ m}^{-3}$
ψ	Soil water potential ($\psi = \psi_{sat}(\theta/\eta)^{-b}$)	MPa

Table 2. List of constant parameters and their values (or ranges). State variables are defined in Table 1 and Figure 1.

Symbol	Description	Value	Units	Sources and notes
b	Exponent of the water retention curve	Soil-dependent	-	(Rodriguez-Iturbe and Porporato, 2004)
$C_{E,0}$	Enzyme concentration outside the microbial cell	-	gC m^{-3}	Numerical values not required
$D_{S,0}$	Diffusivity of substrate in pure water	8.1×10^{-10}	$\text{m}^2 \text{s}^{-1}$	For amino acids and simple sugars (Jones et al., 2005; Anslyin and Dougherty, 2006)
$D_{E,0}$	Diffusivity of enzymes in pure water	8.1×10^{-11}	$\text{m}^2 \text{s}^{-1}$	≈ 10 -fold lower than $D_{S,0}$ (Vetter et al., 1998; Anslyin and Dougherty, 2006)
ε_{max}	Maximum evaporation rate per unit soil volume	0.05	d^{-1}	Estimated to have a drying rate comparable to experiments
f	Fraction of soil moisture in mobile zones	0.85	-	(Griffioen et al., 1998)
k_E	Enzyme de-activation rate	10^{-2} to 1	d^{-1}	(Allison, 2006; Becerra et al., 2014)
k_m	Michaelis-Menten constant	-	gC m^{-3}	Numerical values not required
k_{sat}	Soil hydraulic conductivity at saturation	Soil-dependent	m d^{-1}	(Rodriguez-Iturbe and Porporato, 2004)
m_1	Empirical exponent	1.5	-	(Hamamoto et al., 2010)
m_2	Empirical exponent	2	-	(Hamamoto et al., 2010)

u	Microbial uptake rate	0.1 to 10	d^{-1}	$>1 \text{ d}^{-1}$ (Hobbie and Hobbie, 2012)
ν, V_{max}	Kinetic constants	-	d^{-1}	Numerical values not required
Z	Soil depth	0.1	m	Assumed
$\alpha = \alpha_E$ $= \alpha_S$	Rate of mass transfer to sorbed/immobile phases	10^{-2} to 10	d^{-1}	(Padilla et al., 1999; Toride et al., 2003)
δ	Microbe-substrate characteristic distance	10^{-5} to 10^{-3}	m	(Manzoni et al., 2014)
η	Soil porosity	Soil- dependent	-	(Rodriguez-Iturbe and Porporato, 2004)
ν	Scaling coefficient	6	-	Calculated for a cubic lattice
ρ_b	Soil bulk density	1.2×10^6	g m^{-3}	Assumed
θ_t	Diffusion threshold	$\eta(\psi_{sat}/\psi_t)^{1/b}$	-	Water retention curve after Campbell (1974)
ψ_{sat}	Water potential at saturation	Soil- dependent	MPa	(Rodriguez-Iturbe and Porporato, 2004)
ψ_t	Diffusion threshold	-15	MPa	Sieved soil (Manzoni and Katul, 2014)
		-1	MPa	Intact soil cores (Manzoni and Katul, 2014)

Table 3. Summary of derived equations for the coefficient $\kappa(\theta)$ (Equation (11)), for different simplifying assumptions.

Assumptions		Moisture-dependent coefficient $\kappa(\theta)$
Full model		$\frac{uh_Eh_S}{(h_E+l_E+\alpha+f\theta k_E)[u(l_S+\alpha)+h_S(l_S+\alpha+u)]}$
Negligible leaching		$\frac{uh_Eh_S}{(h_E+\alpha+f\theta k_E)[u\alpha+h_S(\alpha+u)]}$
Negligible leaching in addition to:	Unlimited uptake capacity	$\frac{h_Eh_S}{(h_E+\alpha+f\theta k_E)(\alpha+h_S)}$
	Negligible transfer to sorbed/immobile phases	$\frac{h_E}{h_E+f\theta k_E}$
	Limiting diffusion (low θ)	$\frac{h_Eh_S}{(\alpha+f\theta k_E)\alpha} \propto h_Eh_S \text{ for } \theta \approx 0$
	Limiting microbial activity	$\frac{uh_E}{(h_E+\alpha+f\theta k_E)\alpha} \propto uh_E \text{ for } \theta \approx 0 \text{ and } h_E \ll \alpha$

Figure 1

A) Geometry

B) Carbon pools and fluxes

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7