**An overlooked mechanism underlying the attenuated temperature response of soil heterotrophic respiration**

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**Abstract**

Biogeochemical reactions occurring in the pore space of soil underpin gaseous emissions measured at macroscopic scales but are difficult to quantify due to their complexities and heterogeneity. We develop a volumetric-average method to calculate aerobic respiration analytically from soil with microscopic soil structure represented explicitly. Soil water content in the model is the result of the volumetric average of the microscopic processes and it is nonlinearly coupled with temperature and other factors. Since biogeochemical reactions are driven by oxygen (O2) which must overcome various resistances before reaching microsites from the atmosphere, the volumetric average results in negative feedback between temperature and soil respiration, with the magnitude of the feedback increasing with soil water content and substrate quality. Comparisons with various experiments show that the model reproduces the variation of CO2 emission from soils under different water content and temperature gradients, indicating that it captures the key microscopic processes underpinning soil respirations. We show that alongside thermal microbial adaptation, substrate heterogeneity and microbial turnover and carbon use efficiency, O2 dissolution and diffusion in water constrained in the pore space is another key explanation for the attenuated temperature response of soil respiration and should be considered in developing soil organic carbon models.

***Keywords***: *Soil* *respiration; oxygen dissolution and diffusion; temperature response of soil respiration; soil emission model.*

1. **Introduction**

Soil contains more organic carbon than vegetation and atmosphere combined, and as a consequence, small shifts in soil organic carbon (SOC) could have significant consequences for global warming [1]. Understanding the mechanisms underlying its dynamics processes and representing them adequately in SOC models is hence crucial to predicting its response to climate warming and land management practices [2], but challenging due to the complexities of biogeochemical reactions involved in SOC cycling [3].

Of all biotic and abiotic factors, temperature and water have the greatest influence upon biogeochemical processes in soil [4]. Although their roles in homogeneous media are fairly well understood, controversy arises when applying this understanding to soils because of their complexity and heterogeneity [5]. Microbial activity in soil is patchy and biogeochemical reactions proceed only at sites with coexistence of all substrates and enzymes [6]. Soil variables measured from sampling without information on substrate accessibility can thus give rise to erroneous conclusions when used to calculate biogeochemical reactions [7]. One example is the observed reduced temperature response of aerobic microbial respiration, where the underlying mechanisms have been a contentious issue for decades [8-11]. Changes in microbial physiology with temperature and substrate heterogeneity have been postulated, but there is little consensus about their relative significance [3].

Previous studies on the temperature response of soil respiration have focused on biological processes, overlooking the fact that biogeochemical reactions are driven by bioavailability of oxygen (O2) and substrate accessibility in the pore space. The resistances against O2 dissolution in soil water and its subsequent movement limit its delivery to microbes, evident as the pervasiveness of anaerobicity in relatively dry soils [12]. While soil water was believed to keep microbes hydrated and carry dissolved substrates and enzymes away and towards the microsites [13], experiments showed that the reduced substrate availability due to soil water decrease is far more important than the dehydration [14]. Within the pore space, gaseous O2 first dissolves at the water-air interface before diffusing to aerobic microbes (reactive sites). Respiration generates localized O2 concentration gradients between the water-air interface and reactive sites. Such gradients vary with temperature and soil water content, as rising temperature reduces O2 dissolution while change in soil water reshapes the water-air interface and its distance to the microsites for O2 to dissolve and move, respectively. Therefore, the influence of temperature and soil water on microbial activity have been postulated to be coupled: A change in one factor would alter the response of soil respiration to the other. However, because of their complexities, most SOC models decouple them using separate moisture and temperature functions to describe their respective influence [15, 16]. There are no systematic studies on the potential errors associated with such approaches; indirect evidence indicates that Q10 (or activation energy) which characterizes the temperature sensitivity of soil respiration, is not constant but varies with soil moisture [17]. A small change in Q10 can lead to substantial differences in predicting the response of soil respiration to global warming [18].

The temperature function used in most SOC models is Q10 or the Arrhenius kinetic model [4]. In contrast, the moisture functions are diverse including empirical formulae and mechanistic models [15]. While the empirical functions are phenomenological, most mechanistic models are based on the influence of soil water on diffusion of gaseous O2 and aqueous substrates and overlook that biogeochemical reactions alter local O2 concentration gradients. For a given soil, these models predict a fixed optimal moisture content where respiration peaks, inconsistent with experimental results which showed that the optimal soil water content for soil respiration varies with temperature[19-22], microbial activity[23], and even soil depth[24]. These observations suggest that the moisture effect on respiration is modulated by temperature [25], and their combined influence is more complicated than described by the separated moisture and temperature functions [3].

Apart from mediating O2 dissolution and diffusion, soil water also controls substrate accessibility [13, 26, 27]. The convergent view of decades of study is that increasing soil water content renders pores easier for substrates to move, increasing their accessibility [4, 28]. Some models describe this by introducing a moisture-dependent barrier between substrates and reactive sites [22, 29]. While this is rational for substrates and enzymes, it does not apply to O2 because O2 must dissolve at the water-air interface before becoming bioavailable for respiratory microbes. Increasing water content of a dried soil increases O2 dissolution initially, but after a threshold, increasing soil water content further reduces O2 supply because of the reduced water-air interface for O2 to dissolve and the increased distance for dissolved O2 to reach reactive sites [30]. It is known that O2 dissolution and diffusion control biogeochemical reactions in soil [31], but they are difficult to model due to their complexity [32]. Consequently, most SOC models do not consider O2 explicitly, probably based on an erroneous perception that O2 in the topsoil is not a limiting factor [33]. Decades of studies have demonstrated anaerobic reactions persist even when soil is relatively dry, especially in the rhizosphere [31, 34, 35].

Given the importance of soil structure in modulating water distribution and O2 dissolution and diffusion, as well as the control of biogeochemical reactions control on local O2 concentration gradients between water-air interface and reactive sites, we hypothesize that the reduced O2 dissolution and increased microbial metabolism at raised temperatures attenuate the temperature response of soil respiration. We developed a volumetric average method to incorporate microscopic soil structure to calculate soil respiration. Considering that soil structure and its associated microscopic processes are complicated, to make the calculation analytical, we make several rational simplifications. These include: i) gaseous O2 concentration in the soil sample is uniform as O2 diffuses four orders of magnitude faster in air than in liquid water [36]; ii) biogeochemical reactions in the soil sample are in a quasi-steady state where the amount of O2 dissolved at the water-air interfaces in the soil sample is the same as the amount of O2 reduced by aerobic microbes, and that the mass of O2 respired by aerobic microbes is the same as the mass of O2 diffusing from the water-air interfaces to all reactive sites; iii) O2 reduction by aerobic microbial community at hydrated reactive sites in the soil sample is proportional to the dissolved O2 concentration at the reactive sites; iv) the majority of soil microbes adopt a “waiting” strategy to acquire substrates and O2 [37]. Volumetrically averaging the microscopic processes over the hydrated pore space in the soil sample yields an analytical model to calculate respiration; soil water content in the model is the result of the volumetric average and nonlinearly coupled with temperature and other factors. Such coupling has been conjectured since the 1970s [38, 39], and we theoretically demonstrate its existence that a change in one reshapes the response of soil respiration to the other.

1. **Materials and methods** 
   1. **Theoretical analysis**

Figure 1 depicts the microscopic processes considered in the model. Gaseous O2 originating from the atmosphere first dissolves at the water-air interface before moving in water; soil organic matter associated with the matrix is decomposed enzymatically by exoenzymes. At hydrated sites with coexistence of substrates, aerobic microbes take up dissolved O2 and substrates. The movement of dissolved O2 and substrates are constrained to the regions enclosed by the water-air interface and the wetted pore walls. They are described by the following equations with dissolved organic carbon and O2 as the limiting substrates [4, 40]

 (1)

Variable nomenclature is given in the appendix. Oxygen enters the system via dissolution at the water-air interface, and it is described as a first-order kinetic process [41]:

 (2)

where *do* is O2 dissolution rate over a unit water-air interfacial area, α is the dissolution rate coefficient, *c*0 is the dissolved O2 concentration at the water-air interface, and *Ceq* is the saturated dissolved O2 concentration calculated from the Henry’s law [42].

Compared to the timeframe over which soil water changes, pore-scale processes are rapid and are at quasi-steady state over daily temporal scale. The aim of this paper is not to solve Eq. (1), but to develop a method to volumetrically average it over a soil sample to calculate the total reaction rate. To make the calculation analytical while maintaining the key processes illustrated in Figure 1, we made some rational simplifications. The first is that the gaseous O2 concentration in the air-filled pore space is uniform because O2 diffuses four orders of magnitude faster than in water. The second is that, since most microbes live in the areas adjacent to wetted walls [30], we assume the number of microbes in a thin layer proximal to the wetted pore-wall is proportional to the area of the pore-wall. When soil water content is θ, the total area of wetted pore walls in the soil is represented by *Aws*(θ), and the spatial variation of wetted pore wall in the Cartesian coordinate system is described by function. If the number of aerobic microbes on the wetted wall located at is *n*(*s*), the total aerobic microbes on hydrated microsites in the soil is , which reduces to *Aws*(θ)·*n*0 if the microbes are uniformly distributed in that *n*(*s*)=*n*0. Similarly, when soil water content is θ, the total water-air interfacial area is represented by Awa(θ), and the spatial variation of the water-air interface in the Cartesian coordinate system is described by function s’(x, y, z). If the dissolved O2 concentration at the water-air interface located at (*x*, *y*, *z*) is c0(s’), the dissolving rate of O2 in the soil is . The Michaelis-Menten constant associated with O2 in Eq. (1) regulates microbial growth, varying with species and substrate quality [43, 44]. Since we consider microbial communities, it is approximated by[33].

At steady state, microbial O2 reduction rate in the soil sample is balanced by O2 dissolution rate at the water-air interface. When soil water content is θ, we have

 (3)

The dissolved O2 concentration at the water-air interface in the soil sample is approximately constant as the gaseous O2 concentration is constant. In contrast, substrate concentration and the number of aerobic microbes vary over the wetted wall. Eq. (3) is hence calculated by

 (4)

where *CD* and *n*ʹ are the average substrate concentration and the average number of aerobic microbes over the wetted pore walls in the soil, respectively. Diffusion of O2 in water is slow and its concentration over the wetted pore walls, co, varies. The integral in Eq. (4) is approximated by

 (5)

where *Co* is the average dissolved O2 concentration over the wetted pore wall. We thus have

 (6)

Diffusion of O2 from the water-air interface to reactive sites depends on local concentration gradients and the distance between them. If the average hydraulic distance between the air-water interfaces and wetted pore walls is *L*, and the average cross-sectional area in soil water for O2 to diffuse from the water-air interface to the microsites is *AD*, the overall diffusive flux is

, (7)

Approximating *AD* by the mean of the water-air interfacial areas and wetted pore wall areas, i.e., , at steady state, the mass balance requires that the diffusive flux *Q* equates the total O2 respiration rate, i.e.,

 (8)

Solving for *c*0 yields:

 (9)

Substituting Eq. (9) into Eq. (8) gives

 (10)

From Eq. (2), we have the total respiration rate as follows:

, (11)

where *Er* <1 is a feedback factor emerging from the volumetric average, describing the reduction in respiration due to O2 dissolution and diffusion in soil water. Physically, *k* is the potential demand of aerobic microbes over a unit area of wetted pore wall for O2 when O2 is not a limiting factor, and it depends on temperature, substrate quality/quantity, the number of microbes and their metabolic rates. The hydraulic distance between the water-air interface and the microsites increases with soil water content and is approximated by [41]

 (12)

where λ is constant depending upon soil structure.

The effect of O2 dissolution and diffusion on soil respiration is described by the gaseous O2 dissolution rate coefficient and the molecular diffusion coefficient of dissolved O2, respectively. When O2 dissolution is significantly faster than microbial respiration, i.e., , the feedback factor reduces to

 (13)

with O2 diffusion being the limiting factor. When dissolved O2 diffusion is significantly faster than microbial respiration, i.e.,, the feedback factor reduces to

 (14)

with O2 dissolution being the limiting factor.

The influence of pore geometry and water distribution within the pore space is described by a combination of the water-air interfacial area, the wetted pore wall area, and the hydraulic distance between them. Temperature affects soil respiration in two ways. Physiologically, rising temperature increases microbial metabolic rate as described by the Arrhenius kinetic equation [5]:

 (15)

Physically, increasing temperature reduces O2 dissolution and enhances its diffusion. At one atmospheric pressure, the changes in saturated dissolved O2 concentration and its molecular diffusion coefficient with temperature are described by [36]:

 (16)

* 1. **Water-air interfaces and wetted pore walls**

As soil water content increases, the wetted pore-wall area increases monotonically, while the water-air interfacial area first increases and then declines. To investigate if such changes can be described by general formulae, we simulated water distribution in more than one hundred soil samples as shown in our previous work [45, 46]. For all soil samples, the water-air interfacial area and the wetted pore wall area change with soil saturation in similar trends. Normalized by volume of the soil sample, their changes with soil saturation, ϴ, can be described by

 (17)

where ε is a parameter to represent that when the soil surface is opened to the atmosphere, its water-air interfacial area is not zero when the soil is fully saturated; others are soil parameters.

* 1. **The model**

The rate of O2 dissolution is much faster than its diffusion in water as commonly assumed in hydrogen fuel cells [41]. Since microbial reduction of O2 is slower than electrochemical reduction in fuel cells, all analysis in what follows is based on Eq. (13). For a soil sample, combining Eq. (13) and Eq. (17) gives

 (18)

* 1. **Implementation**

Eq. (18) is used to calculate soil respiration. The influence of temperature and substrates is described by the parameter *k*. Apart from *k*, all other parameters are soil structure-related parameters. Mathematically, each parameter in Eq. (18) can take an arbitrary value, but in validating the model against experimental data, we take all soil structure parameters as a set, calculating it by mining a soil-image dataset consisting of more than 100 X-ray images we accumulated over the past decade for soils with various textures ranging from clay loam to sandy soils [45-49]. Using a method we developed previously [50], we calculated liquid water distribution within the pore geometry at different water contents, as well as the associated water-air interfaces and wetted pore walls for each soil image. For each mined soil image, we inserted its water-air interfacial areas and wetted pore walls into Eqs. (17) and (18), and then adjusted parameter *k*, which can be either constant or varies with pore size (see the result section for details), until the calculated respirations from Eq. (18) matched the experimental data.

1. **Results and discussion** 
   1. **Comparison with experimental data**

The first example is an incubation experiment that measured respiration rates under different saturations for a repacked sandy soil [51]. All measured respiration rates were normalized by the maximum respiration at the optimal soil water content. Figure 2A compares the measured and modelled results using the mined soil structure shown in Figure S1A. They agreed well, indicating that the model captures the key processes underpinning respiration at different soil saturations.

The second example is a field experiment designed to investigate the response of soil respiration to changes in soil moisture [52]. Field soil is more heterogeneous than sieved soil and its respiration often shows the “Birch” phenomenon [53], suggesting that microbes in small pores respire less than those in large pores due to pore-scale variation in substrates and microbial composition [54, 55]. We modelled this by allowing *k* to increase with the pore size. Since water progressively fills small pores to large pores as soil water increases, we described this pore-scale substrate and microbe heterogeneity by allowing *k* to increase linearly with soil water content. Figure 2B shows the comparison. The model reproduces the change in respiration from the dry to the wet end, revealing that O2 dissolution and diffusion in soil water regulates the response of heterotrophic soil respiration to soil water change.

The final example is to verify that the model captures the nonlinear coupling of soil water and temperature in their effect on respiration against an incubation experiment that involved both moisture and temperature gradients [56]. The impact of soil water was measured in the experiment by maintaining a constant temperature, while the influence of temperature was measured by keeping the soil moisture content constant. We first calculated the soil-structure parameters by calibrating the model against respiration rates measured at different saturations at 15 °C and then used these parameters and Eq. (15) to predict the variation of the respiration when temperature was increased from 5 to 30 °C. The molecular diffusion coefficient of dissolved O2 and the saturated O2 concentration at different temperatures were calculated from Eq. (16). The potential demand of the reactive sites for O2 at temperature *T* is *kT*; it was calculated as follows based on its value at 15 °C (*T*15) calculated in calibrating the model to obtain the soil structure parameters:

 (19)

where *k*15 is the value of *k* at 15 oC, and *T*15 and *T* are absolute temperature (*K*).

Moriyama et al [56] estimated the apparent activation energy based on respiration rates measured over a range of temperatures. The activation energy estimated in such ways is bulk, representing the collective impact of all factors which, directly or indirectly, influence the temperature response of soil respiration. In our model *E*a is the intrinsic activation energy, determined by microbial composition and molecular structures of the substrates. This is different from the bulk activation energy that depends on all intrinsic and environmental factors which, directly or indirectly, affect the temperature sensitivity of respiration. However, because substates and microbial composition varies with pore size [55, 57], even the intrinsic *Ea* itself is an average value, representing the average of the intrinsic *Ea* of the substrates and microbes on different hydrated reactive sites. In this example, we treated the average intrinsic *Ea*as an unknown and calibrated it to obtain the results that matched the measured respirations at different temperatures.

For comparing respiration rates, we normalized all variables in the model and multiplied the calculated respiration rates using these normalized variables by a single scalar to match the experimental data. As an illustration, Figure 3A compares the measured and calculated respiration rates under different saturations when the temperature was 15 °C; Figure 3B compares the measured and predicted respirations when soil saturation was 60% and the temperature was increased from 5to 35 °C using an intrinsic *Ea* = 48 kJ mol-1. They agree well, indicating that the combined impact of soil water and temperature on respiration is nonlinear and captured by our model. As a comparison, we also calculated the temperature response of respiration directly using the bulk activation energy (45 kJ mol-1) reported in Moriyama et al [56], and plot the results in Figure 5B. The predictions using the intrinsic *Ea* = 48 kJ mol-1 are slightly better. Because of the limited experimental data, in terms of matching the measured data, the difference between the two activation energies is not significant, but it corroborates that the intrinsic activation energy is higher than the bulk activation energy [5].

**3.2 Attenuated temperature response of respiration**

The nonlinear coupling between soil water content and temperature in Eq. (18) indicates that the temperature response of respiration is regulated by soil water content, and the strength of their coupling is modulated by substrate activation energy. This differs from previous studies which attributed the temperature response of respiration to microbial physiology and substrate quality [58-65]. Eq. (18) separates soil structure and its associated physical processes from other factors in their role in mediating the temperature response of soil respiration. This is important but has been overlooked. For example, increasing temperature from 5 to 35 °C reduces the saturated O2 concentration in water from approximately 14 to 7 mg L-1 [36], and omitting this change in data analysis would overestimate the thermal adaptation of soil microbes. The effect of these physical factors is described by the feedback factor (Eq. 16), whose magnitude varies with soil water content, substrate activation energy and temperature (Figures S1, S2).

The attenuated temperature response of respiration is regulated jointly by *Ea* and soil water content. Figure 4 shows the change in the feedback factor with temperature under different soil saturations (Figure 4A) and *E*a (Figure 4B), calculated using soil parameters for the example in Figure 3. To allow comparison, the feedback factor calculated for each saturation (or *Ea*), was normalized by the value of the feedback factor at 5 °C. Depending on soil saturation (or *E*a), increasing temperature from 5 to 35 °C could dampen the respiration by approximately 60% due to the reduced O2 dissolution and the increased hydraulic resistance for O2 to move from the water-air interface to the reactive sites, as the temperature rises. This is consistent with experimental results that reducing O2 supply substantially reduced respiration [52]; it is also corroborated by the results of a whole soil-profile experiment where the activation energy in the subsoil was greater than that in the topsoil [66, 67]. While biological factors such as differences in SOC quality and microbial composition between the top- and sub-soils might play a part [68, 69], O2 dissolution and diffusion is also important because the subsoil is more saturated, and the preferential consumption of O2 by roots and microbes in the topsoil makes it difficult for O2 to diffuse to the reactive sites in the subsoil. As a result, not accounting for the reduced O2 dissolution and diffusion in the subsoil explicitly would lead to its bulk activation energy to increase.

**3.3 Nonunique optimal soil moisture for aerobic respiration**

The analytical model derived from the volumetric average reveals that the impact of soil water and temperature on soil respiration is more complicated than described by decoupled moisture and temperature functions [70]. For each temperature, however, there is still an optimal soil water content at which the respiration peaks when other factors are fixed. Normalizing respiration rates at different soil water contents by this maximum gives a curve which is equivalent to the moisture function used in most SOC models [15]. Taking the soil parameters calibrated for Figure 3 as an example, we calculated the moisture function under different temperatures with other factors kept constant (Figure 5A). It is evident that for the same soil, the optimal soil saturation is not constant but varies with temperature. When temperature increases from 2 to 30 °C for the example shown in Figure 5A, the optimal soil saturation decreases from approximately 80 to 55%; this covers the range of soil saturation which is deemed optimal (60%) for aerobic microbes as used in most incubation experiments [9, 56, 59].

In soils lacking bioavailable carbon substrates, the respiration rate measured in incubation experiments typically increases linearly as soil water increases rather than following the shape shown in Figures 2 and 3 [71]. Even when soils are approximately saturated, a significant amount of CO2 continues to emit from the soils [56, 72]. The likely reason is that the soil surface remains open to the atmosphere. When soil is fully saturated with water, O2 continues to dissolve on the soil surface and moves into the soil. Thus, depending on the quantity and quality of SOC within the soil and the soil surface areas, the change in respiration with soil water content can be either approximately linear, nonlinear, or bell-shaped; all these are captured in our model. As an illustrative example, Figure 5B compares how the soil-surface opening affects the response of respiration to soil water content when microbial activity is low and other parameters are the same.

**3.4 Substrate and microbial heterogeneity**

Aerobic respiration measured in short-term experiments exhibits an exponential increase as temperature rises [10, 38], while the change in respiration with soil moisture is inconsistent, ranging from a linear increase [73, 74], concave increase [51], convex increase [72], to convex increase followed by a plateau before declining [52]. Even repacking a sieved soil can dramatically change the moisture response of respiration compared to those measured with the soil kept undisturbed [72]. In undisturbed soils, physical constraints often prevent microbes from entering small pores, and there is evidence that substrate quality in small pores is less favourable energetically than those in large pores [57]. These heterogeneities are likely a cause of the broader variation in the moisture response of soil respiration. Representing all these heterogeneities in a single analytical model is a formidable challenge, but their effects can still be quantitatively accounted for by allowing the density of microbial numbers in the wetted pore wall and substrate concentrations to vary with pore size [54]. For example, when soil water content is low, only small pores are filled by water in which microbes are less active as most microbes in larger pores are in dormancy [75]. With soil water content increasing and larger pores progressively refilled by water, a great number of dormant microbes become more active and substrate availability increases. Allowing the density of microbe numbers over the wetted pore wall and substrate concentration to increase with pore size can represent these intricate pore-scale heterogeneities to produce a diverse set of saturation-respiration relationships. Figure 5C shows how including such pore-scale heterogeneity reshapes the moisture response of respiration, in comparison with an experiment which measured respiration from intact and repacked soil cores [72]. We acknowledge that other factors might also play a role in these diverse respiration–saturation relationships such as those in Figure 5C, but we highlight the importance of microscopic soil structure and physical processes which have been overlooked in most data analysis and SOC models. In certain circumstances, they might overwhelm biotic factors and physiological change in microbes in mediating the moisture and temperature response of microbial activity [13].

**5. Conclusions**

We develop a volumetric-average method, with soil structure and microscopic physical processes represented explicitly, to calculate aerobic respiration analytically from soil samples. Soil water content in the model is the result of the volumetric average, and it is nonlinearly coupled with temperature and other factors. Comparison with experimental data shows the model reproduces respiration measured from soils with both water content and temperature gradients. Incorporating microbial and substrate heterogeneities into the model can explain the diverse moisture- and temperature-respiration relationships. The model demonstrates that, alongside thermal adaptation, substrate heterogeneity and carbon use efficiency of microbes, O2 dissolution and diffusion in soil water is another mechanism attenuating the temperature response of soil respiration. Overlooking these mechanisms in data analyses would risk ascribing their impacts mistakenly to biological factors, thereby overestimating the role of microbes and substrate heterogeneity in regulating the temperature response of soil respiration. The next generation of SOC models therefore should consider soil structure and microscopic physical processes.

**Data accessibility.** The Matlab script and soil images for calculating the results presented in this paper are available from the corresponding author upon request.

**Authors’ contributions. X.Z.:** conceptualisation, investigation, methodology, software, validation, writing – original draft, writing - review and editing; P.A.W.: methodology, investigation, writing – review and editing; A.S.G.: methodology, investigation, writing – review and editing; W.R.W.: conceptualisation, methodology, supervision, writing-review and editing; K.C.: investigation, writing – review and editing; A.L.N.: investigation, methodology, writing – review and editing; S.J.M.: conceptualisation, methodology, writing – review and editing; K.S.: conceptualisation, methodology, supervision; T.H.I.: conceptualisation, methodology, supervision, writing – review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed herein.

**Conflict of interest declaration**. We declare that we have no competing interest.

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**Appendix**

Nomenclature

|  |  |
| --- | --- |
| *Aws* | Specific water-pore wall interfacial area (cm²) |
| *Awa* | Specific water-air interfacial area (cm²) |
| *Aw* | Specific water-pore wall interfacial area when soil is saturated (cm²) |
| *Aa* | Parameter in the specific water-air interfacial area (cm-1) |
| *Ceq* | Saturated dissolved oxygen concentration at water-air interface (mg L-1) |
| *Co* | Average dissolved oxygen concentration at wetted pore-wall (mg L-1) |
| *co* | Dissolved oxygen concentration (mg L-1) |
| *cD* | Dissolved organic carbon concentration (mg L-1). |
| *D* | Diffusion coefficient of dissolved oxygen (cm2 s-1) |
| *DD* | Diffusion coefficient of dissolved organic carbon (cm2 s-1) |
| *Ea* | Activation energy (kJ mol-1) |
| *Er* | Feedback factor |
| *kD* | Michaelis-Menten constant for dissolved organic carbon (mg L-1) |
| *ko* | Michaelis-Menten constant for dissolved oxygen (mg L-1) |
| *L* | Average distance between water-air interface and wetted pore-wall (cm) |
| *N* | Number of aerobic microbes in a unit volume of water. |
| *n* | Number of the aerobic microbes associated with a unit area of wetted pore wall |
| *R* | Gas constant (J mol-1) |
| *Rh* | Heterotrophic respiration (μmol g-1 d-1) |
| *sDOC* | Dissolution rate of polymerized carbon to a unit volume (mg L-1 s-1) |
| *T* | Temperature (K) |
| *umax* | Pro-exponential constant (mg s-1) |
| *vmax* | Maximum microbial consumption rate of oxygen (mg s-1). |
| Θ | Saturation |
| α | Dissolution rate of gaseous oxygen at water-air interface (cm s-1) |
| μ | Parameter characterizing wetted pore-wall areas |
| τ | Parameter characterizing water-air areas |
| σ | Parameter characterizing water-air areas |
| λ | Thickness of the thin water layer inhabited by microbes (cm) |
| κ | Constant control microbial uptake of oxygen (L mg-1) |

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**Figure 1**. Schematic representation of microscopic processes controlling aerobic microbial respiration at the pore scale in soil. Brown represents the soil matrix, white regions represent air, blue represents water. Gaseous O2 (red) dissolves at the water-air interface; the dissolved O2 (yellow) moves to aerobic microbes (green) adjacent to wetted pore walls where it is respired.





**Figure 2.** Comparison of experimental data (symbols) and model results (solid lines) using exemplar soil structures under different soil saturations for an incubation experiment using a well-mixed sandy soil where the potential demand of aerobic microbes for O2 was relatively uniform (A); for a field experiment using a loamy soil where microbes and substrates are sparser in small pores than in larger pores (B).





**Figure 3**. (A) Comparison of measured (solid circles) and calculated respiration rates (solid lines) using calibrated soil structure and potential O2 demand when soil saturation is increased from 7 to 80% at 15 °C. (B) The calibrated model was used to predict the variation in respiration rate when temperature was increased from 5 to 35 °C at a constant water saturation of 60%. The increase in microbial metabolic activity with temperature is described by the Arrhenius kinetic model using an intrinsic *E*a = 48 kJ mol-1. As a comparison we also calculated the respirations using the activation energy 42 kJ mol-1 measured from the experimental data [56].



**Figure 4**. The feedback factor describing the attenuation of respiration decreases with the increase in soil saturation (A), and substrate activation energy *Ea* (B). is the ratio between the feedback factor at temperature *T* to the feedback factor at 5 °C.







**Figure 5**. (A) The optimal saturation for maximal heterotrophic respiration (Rh) traditionally used in moisture functions is not unique, but varies with temperature (*T*) because of the nonlinear coupling. (B) Maintaining the soil surface open in incubation experiments alters the response of Rh to soil water saturation at high saturation, and the effects increase from non-open (0 %) to having 5% of pores open. (C) Accounting for microbial and substrate heterogeneities explains the diverse saturation- respiration relationships measured from experiments. Open squares are data measured from a field-structured loamy soil; solid circles are data measured after sieving-repacking; solid lines are results calculated from the model.