## Variable Composition of Microbial Biomass Affects the Accuracy of Microbial Explicit Models Simulation

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Running headline:

Explaining Variable Composition of Microbial Biomass

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## Summary

* Microbial-explicit biogeochemical models become rapidly developing area of soil and earth science. At their current state, they are sometimes criticized for ignoring important controlling factors of element cycling such as diffusion rate of organic substrate in structured soil environment, substrate quality or microbial community composition.
* These models depend on the data soil science can provide. There exist inherent methodological limitations associated with quantification of soil microbial biomass, which cannot be measured directly but only indirectly as various proxy parameters converted to biomass using ad-hoc conversion factors.
* Our results show that microbial-explicit models cannot accurately simulate the dynamic of microbial biomass measured as two different proxy parameters - DNA and cellular proteins, in short-term incubation experiment. The reason is the temporal variability of composition of microbial biomass in respect to both proxy parameters.
* When the variable biomass composition is explicitly accounted for in the microbial-explicit model structure, 75 and 30% of variability in temporal dynamic of DNA and cellular proteins can be explained respectively.
* Our results further suggest significant effect of physical structure of the environment on several model parameters.
* We recommend to integrate variable biomass composition into the microbial-explicit models to effectively harmonize experimental, methodological and modelling efforts.

## Introduction

In recent years, abrupt development of soil biogeochemical models has occurred. Abstract models dominated by cohorts of organic carbon () pools with inherently different decay rates (e.g. Friedlingstein et al., 2006; Heinemeyer et al., 2010; Lawrence et al., 2011; Parton, Stewart, & Cole, 1988) have become replaced by microbial explicit models (Huang et al., 2018; Schimel & Weintraub, 2003; G. Wang, Post, & Mayes, 2013; W. R. Wieder, Grandy, Kallenbach, & Bonan, 2014; W. R. Wieder, Grandy, Kallenbach, Taylor, & Bonan, 2015; William R. Wieder et al., 2015). On one hand, several studies showed the superior simulation accuracy of microbial explicit models over classical cohort models (Hararuk, Smith, & Luo, 2015; He et al., 2014; Huang et al., 2018; William R. Wieder, Bonan, & Allison, 2013). On the other hand, other studies showed the tendency of these models to unrealistic temporal oscillations of and microbial biomass () caused by small perturbations suggesting that the coupled dynamic of and (expressed per unit of organic carbon basis) in soil is not completely understood (Li, Wang, Allison, Mayes, & Luo, 2014; Sihi, Gerber, Inglett, & Inglett, 2016; Y. P. Wang et al., 2014).

Microbial-explicit models use the knowledge of general microbiology and apply it to soil system. Unlike the well mixed liquid batch or continuous culture the general microbiology works with, soil system is complex in respect to physical structure, composition of organic substrate and composition of microbial community consuming the substrate. All these factors have been suggested to considerably affect the coupled and dynamic in soil (Allison, Chacon, & German, 2014; Allison, Lu, Kent, & Martiny, 2014; Don, Rodenbeck, & Gleixner, 2013; Kaiser, Franklin, Richter, & Dieckmann, 2015; la Cecilia, Riley, & Maggi, 2018; Vogel et al., 2015; Xu et al., 2014) but have not been fully considered in microbial-explicit soil biogeochemical models yet. Physical structure controls the diffusion of and extracellular enzymes towards and/or from (Allison, Lu, et al., 2014; Don et al., 2013; M Gharasoo, Centler, Fetzer, & Thullner, 2014; He et al., 2014; Manzoni, Schaeffer, Katul, Porporato, & Schimel, 2014). In mineral soils with low and concentrations, the diffusion limits can lead to physical separation of and increasing turnover time dramatically (Ekschmitt et al., 2008; Schmidt et al., 2011). In contrast to mineral soils, organic substrate composition has been suggested to be a primary control of the turnover time of plant litter and organic material in the top soil horizons (e.g. Berg & McClaugherty, 2014; Currie, 2003; Herman, Moorhead, & Berg, 2008; Schädel et al., 2014). For a long time, it has been considered as the most important factor and therefore all classical cohort models were based on this fact (e.g. Parton et al., 1988). Composition of microbial community is the least established but intensively studied factor regulating and dynamic (Gittel et al., 2014; Kaiser et al., 2015; Schnecker et al., 2014). There are few individual-based microbial community models suggesting a great importance of microbial community composition for turnover or organic nitrogen mineralization (Allison, 2012; Kaiser et al., 2015). Accounting for all these covariates are supposed to improve the capability of microbial-explicit models to project future development of soil organic carbon stocks. Several manipulation experiments were recently designed and conducted to do so (Chen et al., 2018; Liu et al., 2018; Moyano, Vasilyeva, & Menichetti, 2018; Nunan, Leloup, Ruamps, Pouteau, & Chenu, 2017; Oldfield, Crowther, & Bradford, 2018).

One less obvious difference between the liquid batch/continuous cultures and soil is the feasibility of quantification. In soil, cannot be measured directly and quantitatively. Since microbial cells are impossible to detach from soil particles (Ehlers et al., 2008), the most frequently used high-throughput methods target on specific constituents of , typically chloroform labile microbial carbon (i.e. microbial protoplasm), DNA or phospholipid fatty acids (Frostegard & Baath, 1996; Vance, Brookes, & Jenkinson, 1987; Yokoyama et al., 2017), that can be extracted from soil quantitatively. is then calculated using conversion factors (Frostegard & Baath, 1996; Håkan Marstorp, Guan, & Gong, 2000; Vance et al., 1987; Yokoyama et al., 2017). However, the abundance of these constituents in microbial cells varies across different microbial species (H. Christensen, Olsen, & Bakken, 1995; Henrik Christensen, Bakken, & Olsen, 1993; Hanegraaf & Muller, 2001) and changes with changing soil conditions (Dictor, Tessier, & Soulas, 1998; Dilly, 2004; H Marstorp & Witter, 1999; Ross & Täte, 1993; Schimel, Scott, & Killham, 1989; G. P. Sparling, Feltham, Reynolds, West, & Singleton, 1990). For example, Marstrop and Witter (1999) and Dilly (2004) showed that following a substrate addition, the temporal dynamic of DNA, ATP and chloroform labile microbial carbon is significantly different in respect of magnitude and timing of the change. The reciprocal ratios of these cellular constituents vary during the experiment by a factor of four. Yet, most of the studies do not reflect the variability of microbial composition and use one universal conversion factor to calculate implicitely accepting the associated error. Thus, when the composition of microbial cells changes in designated manipulation experiment with time or as a result of experimental treatment, the effect of that treatment on coupled and dynamic is confounded by the error associated with quantitative estimation of . The only method to avoid such a bias is to explicitely account for the variability of composition in the model. Such a possibility offer a Dynamic Energy Budget theory originally developed by Kooijiman (2009) and successfully used by Hanegraaf and Muller (2001) and Hanegraaf, Stouthamer, & Kooijman (2000) to explain and model macromolecular and elemental composition of several pure microbial cultures along the gradient of organic substrate concentration and growth rate. The fundamental assumption of the theory is an existence of two different pools of , reserves () and Structures (). These pools have different function in the organism and different dynamic. Their proportion changes making composition flexible in respect to and . The content of these pools can be defined in respect to cell constituents of interest. This allows to make composition flexible in respect to these constituents too (Hanegraaf & Muller, 2001; Hanegraaf et al., 2000). The provided flexibility should be sufficient to explain the variability of single species molecular composition. However, it has to be expected that different microbial species have different molecular composition and that the microbial species abundance can change with changing soil conditions.

The aim of this study is to quantify the effect of physical structure and quality on the coupled dynamic of and quantified as two different constituents (i.e. Cellular proteins and DNA). To do so, we designed an experiment in which indigenous soil microbial community was incubated with two different substrates (i.e. glucose and cellobiose) and with or without the presence of two types of physical barrier differing in texture (i.e. glass beads and glass wool). We expect that the presence of physical barrier would decrease the rate of uptake by microbial community.

## Material and Methods

### Experimental design

Soil microbial culture (see later) was incubated in liquid batch microcosms at 24°C in the dark for five days under six different experimental treatments. These treatments included two different organic substrates as a sole source of organic carbon for microbial culture and three different levels of microcosm structural complexity. The treatments were arranged in full factorial design. The two organic substrates were glucose and cellobiose. The three levels of structural complexity of the system were represented by 30 ml serum bottles with liquid medium (denoted as **"BROTH"** further in the text), with liquid medium and mixture of 2.7, 0.1 and 0.1 mm glass beads (5 g of 2.7 mm Biospec glass beads, 2 g of 1.0 mm Biospec glass beads and 1 g of 0.1 mm Next-Advanced glass beads; denoted as **"GLASS"** further in the text) and with liquid medium and glass wool (0.2 g of Pyrex fiber glass wool cut to ~0.25 cm pieces; denoted as **"WOOL"** further in the text). A total of 12 replicates were prepared for each condition so that four replicates could be harvested at three time points; 24, 48, and 120 hr.

### Indigenous soil microbial culture

Soil collected from a fertilized prairie plot at the Comparison of Biofuel Systems research site at Iowa State University’s South Reynoldson Farm (Boone County, Iowa) was used to prepare a soil derived microbial inoculum for the microcosm time course experiment. Modified M9 Media was used for soil culturing and was prepared by mixing 300 ml of M9 Minimal Media 10X stock solution (59.623 g anhydrous , 29.938 g , 4.967 g NaCl, 10.003 g , 1.204 g anhydrous and 0.140 g in 1 l of ultra pure water), 10 ml of Hutner's Trace Element Solution (10 g , 7.3 g KOH, 14.45 g , 3.335 g , 0.00925 g , 0.099 g and 50 mL of Hutner's Stock Salt Solution in 1 l of ultra pure water), 1 ml of Biotin (1 g ), 1 ml of Thiamin (1 g ) and ultra pure water to 1 l. Twenty replicates were prepared as follows: one gram of air-dried aggregates (>2 mM) were submerged in 10 mL of sterile modified M9 media and 625 uL of sterile Luria-Bertani (LB) broth (Lennox; Sigma Aldrich) and incubated at 24°C for 7 days. Following 7 days of incubation, the soil cultures were shaken with ~ten 2.7 mm glass beads (Biospec) for ten seconds. The soil cultures were pooled in a sterile glass flask, and particles were allowed to settle for 10 minutes. The liquid phase was further transferred to a new sterile flask and capped with aluminum till microcosm inoculation.

### Microcosm

Microcosms were prepared by adding 3 ml of sterilized modified M9 media enriched with 1 of organic carbon (cellobiose or glucose) and 1 ml of soil culture inoculum to each of the 72 autoclaved 30 mL serum bottles; 24 empty bottles (BROTH), 24 bottles with a glass wool matrix (WOOL), and 24 bottles with mixed glass beads (GLASS). Accounting for the dilution by the inoculum with assumed organic carbon concentration nearly zero, initial organic carbon concentration of the microcosms was 25 mol . Samples were covered with air permeable Breath Easier (BE) membranes and kept at 24°C in an Innova 42R incubator except when bottle headspace air was sampled for measurements.

### Respiration rate

Microbial respiration rate was measured at the beginning of the experiment and each following day (six times in total). Approximately two hours before the headspace concentration measurement, BE membranes were removed from the incubation vials and replaced by the ruber septa secured with the alluminium cap. Vials headspace was exchanged for the -free air and incubated. Headspace was sampled using 5 ml gas tight syringe. 1.5 ml of well mixed headspace air was sampled and directly injected to Li-Cor 7000 (LI-COR, Inc., Lincoln, Nebraska, USA). concentration was calculated against the calibration standard gas with the concentration 2000 ppm. concentration was corrected for the dissolution in liquid media according to Sparling and West (1990). Respiration rate was calculated as corrected concentration divided by the time between the headspace atmosphere exchange and the measurement.

### Biomass harvest

Immediately after respiration measurement, the whole microcosm culture was transferred to 15 ml conical tubes, and a 2 ml aliquot of phosphate buffer solution (PBS) was dispensed into each bottle. Residual biomass was dislodged from bottle and glass matrix with individually wrapped sterile cell scrapers and transferred to corresponding 15 ml conical tubes. An additional 2 ml aliquot of PBS was dispensed into each bottle, and the biomass recovery step was repeated. Recovered biomass was centrifuged at 4,700 g, 4°C for 10 minutes. Cell pellets were suspended in 500 l of PBS and transferred to 1.7 ml locking conical tubes. Samples were stored at -80°C.

### Cell lysis, DNA and protein quantification

To lyse cells, 100 g of 100 m glass beads (Next-Advanced) were dispensed in each tube and lysed by two consecutive five minutes runs in a bead beater (Next-Advanced) staged in a 4°C cold room. Lysates were centrifuged at 8,000 x g, 4°C for five minutes, and supernatant was transferred to new 1.7 ml conical tubes. DNA from cell lysate was isolated using Qiagen power soil kit following the standard protocol. DNA concentration was measured by Nanodrop meter (***company***). A bicinchronic acid assay (Pierce BCA Protein Assay kit) was used as instructed by manufacturer to determine protein concentration. A SpectraMax Plus 384 Microplate Reader was used to measure absorbance at 562 nm following a 30 minute incubation at 37°C. The DNA and protein concentration was converted to C base concentration (mol or mol ) using the conversion factors 0.45 and 0.51 respectively (Vrede, Dobberfuhl, Kooijman, & Elser, 2004).

### Mathematical description

Three different models (Fig. 1) were used to simulate the changes in respiration rate () and microbial biomass () in time. The Monod model (Fig. 1a) represents the microbial part of the microbial-explicit soil biogeochemical model presented by Allison, Wallenstein, & Bradford (2010). The MEND model is its adaptation presented by Wang et al. (2013). The DB model is a simplified adaptation of Dynamic Energy Budget theory derived by Hanegraaf and Muller (2001) for microbial populations. Here we present all models per molar C basis and use the terminology adopted from microbial- explicit biogeochemical models for consistency.

#### Monod model (Fig. 1a)

In this model, organic substrate () is consumed by microbial biomass and converted to or respired. Microbial biomass is dying at constant rate returning used organic carbon to pool. Mass balance equations for pools and are respectively defined by following equations:

[1] ,

[2] .

in which CUE is the carbon use efficiency (defined as an amount of converted to biomass over the amount of taken up) and is the death rate constant of microbial biomass decay process. Microbial carbon uptake is defined by the equation:

[3] ,

in which is maximum velocity constant and is affinity constant. Respiration rate is defined as a growth respiration rate (), which is the constant fraction of uptake:

[4] .

#### MEND model (Fig. 1b)

In contrast to Monod model, in this model consists of two different processes, growth respiration and maintenance respiration (). and are respectively defined by following equations:

[5] ,

and

[6] .

In eq. 6, is biomass specific maintenance rate constant. Since and represent a constant fraction of organic C uptake, the equation defining an uptake rate has to take both fluxes into an account and the eq. 3 is rewritten to:

[7] .

The overall mass balance equations for pools and are respectively defined by the equations:

[8] ,

[9] .

The last term of eqs. 8 and 9 indicates that the rate of loss due to decay is controled by .

#### DB model (Fig. 1c)

In contrast to previous models, microbial biomass consist of two functional parts, reserves () and structures (). Whereas is functionally similar to , represents a "buffering zone" of . is assimilated into reserves by a rate proportional to and :

[10] .

For simplicity, we assume that the is assimilated into R with efficiency equal to one. Organic C is released from reserves by a rate proportional to R. The overall mass balance equation for pool R is thus:

[11] ,

where is the constant controlling the rate of release of organic C from reserves. The released organic C is used to maintain structures or to grow (i.e. increase pool ). However, maintaining structures have a priority over the growth (@Hanegraaf2001). The growth is realized only when reserves contain enough organic C. When pool doesn't contain enough organic C to maintain , proportional part of is lost via respiration. The respective mass balance equations for pools and are defined as:

[12] ,

and

[13] .

In eq. 12, is yield or efficiency of structures production (functionally similar to ) and is the organic C flux available to construct . Depending on the size of the pool and the amount of organic C needed to maintain . This flux can be positive or negative. If the flux is positive, increases, while if it is negative, is lost via respiration (i.e. to cover required maintenance costs). The flux is defined by the equation:

[14] .

consists of two different processes, growth and maintenance respiration:

[15] .

### Parameters estimation and models evaluation

All three models include parameters, whose value can be adjusted to maximize the correspondence between model simulations and observations. Models were calibrated against the measured respiration rate, cellular protein concentration and/or DNA content. Model parameters were adjusted to minimize the objective function using the Differential Evolution algorithm (Mullen, Ardia, Gil, Windover, & Cline, 2011). The objective function was defined for each measured variable (i.e. respiration rate, cellular protein concentration and DNA content) as:

[16] ,

where and stand for observation i and its corresponding value predicted by the model, and is the mean of all observations. The objective functions calculated for each measured variable were summed up. Uncertainty of parameters estimates were determined by Constrained Markov Chain Monte Carlo simulation on 5000 iterations (Soetaert & Petzoldt, 2010). To evaluate the goodness of correspondence between models simulations and observations, Akaike Information Criterion (AIC) and coefficient of determination () were calculated. To evaluate the effect of experimental treatments, model parameters and corresponding goodness of fit were calculated across all treatments or for each substrate (glucose and cellobiose), each level of structural complexity (BROTH, GLASS, WOOL) or each experimental treatment (combination of the substrate and level of structural complexity) separately. The conversion factor between cellular protein concertation or DNA content and , and was estimated together with model parameters. Monod and MEND model were calibrated against cellular protein concentration and DNA content separately. DB model was calibrated against both measured variables at once (Hanegraaf & Muller, 2001). Cellular proteins were assumed to be part of and and DNA was assumed to be part of only (Hanegraaf & Muller, 2001). To produce Fig. 3, Monod and MEND models were also calibrated against observed respiration rate only.

Models were compared by AIC calculated for each measured variable. Because the total number of estimated parameters dramatically increases when all parameters are estimated for each experimental treatment separately, the model parameters with no variability across treatments were kept fixed to avoid artificial AIC increase. The significance of the difference between goodness of fit of two models were calculated using the equation:

[17] ,

in which is the difference between AIC calculated for two different models and particular variable (i.e. respiration rate, cellular protein concentration and DNA content). All analyses were done in statistical program R (R Development Core Team, 2014).

## Results

### Models comparison

The selected models simulated temporal dynamic of , and with different adequacy (Tab. 1). The model comparison is complicated by the fact that the goodness of model simulation was different for different variables and furthermore it was significantly affected by the type of constituent used to calibrate the model along with . In general, was simulated by all three models well as seen from around 0.9 and higher (Tab. 1). The lowest AIC (i.e. the best correspondence given the number of model parameters) was calculated for simulated by Monod model with parameters estimated across all experimental treatments to simulate dynamic of measured and . The goodness of the model fit was significantly better when compared to MEND model with parameters estimated the same way ( = 7.4, p = 0.025) and DB model ( = 7.9, p = 0.019). However, when the Monod model parameters were estimated to simulate dynamic of measured and instead of across all experimental treatments, the difference between models in respect to goodness of fit diminished (Tab. 1). The temporal dynamic of and was simulated by all models significantly worse than . The maximum explained variability for and variable was 76% and 30% respectively. DB model simulated the temporal dynamic of and significantly better as compared to Monod and MEND models with one exception. Monod model with parameters estimated for each level of structural complexity separately simulated the dynamic better than DB model, however, non-significantly at p<0.05 ( = 4.8, p = 0.091).

### Effect of experimental treatments on models simulation accuracy

Experimental treatments affected temporal dynamic of , and differently. The effect of experimental treatments on was small. When parameters of all models were estimated for each experimental treatment separately, of simulation increased but the corresponding AIC increased disproportionally making the effect of structural complexity and quality statistically non-significant. and dynamic, however, was significantly affected by the structural complexity (Fig. 2). When parameters of all three models were estimated for each level of structural complexity separately, AIC calculated for both constituents decreased (Tab. 1). The of DB model simulation increased to 0.75 when the model parameters were estimated for each experimental treatment separately. However, the required number of parameters was so large that the AIC did not decrease but doubled.

### Effect of constituents on Monod and MEND model parameters

Especially in BROTH and GLASS treatments, the temporal dynamic of and differed (Fig. 2). For this reason, it was not possible to calibrate Monod and MEND model against both constituents simultaneously. Depending on the constituent used to calibrate the model, calibration yielded different set of parameters (Fig. 3). The absolute value of model parameters and the patterns of variability observed across BROTH, GLASS and WOOL treatments were affected. For example, the parameter of MEND model decreased in order BROTH > GLASS > WOOL when was used to calibrate the model but it did not change when was used to calibrate the model. In contrast to Monod and MEND, DB model was calibrated against both constituents simultaneously so the calibration yielded one unique set of parameters (Tab. 2).

### Effect of structural complexity on DB model parameters

Structural complexity affected the estimate of all DB parameters except . was the only parameter, which was possible to keep constant across structural complexity treatments without decreasing the correspondence between model simulations and observations. was higher in BROTH treatment compared to GLASS and WOOL treatments (Tab. 2). parameter decreased in order BROTH > GLASS > WOOL (Tab. 2). The distribution of and parameters were highly correlated (data not shown). The estimated proteins content of reserves decreased in the same order as (Tab. 2) but the distribution of this parameter was not correlated with and . The differencess between BROTH, GLASS and WOOL treatments in other parameters were small and the associated uncertainty large. Therefore, no specific pattern can be identified.

## Discussion

### Models comparison

The growth of microbial biomass is regulated by concentration and the decay rate leading to production (i.e. ) is regulated by the size. This reciprocal relationship is the fundamental part of microbial-explicit biogeochemical models. In pure microbial cultures laboratory studies, and are both simulated by a suite of available models that are based on this reciprocal relationship with high accuracy ( ~ 0.9; e.g. Bhunia, Basak, Bhattacharya, & Dey, 2012). In laboratory studies conducted with soils, however, is always simulated accurately whereas simulation accuracy is much lower (Huang et al., 2018; Salazar, Sulman, & Dukes, 2018; G. S. Wang et al., 2015). The temporal dynamic of is different than the models expect based on observed . Such an obvious inconsistency between the theory and observations could be explained by the presence of the dormant microbial cells (G. S. Wang et al., 2015), changing microbial community structure (Buchkowski, Bradford, Grandy, Schmitz, & Wieder, 2017; Kaiser et al., 2015), diffusion limits (Mehdi Gharasoo, Centler, Regnier, Harms, & Thullner, 2012; He et al., 2014) or the methodological bias of quantification. In our study, Monod and MEND models were able to explain roughly 90% of variability in , but almost no variability in measured by two proxy parameters, DNA and cellular proteins. In line with Hanegraaf & Muller (2001) and Hanegraaf et al. (2000), our modelling exercises suggest that the composition of is not constant (Fig. 4) and thus, cannot be ambigously substituted by and measured as specific constituent as it is normally done. When do so, the microbial-explicit models are implicitely required to simulate the dynamic as well as the dynamic of the measrued constituent within the cell (Tab. 1). In contrast, the DB model do it explicitly and therefore, it simulates the and dynamic along with with higher accuracy. As shown previously, two abstract pools (i.e. Reserves and Structures) provide a sufficient flexibility to explain the changing composition of in respect to and along the gradient of concentration (Fig. 5; Hanegraaf & Muller, 2001; Hanegraaf et al. 2000).

In one case, Monod model outperformed DB model in goodness of simulation of when AIC metric was used to compare the models. When metric was used instead, DB model performed better. The difference here is given by the different number of parameters both models have. The amount of explained variability of by Monod model was significantly higher as compared to second measured constituent - . The reason is that unlike , proportion between and is relatively stable over time (Fig. 4). Moreover, DNA is present only in S pool (Hanegraaf & Muller, 2001) and as such its concentration is related to consumption and maintnance respiration, which dominates the respiratory flux when concentration is low. Cellular proteins, on the other hand, make a dominant part of R pool (Tab. 2). R pool controls the growth respiration, which dominates the respiratory flux only during the brief period of time when concentration is high. Its proportion to changes in time dramatically (Fig. 4) and as a result, neither Monod nor MEND model were able to simulate dynamic accurately (Tab. 1). Thus, different constituents have different dynamic within the cell and different relationship to cellular processes depending on their abundance in R and/or S pool (Fig. 5). According to it, they can fulfill the function of in Monod and MEND model with different efficiency. Important consequence of this fact is that the Monod and MEND model calibration against different constituents, becuase of their different functions, yields different sets of parameters (Fig. 3).

### Effect of constituents on Monod and MEND model parameters

The effect of experimental treatment is usually complex, i.e. more than one model parameter is typically affected (G. S. Wang et al., 2015). Nevertheless, to identify this effect unambiguously is problematic because several combinations of model parameters can give equally good correspondence between simulations and observations (Sierra, Malghani, & Müller, 2015). For that reason, the calibration of microbial-explicit models is advised to be constrained by several variables (Sierra et al., 2015). However, when different constituents are used as a proxy for , the unambigeous identification of the effect of experimental treatment on model parameters is problematic too. Our results show that the effect of constituents on model parameters is larger than the effect of experimental treatment itself (Fig. 3) and different conclussions could be reached (Fig. 3). In existing studies, different proxy parameters of are used to calibrate microbial-explicit models. For example, the MEND model was originally calibrated against the measured as a chloroform labile microbial carbon (G. Wang et al., 2013). Its adapted version - MEND\_wod, that implements microbial dormancy was, however, was calibrated against measured as DNA (Jagadamma, Mayes, Steinweg, & Schaeffer, 2014; G. S. Wang et al., 2015).

### Effect of experimental treatments on DB models parameters

In contrast to Monod and MEND, DB model calibration yields one unique set of parameters because both and are used to constrain the model calibration. Moreover, DNA represents the variable closely related to S whereas cellular proteins are mainly related to R (Hanegreaf2001). Thus, these two biomass constituents provide robust base for identification of the effects of experimental treatments on DB model parameters.

There was no significant effect of quality on DB model parameters. Cellobiose uptake, in contrast to glucose uptake, requires to break cellobiose into two molecules of glucose by -glucosidase enzyme first. Our data suggest that this enzymatic reaction is not the limiting step of the whole process. -glucosidases are presumably located on the cell surface (Burns et al., 2013) and their reaction kinetic is fast (German, Marcelo, Stone, & Allison, 2012). Therefore, no effect of the quality or combination of quality and structural complexity was found (Tab. 1).

Structural complexity itself, however, affected model parameters significantly. As can be expected, was higher in BROTH treatments as compared to GLASS and WOOL treatments. It implies that the structural complexity of the environment significantly decrease the difussion of substrate towards microbial cells as previously suggested by Evans, Dieckmann, Franklin, & Kaiser (2016). Nevertheless, estimated for GLASS and WOOL treatments were the same (Tab. 1) suggesting that the texture or porosity of the structured environment doesn't affect substrate diffusion significantly at least under water saturated conditions. Under lower water content of the porous material, the material porosity is likely to affect diffusion of substrate to microbes significantly (Evans et al., 2016; He et al., 2014; Manzoni et al., 2014).

Our analysis further showed decrease of parameter in order BROTH > GLASS > WOOL. We believe that this decrease is consequence of decreasing parameter whose distribution strongly correlates with the distribution parameter (data not shown). The decrease of implies increase of microbial affinity to in structurally more complex environment. However, opposite trend could be expected. Microbial cells attach to surfaces and create microbial biofilms. As a result, smaller surface of microbial biomass is exposed to liquid phase and thus, affinity of microbial community to is expected to decrease, which is in direct contrast to observed pattern.

Surprisingly, the same decrease in order BROTH > GLASS > WOOL was also observed for estimated abundance of cellular proteins in R pool (Tab. 2). In contrast to parameter, this estimate was not affected by . In BROTH treatments, R pool was entirely composed of cellular proteins. In GLASS and WOOL treatments, cellular proteins made 59 and 29% of R pool respectively. Without additional analyses, any unambiguous explanation of this pattern cannot be provided. It can be argued that the observed pattern is caused by the difference in microbial community composition that selected during incubation. As reported by Hanegraaf2001, different microbial species have different abundance of cellular proteins in R pool and thus, the change of microbial community composition can affect the estimated abundance of cellular proteins in R pool. However, the microbial community composition did not change with the structural complexity (data not shown). Therefore, we speculate that the observed difference is caused by the attachment of microbial cells and creation of biofilm, which tend to affect physiology and proteins expression of microbial community (e.g. Belas, Simon, Silverman, Costerton, & Davies, 1986; Chua et al., 2014; Mauter, Fait, Elimelech, & Herzberg, 2013; SvensÃ¤ter, Welin, Wilkins, Beighton, & Hamilton, 2001). Estimation of other model parameters was associated with substantial uncertainty and therefore, the effect of structural complexity treatment cannot be identified. Except , however, the model parameters couldn't be fixed across treattments without decreasing the correspondence between model simulations and observations. represents the efficiency of biomass production, which largely depends on substrate quality (e.g. Stouthamer, 1973). Since the metabolized substrate is in fact glucose molecule in all treatments, the invariable is reasonable expectation. The estimated value 0.6 corresponds well with the theoretical attainable maximum (Roels, 1980; Sinsabaugh, Manzoni, Moorhead, Richter, & Elser, 2013).

### Residual variability

Even though DB model simulated and with greater accuracy than Monod and MEND model, substantial part of the variability remained unexplained. When DB model parameters were estimated for each experimental treatment separately, the amount of explained variability increased for (to 75%) but remained 30% for . The unexplained variability can be associated with methodological issues or DB model assumption:

1. Especially DNA quantification is problematic. UV spectrophotometry can overestimate DNA concentration when UV absorbing compounds such as RNA, proteins, nucleotides etc. are present in the solution (Zipper et al., 2003). An alternative approach offers fluorescence method based on selective reaction of SYBRGreen with DNA, which is calibrated against standard curve of known amounts of Escherichia Coli DNA (Leininger et al., 2006).
2. DNA and proteins are measured as a macromolecular concentration, which have to be converted to organic C. Here we used the conversion factors reported in Vrede et al. (2004) but the C content of DNA and proteins can change among microbial species and through time.
3. DB model assumes invariant composition of R and S pool. If the composition is not invariant (i.e. cellular proteins become more abundant in S pool in time), the model fails to predict and dynamic. For example, we observed unpredicted increase of at the end of the incubation in BROTH treatments, which might suggest the change of macromolecular distribution in R and S pools (Fig. 2).

### Variable composition in microbial explicit models

There are unavoidable physical barriers that do not allow to measure in soil quantitatively so all available estimates based on quantification of various constituents are associated with an error of unknown magnitude (Cleveland & Liptzin, 2007). Although represents important variable, which microbial-explicit models needs to constrain possible combinations of parameter values in order to make reasonable projections (Sierra et al., 2015). Our modelling exercise suggest that the discrepancy between models' requirements and the physical limitations of available methods can be efficiently circumvented by defining two pools of allowing to explain variable composition of . The microbial-explicit model with two pools can be calibrated against any or all possible constituents of that can be measured quantitatively. Ideally, at least two constituents should be measured at the same time in designated experiments.

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## Tables

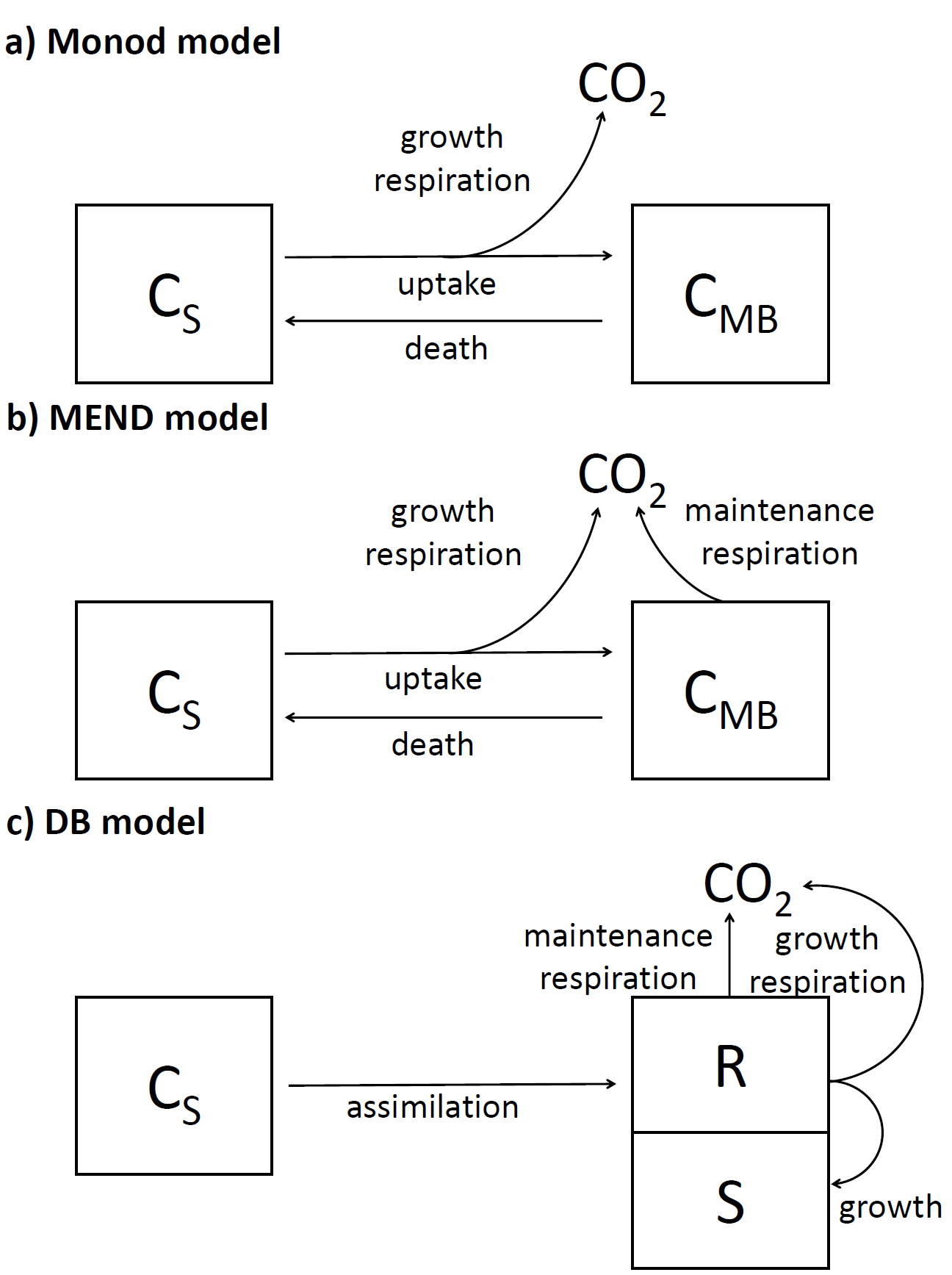
**Tab. 1:** Goodness of correspondence between the observed respiration rate (RH), DNA concentration (per organic carbon basis, CDNA)and cellular proteins concentration (per organic carbon basis, CProteins) and corresponding simulation of three different models (i.e. Monod, MEND and DB, see Material and Methods for details). Two different metrics of the goodness of correspondence (R2 and Akaike Information Criterion (AIC)) are calculated for each model with parameters estimated across all experimental treatments, for each substrate and structure treatment separately and for all combinations of substrate and structure treatments separately. Monod and MEND models were calibrated against DNA or cellular proteins, whereas DB model was calibrated against both variables simultaneously.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Monod | | | MEND | | | DB | | |
|  |  |  | RH | CProteins | CDNA | RH | CProteins | CDNA | RH | CProteins | CDNA |
| All treatments | | *R2* | *0.90* | *0* |  | *0.86* | *0* |  | *0.90* | *0* | *0* |
| *0.86* |  | *0* | *0.86* |  | *0* |
| AIC | 29.9 | 140.0 |  | 37.3 | 246.0 |  | 37.8 | 80.7 | 98.4 |
| 37.4 |  | 325.0 | 37.7 |  | 140.0 |
| Substrate | | *R2* | *0.90* | *0* |  | *0.90* | *0* |  | *0.91* | *0* | *0* |
| *0.88* |  | *0* | *0.87* |  | *0* |
| AIC | 42.0 | 333.0 |  | 42.1 | 121.0 |  | 57.7 | 99.1 | 117.0 |
| 45.7 |  | 163.0 | 48.6 |  | 159.0 |
| Structure | | *R2* | *0.95* | *0* |  | *0.95* | *0* |  | *0.95* | *0.51* | *0.30* |
| *0.94* |  | *0.19* | *0.91* |  | *0* |
| AIC | 45.0 | 123.0 |  | 46.1 | 116.0 |  | 50.9 | 68.7 | 88.8 |
| 40.3 |  | 84.0 | 51.8 |  | 155.0 |
| Substrate ᵡ Structure | | *R2* | *0.96* | *0* |  | *0.96* | *0.06* |  | *0.97* | *0.75* | *0.30* |
| *0.97* |  | *0* | *0.93* |  | *0* |
| AIC | 78.8 | 184.0 |  | 79.2 | 124.0 |  | 102.0 | 110.0 | 143.0 |
| 80.0 |  | 157.0 | 84.4 |  | 166.0 |

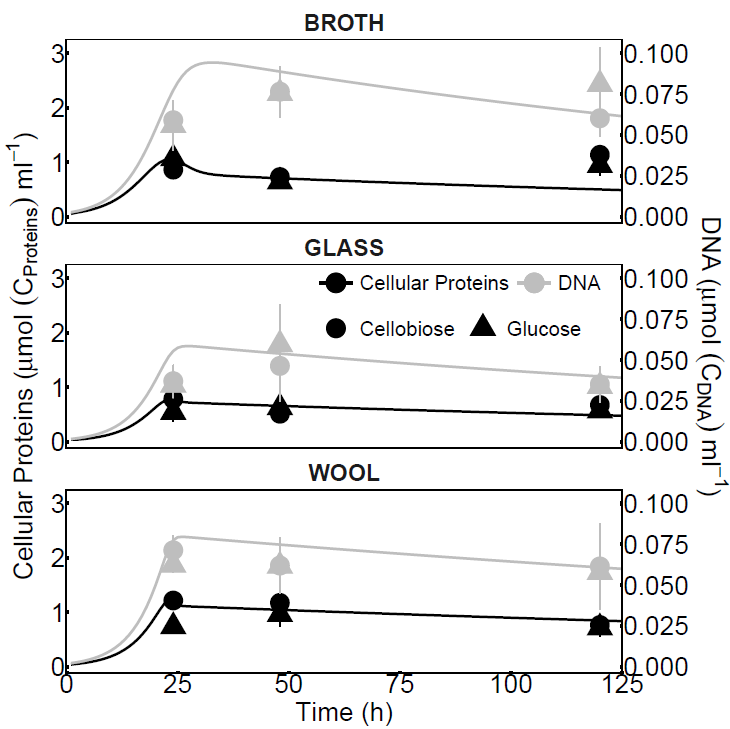
**Tab. 2:** DB model parameters (VMAX – maximum velocity constant, KM – affinity constant, f0 – reserves release rate constant, YS – efficiency of structures production, mR – specific maintenance rate constant, RProteins – abundance of proteins in reserves, SProteins – abundance of proteins in structures, SDNA – abundance of DNA in structures) estimated separately for three levels of structural complexity experimental treatment (BROTH, GLASS and WOOL). Best model parameters (plain numbers) estimated by the Differential Evolution Algorithm and standard deviation of posterior parameter distribution (italic numbers) estimated by Constrained Markov Chain Monte Carlo simulation are reported.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | *VMAX* | *KM* | *f0* | *YS* | *mR* | *RProteins* | *SProteins* | *SDNA* |
|  | µmol (CS) ml-1 [µmol(CMB)ml-1]-1 h-1 | µmol ml-1 | µmol (R) h-1 | unitless | nmol (CO2) [µmol(S)]-1 h-1 | % | | |
|  |
| BROTH | 0.5 | 17.7 | 3.2 | 0.6 | 4.8 | 99.8 | 4.4 | 0.6 |
| *0.1* | *3.6* | *2.2* | *0.1* | *2.5* | *27.5* | *1.4* | *0.2* |
| GLASS | 0.3 | 5.0 | 7.5 | 0.6 | 4.2 | 58.8 | 4.1 | 0.3 |
| *0.0* | *2.6* | *3.4* | *0.1* | *2.7* | *26.6* | *2.3* | *0.1* |
| WOOL | 0.3 | 2.5 | 3.1 | 0.6 | 2.9 | 29.4 | 6.3 | 0.5 |
| *0.0* | *1.5* | *2.8* | *0.1* | *2.6* | *21.4* | *2.2* | *0.2* |

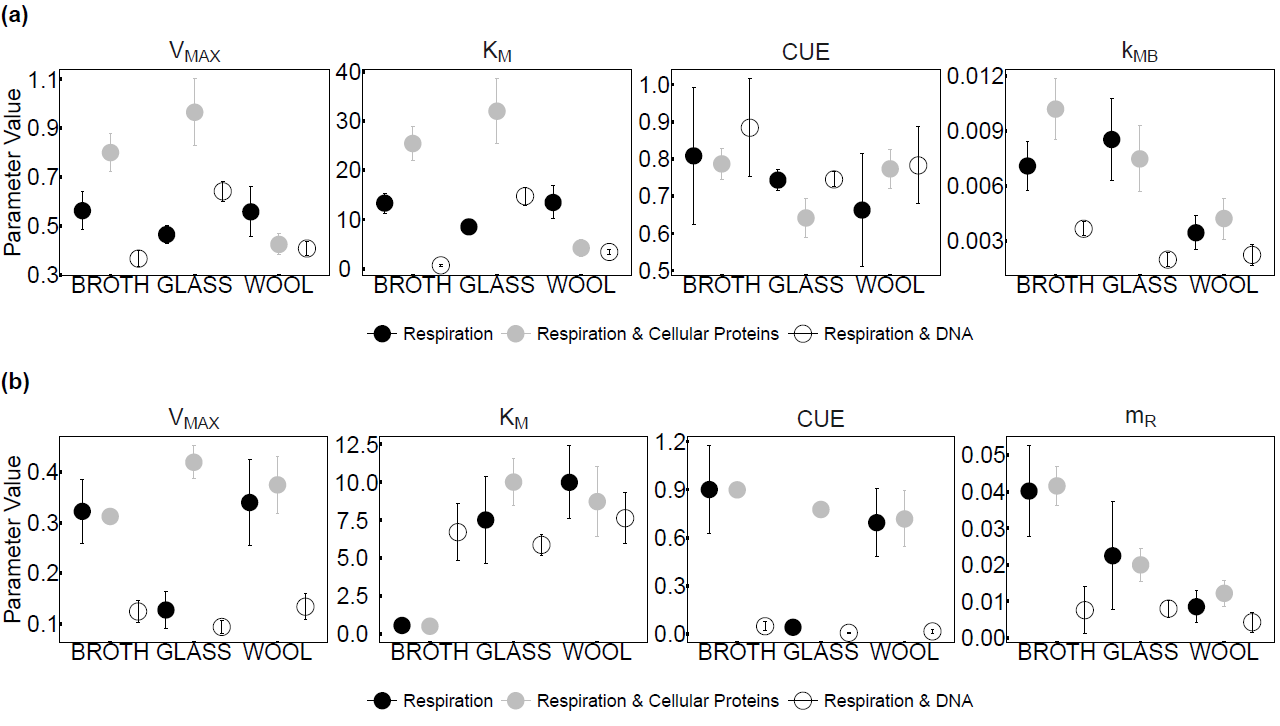
## Figures



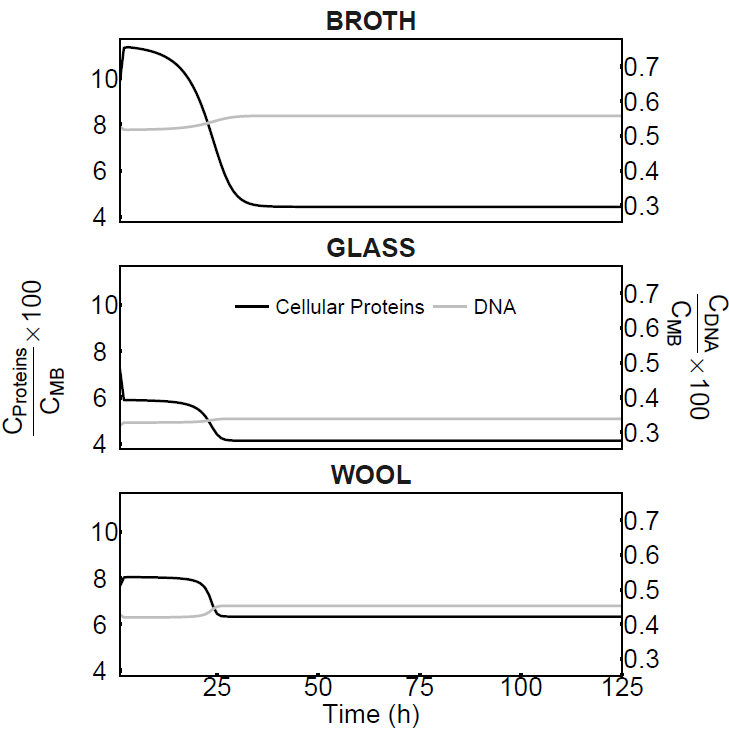
**Fig. 1:** Schematic representation of three models (Monod, MEND and DB) simulating coupled dynamic of organic substrate (CS) and microbial biomass (CMB) on per carbon basis leading to CO2 production. Squares represent pools and arrows represent. DB model defines two pools of microbial biomass, i.e. Reserves (R) and Structures (S).



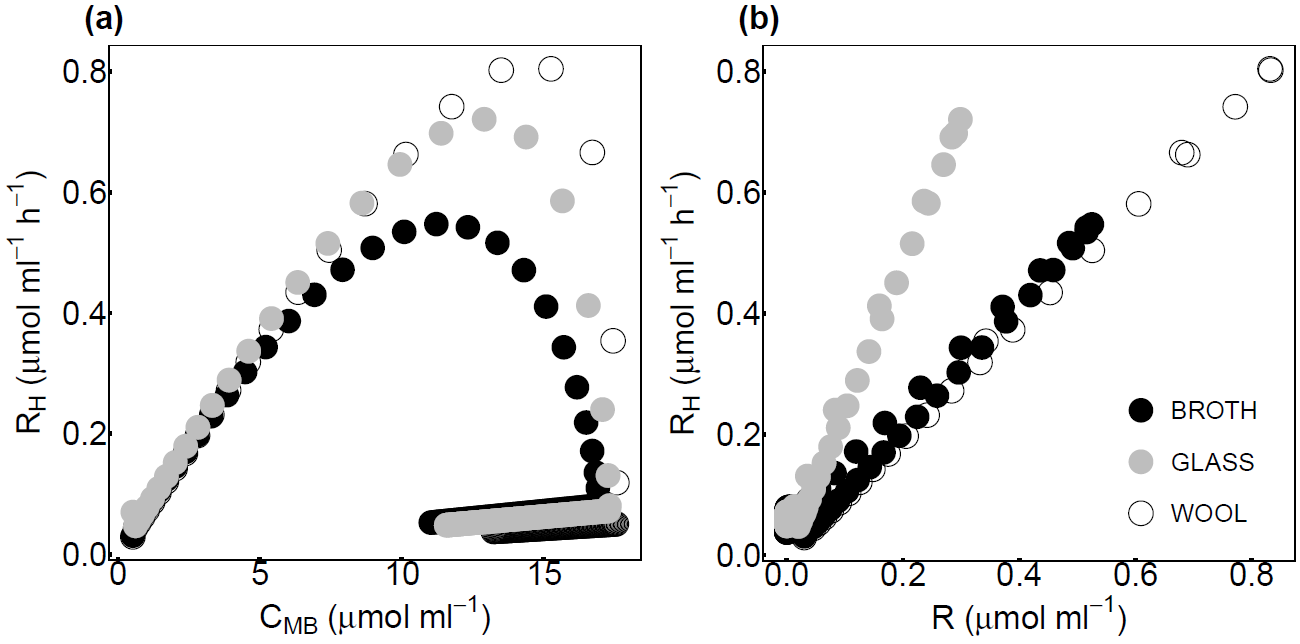
**Fig. 2:** The dynamic of cellular proteins (black symbols) and DNA (grey symbols) in time (mean and corresponding standard deviation are shown). Filled circles and triangles represent measured cellular proteins and DNA concentration in Glucose and Cellobiose treatments respectively. Black and grey lines represent DB model simulation of DNA and cellular proteins temporal dynamic respectively. Data and model simulation is shown for three different levels of structural complexity treatment (BROTH, GLASS and WOOL) separately. Note that the left (cellular proteins) and right (DNA) y-axis have different scales.



**Fig. 3:** Monod (a) and MEND (b) model parameters (VMAX – maximum velocity constant, KM – affinity constant, CUE – carbon use efficiency, kMB – microbial biomass decay rate constant, mR – specific maintenance rate constant) estimated separately for three different levels of structural complexity treatment (BROTH, GLASS and WOOL). Symbols represent best parameters values estimated by the Differential Evolution Algorithm and error bars represent standard deviation of posterior parameters distribution estimated by Constrained Markov Chain Monte Carlo simulation. Parameters estimation was constrained by either observed respiration rates (black full circles), observed respiration rates and cellular proteins concentration (grey full circles) or observed respiration rates and DNA concentration (empty circles).



**Fig. 4:** Temporal dynamic of conversion factors between cellular proteins (CProteins) or DNA (CDNA) and microbial biomass (CMB) as predicted by DB model for three different levels of structural complexity treatment (BROTH, GLASS and WOOL). Note that the left (cellular proteins) and right (DNA) y-axis have different scales.



**Fig. 5:** Relationship between respiration rate (RH) and microbial biomass (CMB) or reserves (R) predicted by DB model for three different levels of structural complexity treatment (BROTH, GLASS and WOOL).

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