**Title**

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## Abstract

## Introduction

## Material and Methods

### Experimental design

Soil microbial culture (see later) was incubated in liquid batch culture microcosms at 24°C in the dark for five days under six different experimental treatments. These treatments include two different organic substrates as a sole source of carbon for microbial culture and three different levels of microcosm structural complexity. The treatments are in full factorial design. The two organic substrates were glucose and cellobiose. The three levels of structural complexity of the system were represented by ***??34 ml??*** incubation vials 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 cutted to ~0.25 cm pieces; denoted as **“WOOL”** further in the text). All experimental treatments were replicated four times ***but this is probably not true since the sampling was destructive. Could you please describe it?***.

### Soil microbial culture

Soil microbial culture used in the experiment was obtained by imbibement of the ***??prosser soil?? (is it correct)***, *short characteristic of soil* … with the growth medium. One gram of air dried soil was supplemented by the 10 ml of sterile Cellulose Degrader M9 Media (see later) with 6.25 ml of lysogeny broth. The final organic carbon concentration of added medium was ~40 mmol l-1 (0.4 mmol g(DW)-1). The soil with medium was incubated seven days at 24°C in dark. At the end of incubation, soil-medium suspension was shaken with ~ten 2.7 mm glass beats for ten minutes. The suspension was transferred to sterile flask and soil particles were allowed to settle down. The liquid phase was further transferred to new sterile flask and used as a microbial culture inoculum.

### Microcosm

***??34 ml??*** empty incubation vials or vials with glass beads and glass wool were filled with 3 ml of sterile medium and 1 ml of microbial culture. The medium was composed of Cellulose Degrader M9 Media and organic substrate (the final concentration 1 g of organic substrate per one liter of medium). Cellulose Degrader M9 Media 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. Accounting for the dilution by the inoculum with assumed organic carbon concentration nearly zero, initial organic carbon concentration of the microcosms was 25 mmol . Incubation vials were kept covered by Breathe-Easy sealing membrane secured with the aluminium cap at all times the respiration rate was not measured. ***(short decription of the incubator)***.

### 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, Breathe-Easy sealing 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 Li 7000 (LI-COR, Inc., Lincoln, Nebraska, USA). concentration was calculated against the calibartion 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.

### Cellular protein quantification

To quantify microbial biomass in the microcosms, protein content of microbial cells was isolated on the first, second and last day of incubation. The sampling was destructive. The whole volume of microcosm was quantitatively transfered to 15 ml falcon tubes. Cell scraper was used to detach microbial cells from surface of vials or from glass beads/wool. Two ml of phosphate buffer was added and the final solution thoroughly mixed. The final solution was centrifuged at 4,700 g for ten minutes at 4°C and pelet was separated from supernatant. Pellet was resuspended in 0.5 ml and kept deep frozen (-80°C) until the assay was conducted. Cell pelet was bead beated in ethanol solution for 5 minutes to lyse the cells.Protein concentration was assesed in cell lysate by bicinchoninic acid assay. ***(specification of spectrophotometer)*** was used to measure protein concentration.

### Mathematical description

Three different models (Fig. 1) were used to predict the changes in respiration rate () and microbial biomass () expressed per molar C basis in time. The Monod model is a fundamental part of microbial explicit biogeochemical models (S. D. Allison, Wallenstein, and Bradford 2010; W. R. Wieder et al. 2014; William R. Wieder, Bonan, and Allison 2013).

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

In this model, organic substrate () is consumed by microbial biomass and is transformed to or respired. Microbial biomass is dying at constant rate returning used organic carbon to pool:

[1] ,

[2] .

In eqs. 1 and 2, CUE is the carbon use efficiency (defined as an amount of C incorporated to biomass over the amount of C taken up) and is the death rate constant of microbial biomass decay process. Microbial carbon uptake is defined as hyperbolic function of and :

[3] ,

in which is maximum velocity constant and is affinity constant. Respiration rate is given by .

#### Microbial enzyme models (Fig. 1c)

In microbial enzyme models, microbial biomass produce extracellular enzymes. The way the carbon is partitioned between , and can follow different rules.

* Classical microbial enzyme model (MEM)

Microbial enzyme model presented by Allison et al. (2010) assumes that extracellular enzymes are produced constitutively by the rate (), which is proportional to biomass:

[5] .

When extracellular enzymes are produced, microbial biomass is loosing carbon. Therefore, the mass balance equation for microbial biomass is:

[6] .

## Results

## Discussion

## References

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