



Kinetic analysis of microbial respiratory response to substrate addition

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Heterotrophic component of CO₂ emitted from soil is mainly due to the respiratory activity of soil microorganisms. Field measurements of microbial respiration can be used for estimation of C-budget in soil, while laboratory estimation of respiration kinetics allows the elucidation of mechanisms of soil C sequestration. Physiological approaches based on 1) time-dependent or 2) substrate-dependent respiratory response of soil microorganisms decomposing the organic substrates allow to relate the functional properties of soil microbial community with decomposition rates of soil organic matter. We used a novel methodology combining (i) microbial growth kinetics and (ii) enzymes affinity to the substrate to show the shift in functional properties of the soil microbial community after amendments with substrates of contrasting availability. We combined the application of ¹⁴C labeled glucose as easily available C source to soil with natural isotope labeling of old and young soil SOM. The possible contribution of two processes: isotopic fractionation and preferential substrate utilization to the shifts in $\delta^{13}\text{C}$ during SOM decomposition in soil after C₃-C₄ vegetation change was evaluated.

Specific growth rate (μ) of soil microorganisms was estimated by fitting the parameters of the equation $v(t) = A + B * \exp(\mu * t)$, to the measured CO₂ evolution rate ($v(t)$) after glucose addition, and where A is the initial rate of non-growth respiration, B - initial rate of the growing fraction of total respiration. Maximal mineralization rate (V_{max}), substrate affinity of microbial enzymes (K_s) and substrate availability (S_n) were determined by Michaelis-Menten kinetics. To study the effect of plant originated C on $\delta^{13}\text{C}$ signature of SOM we compared the changes in isotopic composition of different C pools in C₃ soil under grassland with C₃-C₄ soil where C₄ plant *Miscanthus giganteus* was grown for 12 years on the plot after grassland.

The shift in $13\delta\text{C}$ caused by planting of *M. giganteus* was not equal in different C pools and was most pronounced in microbial biomass (from -26.5‰ to -15.8‰), while was rather weak in CO₂ (from -27 ‰ to -20.5‰) and in dissolved organic carbon (from -26.7‰ to -22.1‰). The contribution of «old» C to total CO₂ was not constant and permanently decreased during 54 days of incubation from 60 to 40%. Contrary to that, the contribution of «old» C in microbial biomass pool increased from 20 to 40% during the incubation. Thus, preferential mineralization of «young» C, while preferential usage of «old» C for building up the microbial biomass was observed in course of two-month incubation. It is crucial to consider the opposite trends in dynamics of $\delta^{13}\text{C}$ in microbial and in respired C for estimations of C mineralization in soil incubation studies.

Input of small amounts of easily available substrates (i.e. glucose or root exudates) significantly increased the specific growth rates of soil microorganisms by up to 13 and 20%, respectively. This increase, showing the shift from K to r strategies, was confirmed by a 50% decrease in the affinity of microorganisms to the easily available substrates. In contrast, plant residues lowered specific growth rates by 16–30% and increased the affinity of microorganisms to the substrate by 23–131% compared with untreated soil. The generation time of the actively-growing fraction of microbial biomass in soil was 1.8 to 2.8 hours, which was 100 to 1000 times faster than that of the whole microbial community.

Combination of two complementary physiological approaches based on microbial growth kinetics and substrate affinity showed contrasting effects of easily and less available substrates on the shift of growth strategies (r vs. K) of the whole microbial community. We would like also to discuss the physiological restrictions which are necessary to be considered for successful application of respiratory-based approaches for soil ecological studies