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Review paper

Active microorganisms in soil: Critical review of estimation criteria and approaches

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ABSTRACT

Microbial functioning refers to microbial activity because only the active microorganisms drive biogeochemical processes. Despite the importance of *active* microorganisms, most methods focus on estimating *total* microbial biomass and fail to evaluate its active fraction. At first, we have described the differences among the *active*, *potentially active*, and *dormant* microbial states in soil and suggested threshold values of parameters for their identification. Secondly, we critically reviewed the ability of a broad range of approaches to estimate and characterize the active and the potentially active microorganisms in soil. Following approaches were evaluated: plate count and microbial cultures; direct microscopy combined with cell staining; ATP, PLFA, DNA and RNA content; microarray analyses; PCR-based approaches; stable isotope probing; soil proteomics, enzymes activity; and various approaches based on respiration and substrate utilization. The "static" approaches, mainly based on the single-stage determination of cell components (ATP, DNA, RNA, and molecular biomarkers), detect well the presence of microorganisms and total biomass, but they fail to evaluate the active part and consequently the functions. In contrast, the dynamic approaches, estimating the changes of these parameters during microbial growth and based on process rates: substrate utilization and product formation, e.g., respiration, help to evaluate active microbial biomass and relate it to specific process rates. Based on a comparison of all approaches for their universality (possibility to analyze active, potentially active and dormant microorganisms), we concluded that 1) direct microscopy with complementary stains, 2) a combination of RNA-based FISH with staining of total microbial biomass, and 3) approaches based on microbial growth were the most advantageous and allowed simultaneous quantitative estimation of *active*, *potentially active*, and *dormant* microorganisms in soil.

The *active* microorganisms compose only about 0.1–2% of the *total* microbial biomass and very seldom exceed 5% in soils without input of easily available substrates. Nonetheless, the fraction of *potentially active* microorganisms (ready to start utilization of available substrates within few hours) is much higher, contributing between 10 and 40% (up to 60%) of the total microbial biomass. Therefore, we emphasize the role of *potentially active* microorganisms with quick response to fluctuating substrate input in soil microhabitats and hotspots.

The transition from the potentially active to the active state occurs in minutes to hours, but the shift from dormant to active state takes anywhere from hours to days. Despite very fast activation, the reverse process – fading to the potentially active and dormant stage – requires a much longer period and is very different for individual criteria: ATP, DNA, RNA, enzyme production, respiration rates. This leads to further difficulties in the estimation of the active part of microbial community by methods based on these parameters. Consequently, the standardization, further elaboration, and broad application of approaches focused on the portion of active microorganisms in soil and their functions are urgently needed. We conclude that because active microorganisms are the solely microbial drivers of main biogeochemical processes, analyses of the active and potentially active fractions are necessary in studies focused on soil functions.

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1. Introduction: why consider active microorganisms?

Studies that refer to microbial biomass are central not only in soil science but also in all biogeochemistry-related disciplines. Microbial biomass is studied not as end in itself but as a driver of biogeochemical cycles. This requires knowing which microorganisms are responsible for specific processes and, more generally, which portion of the microbial biomass is responsible for the turnover of elements.

Microbial communities in soils consist of a very broad range of organisms in different physiological states. These are frequently termed as active, viable, living, dormant, passive, dying, dead, and so on, states (Johnsen et al., 2001) and are often difficult to differentiate among (Rousk et al., 2009). These terms can be summarized as four physiological states of microorganisms. The first three are living states. The first is the *active* state of the microorganisms. The active microorganisms are involved in the ongoing utilization of substrates and associated biochemical transformations. The second is the *potentially active* microorganisms. This part is in physiological alertness (De Nobili et al., 2001; Raubuch et al., 2010) and can switch to utilization of substrates within minutes to a few hours. The last state of living microorganisms is the *dormant* state. It does not contribute to ongoing processes currently but can contribute under altered circumstances. The fourth state of microorganisms in soil is *dead* (including lysed cells and microbial residues), but also quantified by some methods and does not directly contribute to any ongoing processes. Dead microbial biomass does, however, affect turnover of C and N as a source of easily available substrates. All these parts of *total* microbial biomass are crucial for evaluating soil functions and comparing treatments, environmental conditions, land use, and management practices. However, only active microorganisms are involved in the ongoing processes and consequently, all processes should be related to the mass of *active* microorganisms driving biogeochemical elements cycling in soil.

Most methods for estimating microbial biomass (reviewed by Beck et al., 1997; Nannipieri et al., 2003; Hartmann et al., 2004; Bölter et al., 2006; Joergensen and Emmerling, 2006; Joergensen and Wichern, 2008) were developed to measure *total* microbial biomass, and these reviews are focused on methods for estimating *total* microbial biomass. However, because most processes are driven by *active* microorganisms, it is a current challenge to quantitatively distinguish active and dormant biomass and to assess ecologically relevant microorganisms actively contributing to ecosystem functions (Ellis et al., 2003).

This motivated the present review of one of the most dynamic pools and drivers in soil – the active microbial biomass. After the definition of terms, we evaluated suitable methods to estimate the active part of microbial biomass (note that this review does not focus on presenting analytical details of the methods) and then compared the approaches by their suitability to evaluate separately the three parts of living microbial biomass. Furthermore, we suggested the threshold values or parameter ranges as criteria for differentiation of the three parts of living microbial biomass by various approaches.

It was not the aim of this review to analyze various microbial activities such as respiration, decomposition rates of natural substrates or xenobiotics, transformations of biogenic elements, ATP production, or enzyme activities. However, we refer to some of these approaches if they are directly or indirectly useful to estimate or to characterize the portion of active microorganisms.

2. Definitions: total, dead, dormant, and active microorganisms

The total microbial biomass includes all living and nonliving soil organisms smaller than 150–200 μm (Swift et al., 1979; Coleman and Wall, 2007). The total amount of microbial biomass is relatively small (50–2000 μg C g^{-1} soil). It averages at 2–3% (Anderson and Domsch, 2010) and usually does not exceed 4.5% of organic C content (Anderson, 2003). The **dead** microorganisms are in an irreversible state in which no growth, cell elongation, or protein synthesis can take place (Villarino et al., 2000). Dead cells, or **microbial necromass**, act as an additional pool of available substrate but do not contribute actively to any biogeochemical processes. Microbial necromass is a fraction of easily available SOM and

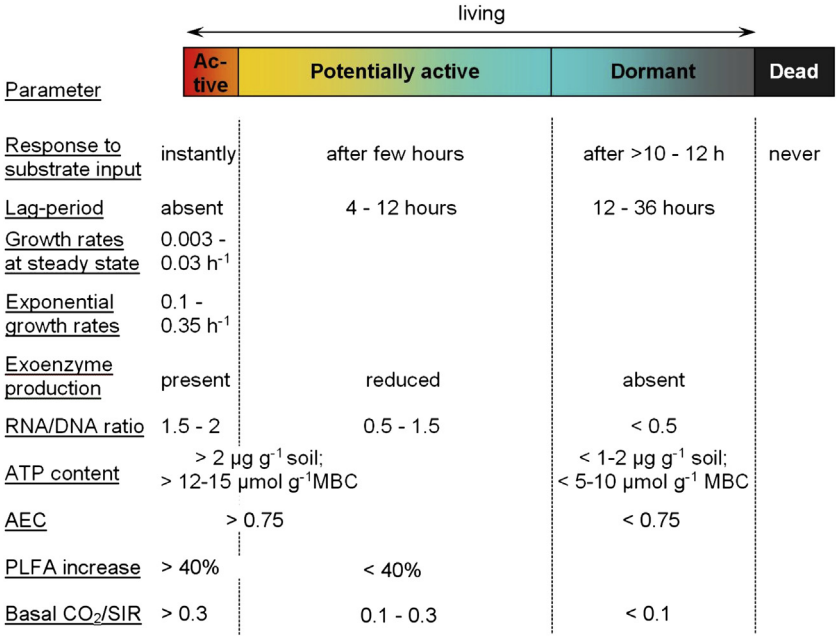

Fig. 1. Various physiological stages of microorganisms in soil: active, potentially active, dormant and dead. Threshold values and ranges for parameters obtained by various approaches to differentiate between the physiological stages are suggested (see text for details and references).