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Research paper

Shift from dormancy to microbial growth revealed by RNA:DNA ratio

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ABSTRACT

Although soil microorganisms spend most of their lifetime at dormant or resting states, they are quickly activated by substrate input and easily switch to growth. Under steady-state, the double-stranded DNA (dsDNA) content is considered as a measure of total microbial biomass while the RNA content mainly indicates the active microbial fraction. In growing population, however, an increase in DNA is solely related to replication of microbial cells, while the RNA, besides growth, is also involved in non-growth processes. Therefore, the dsDNA and RNA content increase differently during microbial growth, applying the RNA:dsDNA ratio as promising indicator of growth-related and non-growth microbial activity. To what extend the ratio can be used to comparatively infer investment in microbial biomass production versus maintenance-related synthesis following substrate induction remains to be studied.

We measured the RNA:dsDNA ratios of representative soil types of four different ecozones before and after glucose addition to prove the prediction capacity of physiological status of soil microorganisms. The RNA:dsDNA ratio remained stable after activation of microorganisms in Retisol and Luvisol indicating balanced growth. In contrast, very moderate increase in DNA in sandy Calcisol was accompanied by disproportionally high increase in RNA. As a result, the RNA:dsDNA ratio in Calcisol with low nutrient status increased by 36-fold after glucose amendment, indicating strong non-growth related processes. The RNA recovery decreased exponentially with increasing clay content, indicating the strong association to the textures of the soil types. This suggests, that the underestimation of RNA yields in clayey soils biased the RNA:dsDNA ratio, and subsequently the physiological state of the microbial community is not adequately represented in soils clay contents exceeding 30%. Monitoring the relative changes in dynamics are required to overcome the restricted applicability of RNA:dsDNA ratio in soils with high clay content.

1. Introduction

Bulk soil is regarded as an oligotrophic environment, as it is generally poor in labile organic compounds directly available for microorganisms (Van Elsas and van Overbeek, 1993). Active microbes utilize the available substrate and therefore maintain biochemical transformations (Gunina et al., 2014). The total microbial biomass consists of only about 0.1–2% of active microorganisms in absence of easily available substrates (e.g. root exudates), whereas potentially active microorganisms contribute up to 60% of the total microbial biomass (Blagodatskaya and Kuzyakov, 2013). The potentially active microbial population permanently exists in soil between the active and dormant physiological states (De Nobili et al., 2001). However, the mechanisms controlling the percentage of microbes being active are poorly defined. The low amount of readily-available organic carbon (C) precludes slow bacterial growth and low activity. Many soil organisms, therefore, have very low metabolic activity and frequently spend most of their lifetime in dormant or resting phases, especially in soils of low C and N contents (Sparling et al., 1981; Van Elsas and van Overbeek, 1993).

The input of readily assimilated C substrates in soil hot spots, such as the rhizosphere, detritusphere and drillosphere shifts microbial population from dormancy to activity, thereby strongly accelerates microbial metabolism, and may induce microbial growth. A recent study showed that total microbial biomass in the rhizosphere was 14–31% higher than that in the root free soil, while the growing (active) part of microbial biomass was 45–83% higher (Blagodatskaya et al., 2014). This switch leads to increasing both DNA- and RNA- content. Microbial activity can have various expressions: 1) Growing cells – actively

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dividing and consequently increasing DNA and RNA contents, and 2) producing proteins and enzymes for metabolism. In the latter case, the DNA remains nearly constant but RNA increases (Blazewicz et al., 2013).

DNA and RNA molecules are responsible for the storage of genetic information and for translation of this genetic information for protein synthesis, correspondingly. The DNA extracted from soil in relatively large amounts represents organisms at any physiological state – dead, dormant and active (Levy-Booth et al., 2007; Blagodatskaya and Kuzyakov, 2013). In contrast to DNA, the RNA content in dormant cells is extremely low, showing often reduced concentrations of lipids, fatty acids and proteins, while it increases dramatically after microbial activation (Lennon and Jones, 2011). Since the RNA amount per cell is proportional to metabolic activity of microorganisms (Mills et al., 2004; Molin and Givskov, 1999), the RNA-based approaches provide information on the metabolically active part of microbial community. More than a hundred studies are available using rRNA to indicate active microorganisms in batch studies but also in the marine and terrestrial environment (Blazewicz et al., 2013; Hunt et al., 2013; Jones and Lennon, 2010; Wu et al., 2011). The response of microbial growth and activity on substrate addition is mainly focused on pure cultures (Scott and Hwa, 2011; Taymaz-Nikerel et al., 2013), e.g. for model microorganisms such as *Escherichia coli* or *Bacillus* sp. (Ferenci, 1999). Substrate addition to pure cultures studies caused multiple regulative responses, such as shifts in metabolism, storage products and osmoregulation (Van Bodegom, 2007). However, knowledge from these studies cannot be directly linked to the response of diverse microbial communities in soils.

For 17 soils and sediments under steady-state, the RNA:DNA ratio ranged between 0.01 and 0.35 with a mean ratio of 0.13 (Hurt et al., 2001). A high ratio reflects enhanced microbial activity, subsequently resulting in increased soil organic matter (SOM) decomposition rates.

Comparison of eco-physiological indices defined by RNA:dsDNA ratio (i.e. where the ratio reflects microbial C mineralization) may help us to understand how microbial physiological responses relate to soil properties like nutrient content, texture, or land use history (Anderson and Domsch, 1993; Bending et al., 2004). The microbial metabolic quotient (q_{CO2}) was determined, as an integrative indicator of substrate availability microbial activity, maintenance requirements and carbon use efficiency (Insam and Haselwandter, 1989). Disturbances or nutrient limitations in soil (e.g. land-use change) have negative impact on microbial properties, and subsequently increases the metabolic quotient (Papst et al., 2016).

Microbially-mediated processes have been incorporated into new generation ecosystem models as a parameter for SOM decomposition (Wang et al., 2014). However, continued development is still required to assess microbial processes into global climate models (Wang et al., 2015) because these models neglect physiological state changes of microorganisms. This could result in incorrect estimates of microbial biomass, which subsequently infers deficiencies in model parameterization and predictions of SOM decomposition. Physiological status indicators such as the RNA:dsDNA ratio under substrate-induced conditions might be useful in modelling organic matter dynamics with land-use changes that shift soil textures and organic input quality.

Great progress in molecular technologies ensured quantitative extraction of DNA and RNA even from soil samples making the RNA:DNA ratio a promising indicator of the physiological status of bacterial (Dell'Anno et al., 1998; Kerkhof and Ward, 1993; Muttray and Mohn, 1998) and of microbial communities as a whole in soils (Hahn et al., 1990; Tsai and Olson, 1991). The sensitivity and applicability of this ratio in various soil types, differing in texture and nutrient status, require experimental prove.

Quantitative determination of DNA content in soil is well established (Marstorp and Witter, 1999; Blagodatskaya et al., 2003) and is possible by application of commercially available kits (Fornasier et al., 2014). Quantitative extraction of microbial DNA from soil (Marstorp et al., 2000) can be used as a measure of total microbial biomass (Joergensen and Emmerling, 2006; Renella et al., 2006). Highly sensitive fluorescent dye PicoGreen (Life Technologies) is applied for dsDNA quantitative analysis (Fornasier et al., 2014; Smets et al., 2016; Terrat et al., 2012). Specifically binding to dsDNA, PicoGreen can be used for quantification of total dsDNA yields even in the presence of co-extracted and co-purified components such as humic acids, cellular debris and organic solvent residues (Bachoon et al., 2001). The positive linear correlation between dsDNA content and total microbial biomass was already confirmed by the number of studies (Blagodatskaya et al., 2003; Anderson and Martens, 2013). Another advantage of DNA-based approach is related to the relatively small contribution of plant dsDNA to total dsDNA in comparison with the contribution of root residues to quantification of microbial biomass by chloroform fumigation-extraction. DNA content is much lower in plants compared to microbial cells, the plant dsDNA never exceeded 2.6% of total dsDNA content for a wide range of soils (Gangneux et al., 2011). Similarly, the extracellular DNA content does not exceed 3% of total DNA (Blagodatskaya et al., 2003; Gangneux et al., 2011). Relatively stable conversion factor from dsDNA units to microbial C in a narrow range of 5.0 (Anderson and Martens, 2013), 5.4 (Blagodatskaya et al., 2003) and 5.6 (Lloyd-Jones and Hunter, 2001) has been frequently revealed. A literature review based the conversion factor from dsDNA into microbial biomass averaged at 6, indicating that approximately 13% of microbial C stems from DNA (Joergensen and Emmerling, 2006).

The RNA is more difficult to extract from soil than the DNA, and quantitative extraction of soil RNA comprises several challenges. The RNA pool of a microbial cell is mainly composed of rRNA (82–90%) and in much lesser extent of mRNA and tRNA (Neidhardt, 1987). The RNA recovery from soil remains very low and rarely exceeds 10% (Duarte et al., 1998). The RNA yields extracted from soil range from tens of nanograms to several micrograms per gram of soil (Borneman and Triplett, 1997; Bürgmann et al., 2003; Mettel et al., 2010; Moran et al., 1993; Sessitsch et al., 2002; Wang et al., 2008,2009). Such a wide range of RNA yield may be caused by activity state of soil microorganisms, contamination of RNA sample by humic substances or the loss of RNA during purification (Wang et al., 2012). Furthermore, the strong RNA losses during isolation may be caused by an RNase activity and by adsorption to the clay fraction. Although, both DNA and RNA could be adsorbed by soil particles (Goring and Bartholomew, 1952), the adsorption of single-strand RNA molecules is much stronger in soils with clay and clay-loamy texture (Tournier et al., 2015).

Despite low recovery, the relative changes in RNA content within the same soil type can shed light on shifts in physiological state of soil microorganisms (Bakken and Frostegård, 2006; Blagodatskaya and Kuzyakov, 2013). Since not all active microorganisms are growing, but all the growing microorganisms are active (Blazewicz et al., 2013), a differentiation between microbial growth and activity in soils remains a great challenge to identify the underlying mechanisms of microbial functioning. To investigate physiological state of microorganisms in soils with contrasting nutrient status, we sampled soils from four zonal soil types in Russia (Fig. 1). We hypothesized that 1) despite the substrate addition, the growth of microorganisms is strongly dependent on the initial C and N status of the respective soils 2) strong growth of microorganisms subsequently indicates high microbial activity.

To test these hypotheses we determined the RNA:dsDNA of the soils varying in soil organic carbon content (C_{org}), soil nitrogen content (N_{tot}) and particle size distribution. Virgin and arable Chernozems were characterized by the highest C_{org}, N_{tot}, soil microbial biomass (C_{mic}) and soil C:N ratios. Retisol and Luvisol had almost similar soil properties, and Calcisol was lowest in C_{org}, N_{tot} and C_{mic}. Thus, these four soil types represent the reduced enrichment gradient of C and N from Chernozems over Retisol and Luvisol to Calcisol. Based on these range of properties, we expected that the highest DNA- and RNA-contents would be found in the C- and N-rich Chernozem, and the lowest in Calcisol. We switched physiological state of soil microorganisms by addition of glucose in