Soil organic matter quality exerts a stronger control than stoichiometry on microbial substrate use efficiency along a latitudinal transect

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ABSTRACT

A substantial portion of soil organic matter (SOM) is of microbial origin. The efficiency with which soil microorganisms can convert their substrate carbon (C) into biomass, compared to how much is lost as respiration, thus co-determines the carbon storage potential of soils. Despite increasing insight into soil microbial C cycling, empirical measurements of microbial C processing across biomes and across soil horizons remain sparse. The theory of ecological stoichiometry predicts that microbial carbon use efficiency (CUE), i.e. growth over uptake of organic C, strongly depends on the relative availability of C and nutrients, particularly N, as microorganisms will either respire excess C or conserve C while mineralising excess nutrients. Microbial CUE is thus expected to increase from high to low latitudes and from topsoil to subsoil as the soil C:N and the stoichiometric imbalance between SOM and the microbial biomass decrease. To test these hypotheses, we collected soil samples from the organic topsoil, mineral topsoil, and mineral subsoil of seven sites along a 1500-km latitudinal transect in Western Siberia. As a proxy for CUE, we measured the microbial substrate use efficiency (SUE) of added substrates by incubating soil samples with a mixture of ^{13}C labelled sugars, amino sugars, amino acids, and organic acids and tracing ^{13}C into microbial biomass and released CO_2. In addition to soil and microbial C:N stoichiometry, we also determined the potential extracellular enzyme activities of cellobiohydrolase (CBH) and phenol oxidase (POX) and used the CBH:POX ratio as an indicator of SOM substrate quality. We found an overall decrease of SUE with latitude, corresponding to a decrease in mean annual temperature, in mineral soil horizons. SUE decreased with decreasing stoichiometric imbalance in the organic and mineral topsoil, while a relationship of SUE with soil C:N was only found in the mineral topsoil. However, contrary to our hypothesis, SUE did not increase with soil depth and mineral subsoils displayed lower average SUE than mineral topsoils. Both within individual horizons and across all horizons SUE was strongly correlated with CBH:POX ratio as well as with climate variables. Since enzyme activities likely reflect the chemical properties of SOM, our results indicate that SOM quality exerts a stronger control on SUE than SOM stoichiometry, particularly in subsoils were SOM has been turned over repeatedly and there is little variation in SOM elemental ratios.

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1. Introduction

A substantial part of soil organic matter (SOM) is of microbial origin, as both plant inputs and microbial products are cycled through the soil microbial community (Miltner et al., 2012; Rumpel and Kögel-Knabner, 2011; Simpson et al., 2007). The carbon (C) taken up by heterotrophic microorganisms is partitioned between biomass production and respiration (del Giorgio and Cole, 1998). This partitioning is described by the microbial carbon use efficiency (CUE, also referred to as microbial growth efficiency (Six et al., 2006), growth yield efficiency (Thiet et al., 2006), or substrate use efficiency (Schimel and Weintraub, 2003)). High CUE therefore increases the amount of microbial products potentially available for storage in soils (Cotrufo et al., 2013). At the same time, high CUE means that more biomass is produced per unit substrate, which may in turn lead to a larger microbial biomass pool and higher rates of SOM decomposition and C mineralisation (Allison et al., 2010; Wieder et al., 2013). The efficiency with which microorganisms can convert available C substrates into biomass is therefore an important factor in determining soil C storage (Xu et al., 2014), and even small changes in CUE can strongly affect model estimates of soil respiration and soil C storage (Six et al., 2006).

While the importance of soil microbial CUE for understanding and modelling soil C cycling and storage is widely recognised (Schimel, 2013), empirical studies investigating its controls across ecosystems and soil horizons are largely lacking. Many biogeochemical models assume CUE to be constant (Manzoni et al., 2012; Sinsabaugh et al., 2013), although studies on aquatic systems, litter, and soil indicate that CUE varies with substrate stoichiometry and chemistry, as well as with environmental conditions, such as temperature and substrate availability (del Giorgio and Cole, 1998; Manzoni et al., 2012; Roller and Schmidt, 2015).

Based on ecological stoichiometric theory as well as litter decomposition studies, CUE in soils is believed to be strongly controlled by the substrate C:nitrogen (N) ratio (Manzoni et al., 2012, 2010, 2008; Sinsabaugh et al., 2016, 2013). Microorganisms need to maintain the stoichiometry of their biomass within physiological boundaries and thus show limited variability in their C:N ratios, i.e. display elemental homeostasis (Cleveland and Liptzin, 2007; Xu et al., 2013; Zhou and Wang, 2015). Ecological stoichiometric theory predicts that microorganisms adjust their CUE in response to substrate imbalances between microbial biomass and substrate C:N ratios (Mooshammer et al., 2014b; Sterner and Elser, 2002), as given by the mass balance equation:

$$CUE = NUE \frac{C: N_{Biomass}}{C: N_{Substrate}} \tag{1}$$

where C:N_{Biomass} is the C:N ratio of the microbial biomass, C:N_{Substrate} is the C:N ratio of the substrate and NUE is the microbial N use efficiency. Similarly to CUE, NUE has been shown to vary in response to substrate stoichiometry and can decrease when N is available in excess relative to C (Mooshammer et al., 2014a). Equation (1) suggests that at low substrate C:N ratios homeostatic microbial communities have high CUE (and low NUE) as microorganisms will be C limited and aim to conserve C. Conversely, when substrate C:N ratios are high, CUE will be low (and NUE high) as excess C is respired through overflow respiration (Larsson et al., 1995; Sterner and Elser, 2002).

For equation (1) to be valid, it needs to be assumed that C assimilation is not limited by the chemical composition of the substrate. However, substrates with similar C:N stoichiometry but with different chemical structure may be converted into biomass with different efficiency. In soils, complex substrates are initially broken down by the activity of extracellular enzymes which can be substrate specific (hydrolytic enzymes) or unspecific (oxidative enzymes). Complex compounds, including phenolic substances such as lignin and humic substances, which require multiple enzymatic steps for decomposition, may be less efficiently converted into biomass (Bosatta and Ågren, 1999). Also, different compounds are assimilated through different

metabolic pathways, which leads to different respiration rates per unit C assimilated (Gommers et al., 1988). Furthermore, C assimilation into biomass is constrained by the chemical energy per unit C, given as the degree of reduction (Manzoni et al., 2012). If the degree of reduction of the substrate is lower than that of the microbial biomass, CUE will remain below a theoretical maximum of approximately 0.8 for the assimilation of individual compounds (Gommers et al., 1988; Roller and Schmidt, 2015). However, Sinsabaugh et al. (2013) have suggested that, when taking the full maintenance costs of microbial metabolism into consideration, the thermodynamic maximum of CUE is around 0.55.

Organic matter chemistry, nutrient status, and productivity of ecosystems are strongly determined by climate and follow latitudinal patterns at a large scale. High latitude ecosystems, such as arctic tundra and boreal forest, display higher soil C:N ratios compared to lower latitudes (Post et al., 1985; Xu et al., 2013). This is attributed to low-quality litter inputs and harsh climatic conditions that limit the activity of microbial decomposers (Hobbie et al., 2000). Substrate properties and nutrient availability also change within soil profiles, since C:N ratios decrease with depth as C is successively respired during decomposition, and the chemical composition of SOM changes from primarily plant-derived to primarily microbial derived compounds (Rumpel and Kögel-Knabner, 2011).

The aim of this study was to investigate changes in microbial CUE in response to changes in C:N stoichiometry across ecosystems as well as within the soil profile. Specifically, we focused on stoichiometric controls of microbial CUE and hypothesized that (i) CUE increases from high to low latitudes with decreasing soil C:N ratios, (ii) this latitudinal effect is less pronounced in the mineral horizons than in the organic topsoil, as environmental fluctuations are attenuated and substrate properties are less dependent on the vegetation, and (iii) CUE increases with soil depth as the C:N of SOM decreases. To this end, we established a 1500-km latitudinal transect through Western Siberia that corresponded to a threefold decrease in organic topsoil C:N ratios. The transect included seven sampling sites and spanned four major biomes: tundra, taiga, forest steppe, and steppe. Soil samples were collected from the organic topsoil, mineral topsoil and mineral subsoil horizons at each site.

Soil samples were incubated with a mixture of ^{13}C-labelled substrates and ^{13}C incorporation was traced into biomass and CO_2 to estimate microbial CUE. While often reported as CUE, such an approach measures the efficiency of the microbial community to incorporate an added substrate and may not fully capture microbial growth and maintenance respiration. We therefore use the term substrate efficiency (SUE) (Sinsabaugh et al., 2013) instead of CUE throughout the manuscript to highlight that for methodological reasons CUE could not be directly measured. This does not compromise, however, the validity of our hypotheses. In addition, we measured soil and microbial C:N stoichiometry to assess possible stoichiometric constraints on microorganisms, and we assessed the potential activities of cellobiohydrolase and phenol oxidase as indicators of the chemical complexity and recalcitrance of the substrates that microorganisms decompose. We expected that with diminishing substrate quality SUE would decrease.

2. Materials and methods

2.1. Site description and sampling

Samples were taken from seven ecosystems along a 1500-km latitudinal transect in Western Siberia that spans a range of climate and vegetation zones, from arctic tundra, to boreal forest to semiarid steppe (Supplementary Fig. 1; see also Wild et al., 2015). Along the transect, mean annual temperature (MAT) displays a near perfect negative correlation with latitude (r = −0.99), that is, MAT increases linearly along the transect from north to south. Mean annual precipitation (MAP) slightly increases from the tundra to the middle taiga and then