Enzymology under global change: organic nitrogen turnover in alpine and sub-Arctic soils

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Abstract
Understanding global change impacts on the globally important carbon storage in alpine, Arctic and sub-Arctic soils requires knowledge of the mechanisms underlying the balance between plant primary productivity and decomposition. Given that nitrogen availability limits both processes, understanding the response of the soil nitrogen cycle to shifts in temperature and other global change factors is crucial for predicting the fate of cold biome carbon stores. Measurements of soil enzyme activities at different positions of the nitrogen cycling network are an important tool for this purpose. We review a selection of studies that provide data on potential enzyme activities across natural, seasonal and experimental gradients in cold biomes. Responses of enzyme activities to increased nitrogen availability and temperature are diverse and seasonal dynamics are often larger than differences due to experimental treatments, suggesting that enzyme expression is regulated by a combination of interacting factors reflecting both nutrient supply and demand. The extrapolation from potential enzyme activities to prediction of elemental nitrogen fluxes under field conditions remains challenging. Progress in molecular ‘omics’ approaches may eventually facilitate deeper understanding of the links between soil microbial community structure and biogeochemical fluxes. In the meantime, accounting for effects of the soil spatial structure and in situ variations in pH and temperature, better mapping of the network of enzymatic processes and the identification of rate-limiting steps under different conditions should advance our ability to predict nitrogen fluxes.

Global change and the soil nitrogen cycle
It is predicted that global warming trends will continue to be most severe in Arctic, sub-Arctic and alpine environments [1]. Moreover, biological processes in these systems are often temperature limited [2,3]. As soils are often close to the freezing point, non-linear effects of temperature around frequent freeze–thaw events can have large impacts [4,5]. Arctic and sub-Arctic biomes constitute a globally significant carbon sink (~30% of the global soil carbon pool) in the form of soil organic matter stored in peatlands and permafrost [6]. If changes to climate affect local biological processes in these ecosystems and consequently modify elemental fluxes, there is a potential for feedbacks to global biogeochemical cycles [7–9]. Understanding the response of these important carbon sinks to higher temperatures is therefore crucial to predicting future biogeochemical cycles and climate.

Carbon storage in soil organic matter depends on the balance between carbon assimilation via primary production, and mineralization via decomposition. Although higher temperatures are expected to increase the rates of both of these processes in the short term, their long-term responses will depend on interactions with other potentially limiting factors such as soil moisture [3,9], oxygen availability [10] and nutrient availability [11] and may also be affected by non-linear irreversible processes such as permafrost melting [12]. Nitrogen availability in particular is expected to constrain the response of primary production and decomposition to global change, given that both are potentially limited by nitrogen availability, particularly in colder biomes [3,13]. Due to the minimal inputs via atmospheric deposition and biological fixation, increased nitrogen supply has been found to relate closely to in situ decomposition rates of soil organic matter in pristine Arctic and sub-Arctic habitats [3,14]. Although plant species-specific factors can complicate models of litter decomposition [15], the close links between carbon and nitrogen cycling underscore the importance of understanding the soil nitrogen cycle when attempting to predict the fate of cold-biome carbon stores in a warming climate.

Plants and microbes, constituting the main sinks for soil nitrogen, are both capable of assimilating low-molecular-mass forms of organic nitrogen such as amino acids [16,17], effectively short-circuiting the nitrogen cycle and leading to minimal levels of complete mineralization of organic nitrogen to ammonia [18]. For this reason, overall nitrogen cycling is regulated by the process of depolymerization, defined as the enzymatic release of labile low-molecular-mass nitrogenous molecules (amino acids, amino sugars and peptides) from the complex forms that make up soil organic matter [19,20].

In contrast to constructed nitrogen budgets, direct measurements of the enzymatic processes that contribute to the total flux provide information about the drivers and modifiers of individual steps in a complex network of
Figure 1 | Simplified schematic diagram of the soil organic nitrogen cycle, including examples of assays available for the quantification of potential rates of each process

<table>
<thead>
<tr>
<th>Soil N Pool</th>
<th>Process</th>
<th>Example Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter</td>
<td>Fragmentation/leaching</td>
<td></td>
</tr>
<tr>
<td>Complex Polymeric N</td>
<td>Proteolysis</td>
<td>Protease</td>
</tr>
<tr>
<td>Peptides</td>
<td>Peptide hydrolysis</td>
<td>Aminopeptidases</td>
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<td>Amino acids</td>
<td>Ammonification</td>
<td>Arginine ammonification, Urease</td>
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<td>Mineral N</td>
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interacting fluxes, information that is useful for gaining a mechanistic and ultimately predictive understanding of soil nitrogen cycling under global change. A range of standardized soil enzyme assays for a large number of reaction substrates has been developed [21,22], which allow the comparison of soil enzymatic potentials across ecosystems [23]. In particular, the use of substrates containing a fluorogenic component have increased sensitivity and lowered detection limits relative to colorimetry-based methods [24]. Figure 1 shows a selection of enzyme assays available for measuring different steps in the pathway from polymeric soil organic nitrogen to mineralization. Unfortunately, soil enzyme assays typically involve incubations of homogenized soil slurries at standardized temperature and buffer conditions with non-limiting amounts of substrate for a specified period of time. As a consequence, their relevance for estimating actual nitrogen fluxes may at times be questionable. In the present paper, we review briefly the experiments that have investigated the effects of global change drivers on soil enzyme activities, with a focus on alpine and (sub-)Arctic environments, so that we can subsequently discuss the challenges for translating enzyme assay data into predictions of soil nitrogen fluxes.

**Global change effects on soil enzyme activities in temperature-limited systems**

Understanding the direct effects of higher temperatures on soil nitrogen cycling requires knowledge of the kinetics and stoichiometry of decomposition processes [25,26] and their responses to temperature. One aspect of this response is the temperature sensitivity of the reaction rate usually expressed as the change in activity per 10°C increase in temperature, $Q_{10}$. Although interpretation of $Q_{10}$ values can be influenced by the temperature range of their application [27,28], they have proven useful in the study of the sensitivity of biological processes to changes in temperature. A study conducted in the Alaskan tundra suggests that carbon-cycle enzymes are more temperature-sensitive than nitrogen-cycle enzymes [29]. This result is supported by experiments with alpine soils that additionally found that enzymes related to hydrolysing labile compounds are more temperature-sensitive than those associated with the breakdown of more stable soil organic matter [30]. Given that many nitrogen-containing compounds are protected by carbon polymers, the responses of soil enzymes associated with both cycles need to be understood: if the differential response of carbon and nitrogen cycles to warming is consistent and long lasting, then non-linear shifts in the stoichiometric balance of soil organic matter cycling, most likely towards enhanced nitrogen limitation, would be expected.

In addition to directly affecting enzyme activities, global warming may also increase nitrogen availability through other pathways. Studies that manipulate temperature in the field have the advantage of combining direct temperature effects on enzyme activities with a range of possible indirect effects mediated by, e.g., longer-term changes in vegetation composition and changes in nutrient supply via litter and grazing. These indirect effects may be even stronger than direct effects of global change drivers (temperature, CO$_2$ and precipitation) [31], and it is therefore surprising that there is a relative lack of enzyme data from this type of study from Arctic and alpine studies, despite the large number of climate manipulation experiments currently underway [32]. Several studies have, however, investigated the effects of increased mineral nitrogen inputs into soils on the dynamics of enzyme...
activities. Originally established to predict ecosystem impacts of anthropogenic increases in nitrogen deposition [33], these studies also provide important insights into the mechanisms of enzymatic regulation of soil nitrogen cycling. In this respect, it is still unclear whether measured soil enzyme activities reflect a response to nutrient deficiency or to availability, in other words, whether enzyme production by the microbial biomass is regulated by nutrient demand or supply. There is evidence available to support both possibilities [22,34], which might be due to the complex enzyme regulation of the soil nitrogen cycle. Chronic nitrogen fertilization of alpine tundra soils reduced leucine-aminopeptidase activities and increased urease activities while not affecting other nitrogen-cycle enzymes [35], whereas in an ombrotrophic bog in Scotland, nitrogen addition reduced potential activities of chitinase and cellulase, and altered the patterns of carbon substrate utilization [36]. Similar complex responses have been found in other systems. Most strikingly, Enowashu et al. [37] found that the activity of different types of peptidases in German spruce forest soils showed opposite responses to changes in mineral nitrogen input. Such disparate responses among enzymes with presumably similar functional roles point to a complex (and hard to predict) regulation of enzyme expression.

Further evidence on the effects of both temperature and nitrogen availability on enzyme activities is given by studies that measured seasonal dynamics, as seasonality can be understood as a driver that integrates simultaneous variance in temperature, moisture, plant and microbial nutrient demand and transport of nutrients into and out of the soil system. Studies that measured seasonal variation in potential enzyme activities have typically found large intra-annual differences [29,34,36], up to 10-fold [36], with such differences often being larger than those detected in response to experimental manipulation of single factors (see above). In alpine, Arctic and sub-Arctic environments, the highest enzyme activities seem to coincide with the period of microbial and nutrient turnover around the spring thaw, during which frequent soil freeze–thaw cycles cause rapid drastic shifts in the soil physiochemical conditions [4], with consequences for the soil microbial community structure and associated functions [38].

This implies that global change effects on enzyme activities are: (i) likely to involve interactive (i.e. non-additive) effects of changes to temperature and nutrient supply, and (ii) be mediated through changes to nutrient demand and supply both within the soil organic matter decomposing community, as well as in the associated vegetation [39].

Extrapolating enzyme measurements to flux predictions: the missing link

Soil enzyme activity measurements are useful only insofar as they allow us to predict elemental fluxes and, e.g., their responses to global change. Despite this, studies that explicitly link enzyme measurements to measurements of fluxes in the same experimental units are rare (e.g. [40,41]) and, to our knowledge, non-existent in (sub-)Arctic and alpine environments. To illustrate what this link may look like, we present a synthesis of data from alpine [30] and Arctic tundra [34,42] in Figure 2. The measured activities of some enzymes were correlated with flux rates, i.e. nitrogen mineralization with xylanase activity ($r^2 = 0.58$, $P < 0.05$) and β-glucosidase ($r^2 = 0.52$, $P < 0.05$), and carbon mineralization with protease activity ($r^2 = 0.94$, $P < 0.05$). However, not all enzyme activities seem to translate into elemental fluxes, as with nitrogen mineralization against protease activity (Figure 2B) and carbon mineralization against β-glucosidase and xylanase in Figure 2(A) (i.e. none of these correlations were significant). This latter result is all the more surprising as β-glucosidase activity is traditionally considered to be a reliable proxy of soil microbial activity [43].

Connecting potential enzyme activities, as reflected by enzyme assays, to actual enzyme activities in situ and, more crucially, realized elemental fluxes requires clarifying the influence of some important complicating factors. For instance, soil processes are spatially structured at multiple scales [18]. Diffusion of enzymes and substrates within and between microhabitats [44] could potentially limit soil enzyme activities in ways that are not detectable by traditional soil enzyme assays that incorporate a homogenization step, particularly given the potentially important role of enzymes stabilized on soil particles [21]. Furthermore, enzymatic processes are sensitive to solution pH and temperature conditions. Standardized assay conditions, although useful for facilitating cross-system comparisons [23], may lead to under- or over-estimates of in situ potential rates [24]. Moreover, given that temperature sensitivities have been shown to vary considerably between enzymes and across seasons [29,30], extrapolations from assay data collected at a single temperature are potentially unreliable.

Probably the most serious factors complicating translation of potential enzyme activities to fluxes are the two (interrelated) problems of (i) the unclear role of substrate availability, and (ii) the complex multi-step pathways of enzymatic intermediate reactions, which ultimately lead to elemental fluxes. As mentioned above, it remains uncertain whether enzyme production is induced by nutrient deficiency (demand driven) or by availability (supply driven) [22,34]. This makes using potential activities as a guide for flux predictions problematic, as realized fluxes are a function of the actual substrate supply rate, as well as potential activity. If enzymes are produced in response to nutrient limitation, then assays that measure potential activities will have an inverse relationship with real fluxes in the field. Indeed, experimental approaches give support for an ‘economic’ or demand-driven model of enzyme regulation [45], and this has also been incorporated into a modelling framework [46]. Further investigation into this problem across a range of ecosystems will be an important step towards allowing us to make well-informed links between enzyme measurements and field-level nitrogen availability, particularly given the interaction between the latter and other global change factors.
Similarly, the complex multi-step pathway from polymeric organic nitrogen to mineral nitrogen (Figure 1) implies that measurements of the activity of a simple step in a complex network will not be sufficient for predicting total flux rates. Indeed, multiple soil nitrogen-cycle networks are possible, each with different dominant nitrogen forms and different critical rate-limiting steps [18,19]. Given the multiple, indirect effects of global change drivers, it is conceivable that they can alter the relative importance of different nitrogen-cycle pathways, and therefore the critical rate-limiting enzymatic processes determining elemental flux rates. All this argues for simultaneous measurements of enzymes in different positions of the nitrogen-cycling network [37] (Figure 1) and further research into how global change alters the different pathways in such soil nitrogen flux networks [19].

Ultimately, the effects of global change on the soil nitrogen economy will always be filtered through the soil microbial community, the proximate driver of soil enzyme pool sizes and elemental fluxes [21,22]. Whether this filtering is best examined at the level of the composition of the soil microbial community (as detectable via metagenomics [47]), their transcriptional activity (metatranscriptomics [48]), their enzyme production (proteomics [49]) or through soil enzyme assays remains an open question, although a combination of approaches will probably be the most fruitful approach. Molecular tools to predict enzyme activities have therefore been strongly advocated [21,22]. Indeed, in the long run, molecular methods are likely to offer considerable explanatory power in understanding soil community function once it has been established to what extent microbial communities are functionally and kinetically redundant [50], and once bioinformatic tools are sufficiently advanced to reliably predict functional characteristics of communities from environmental sequence data [22,47].

Given the current state-of-the-art, we propose that a greater focus on the direct effects of environmental conditions and resource supply rates on elemental fluxes, and the enzyme processes that underlie them, will help to provide important information in the short term that should lead to considerable advances in the understanding of the global change effects on soil biogeochemical cycling.

Figure 2 | Relationships between measured enzyme activities and carbon and nitrogen mineralization rates as reported in (A) Koch et al. [30] and (B) Weintraub and Schimel [34,42].

Closed symbols and continuous lines, carbon mineralization (C min); open symbols and broken lines, nitrogen mineralization (N min). For (A), each data point represents a season x soil combination (summer wet fen soils excluded from linear trend line). Triangles and grey lines, xylosidase (Xylo) activity; squares and black lines, β-glucosidase (Beta-gluc) activity. For (B), each point represents four separate soil types; protease activity was calculated as cumulative potential activity by interpolation. See the text for further statistical information.
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References


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