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Linkages between extracellular enzyme activities and the carbon and nitrogen content of grassland soils

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ABSTRACT

Important biochemical reactions in soils are catalyzed by extracellular enzymes, which are synthesized by microbes and plant roots. Although enzyme activities can significantly affect the decomposition of soil organic matter and thus influence the storage and cycling of carbon (C) and nitrogen (N), it is not clear how enzyme activities relate to changes in the C and N content of different grassland soils. Here we address whether the activity of C-acquiring (β-1,4-glucosidase, BG) and N-acquiring (L-leucine amino-peptidase (LAP) and β-1,4-N-acetyl-glucosaminidase (NAG)) enzymes is linked to changes in the C and N content of a variety of human-managed grassland soils. We selected soils which have a well-documented management history going back at least 19 years in relation to changes in land use (grazing, mowing, ploughing), nutrient fertilization and lime ($CaCO_3$) applications. Overall we found a positive relationship between BG activity and soil C content as well as between LAP + NAG activity and soil N. These positive relationships occurred across grasslands with very different soil pH and management history but not in intensively managed grasslands where increases in soil bulk density (i.e. high soil compaction) negatively affected enzyme activity. We also found evidence that chronic nutrient fertilization contributed to increases in soil C content and this was associated with a significant increase in BG activity when compared to unfertilized soils. Our study suggests that while the activities of C- and N-acquiring soil enzymes are positively related to soil C and N content, these activities respond significantly to changes in management (i.e. soil compaction and nutrient fertilization). In particular, the link between BG activity and the C content of long-term fertilized soils deserves further investigation if we wish to improve our understanding of the C sequestration potential of human-managed grassland soils.

1. Introduction

Soil ecosystems represent the largest terrestrial reservoirs of carbon (C) and nitrogen (N) and could act either as sources or sinks of C and N depending on the net effect of multiple environmental factors including soil management by humans (Post and Kwon, 2000; Lal, 2004). Changes in the storage and cycling of C and N are strongly dependent on the rate of decomposition of organic detritus returned to soils and the incorporation of C and N into stable soil organo-mineral fractions. Soil organic matter (SOM) decomposition is a predominantly microbial-mediated process whereby soil microorganisms produce a variety of extracellular enzymes, which serve a dual function: (1) to catalyze a set of chemical reactions involved in the degradation of SOM, and (2) to acquire the necessary energy and nutrient resources for the enzyme producers (Sinsabaugh et al., 2009; Brzostek and Finzi, 2011; Wallenstein et al., 2011). Enzyme activities in soils represent key biological processes that link the quality of SOM (e.g. the relative availability of C and N) with the ability of microbes to assimilate nutrients and use C for their own metabolism (Allison et al., 2011). This suggests that shifts in C- or N-acquiring enzyme activities may be linked to changes in the availability and/or storage of C and N within SOM pools.

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Recent studies show that soil enzyme activity is associated with changes in SOM concentration across different terrestrial ecosystems (Sinsabaugh et al., 2008; Trasar-Cepeda et al., 2008; Stursová and Baldrian, 2011; Wallenius et al., 2011). Despite this evidence, our mechanistic understanding of whether shifts in the activity of C- or N-acquiring enzymes might be linked to changes in soil C and N content is still limited. Greater SOM accumulation can lead to increases in the activity of extracellular enzymes ‘simply’ by providing a wider range of C and N substrates that can be accessed and utilized by a variety of soil microbial groups. In addition, increased stratification of SOM can lead to higher spatial heterogeneity of the soil matrix, which in turn can be associated with a larger number of niches available to microbial enzyme foraging (Allison, 2005).

Changes in microbial enzyme activities in response to land management practices may themselves actively influence SOM concentration and thus soil C and N content. For example, the stimulation or repression of C- or N-acquiring enzymes could influence rates of SOM decomposition (Burns et al., 2013). Furthermore, once released into the soil, these extracellular enzymes may accumulate, adsorb and stabilize onto mineral surfaces thus contributing to increases in the C and N content of SOM (Kandeler et al., 1999; Cotrufo et al., 2013). When enzymes are sorbed to mineral surfaces they become less effective in degrading C and N compounds thus contributing to greater C and N accumulation in mineral soil pools (Allison and Jastrow, 2006; Grandy et al., 2008).

The main goal of our study is to address whether and how changes in soil C and N content correlate significantly with changes in the activity of C- and N-acquiring enzymes in grasslands characterized by very different human management histories. Based on previous studies (Sinsabaugh et al., 2008; Trasar-Cepeda et al., 2008; Stursová and Baldrian, 2011; Wallenius et al., 2011), we hypothesize that soil C and N content should be positively related to the activity of C-acquiring and N-acquiring enzymes in most grassland soils. In particular we hypothesize the existence of a positive feedback between soil C (or N) content and the activity of the C-acquiring (or N-acquiring) enzyme. Because we included in our analyses soil types characterized by very different pH values and because soil pH can strongly influence both enzyme activity (Turner, 2010) as well as soil C and N (Fornara et al., 2011) we also ask whether enzyme activities might relate to changes in C and N content across soils with different pH values.

Secondly, we ask whether and how past and current management practices (i.e. management history) have influenced the relationship between enzyme activity and soil C and N content. Here we compare two sets of grasslands, one of which has been historically managed as permanent grassland and the other, which had been regularly ploughed until the 1960s. We also compare permanent grasslands, which are distributed along a gradient of use intensity (i.e. management intensity) as determined by livestock density, nutrient fertilizer and liming applications (i.e. $CaCO_3$).

Finally, we compare grasslands, which have received long-term applications of N (i.e. nutrient fertilization), asking how the increased availability of N relative to C has ultimately influenced the activity of C- and N-acquiring enzymes as well as the relationship between enzyme activities and the C and N content of these grassland soils.

2. Methods

We selected grassland soils, which had a clear history of human management and are established across three European regions. Our aim was to select soils that have been undergoing specific treatments (e.g. nutrient fertilization, grazing, liming) for ≥19 years, as follows.

2.1. Nash's field (England)

Nash's Field is a long-term grassland experiment that was set up in 1991 at Silwood Park, Berkshire, England. This area is characterized by a temperate oceanic climate with average annual temperature of 9.6 °C and annual precipitation of 754 mm (www.climatedata.eu). Nash's Field is a species-poor grassland lying on acid, sandy soil. The experiment is a split-plot design, which includes large (randomly distributed) blocks (each 900 m^2) within which are nested multiple experimental treatments (see Edwards and Crawley, 1999). In our study we specifically tested for the effects of (1) rabbit grazing (±rabbit fencing), (2) liming (±lime, i.e. $CaCO_3$), and (3) nutrient treatments (±N, P, K and Mg fertilizers nutrients) on enzyme activities and soil C and N content. Rabbit grazing was excluded by using 1 m high fences with wire mesh (3 cm diameter mesh size). Each fenced and grazed plot (400 m^2) was divided in two equal plots, one of which was randomly selected to receive lime applications (5 tons $CaCO_3$ ha^{-1}) and the other not. Each of the limed and non-limed plots (144 m^2) was then split into subplots (4 m^2) for the application of five different nutrient combinations (N-only, P-only, N and P together, all nutrients-N, P, K and Mg) plus a control plot without any nutrient treatment. Mineral nutrients have been applied yearly with the following concentrations: 100 kg ha^{-1} of N (as ammonium nitrate- NH_4NO_3), 35 kg ha^{-1} of P (as triple superphosphate), 225 kg ha^{-1} of K (as muriate of potash) and 11 kg ha^{-1} of Mg (as Epsom salts). Total atmospheric N deposition is estimated as ~22 kg N ha^{-1} y^{-1} (www.apis.ac.uk). In summary, the experiment has 4 large blocks (each 900 m^2), 8 rabbit grazing plots (each 400 m^2), 16 liming plots (each 144 m^2), and a total of 80 nutrient plots (each 4 m^2), which were sampled in 2011. Four soil cores (3 cm diameter) were collected from each nutrient plot between 0 and 20 cm soil depth.

2.2. Lautaret (France)

The Lautaret experimental site is located in the Central French Alps on the south-facing slope of the upper valley of the Romanche River (Villar d'Arène, 45°03′N, 6°24′E). Winters are cold and snowy (mean February temperature of −7.4 °C), summers are cool (mean July temperature of 13 °C), while average annual precipitation is 956 mm (see Lavorel et al., 2007, 2011 and Robson et al., 2007 and for more details). The total area is 13 Km^2 and the elevation ranges between 1552 and 2500 m a.s.l. Historical land use trajectories, representing combinations of past and present land use were defined based on historical records and aerial photography (Quétier et al., 2007). Former arable fields (1650–2000 m) were converted to grasslands fifty years ago and are currently used for hay or grazing by sheep or cattle; former (never ploughed) hay meadows (1800–2000 m) are either still mown or grazed by sheep; summer grasslands (>2000 m) have remained under pastoral use over history. In this study we investigated 5 trajectories: three representing previously (until the 1960s) cultivated terraces (1) currently fertilized (mainly animal manure) and mown, (2) mown, (3) unmown and grazed in spring and autumn, and two representing never cultivated (never ploughed) permanent grasslands with a multi-century history of mowing (4) currently mown, and (5) unmown (for > 30 years) and summer grazed. Applications of organic nutrient manure generally occur between May and June each year and on average correspond to the addition of 8 kg N ha^{-1} yr^{-1} (Robson et al., 2007). Here we randomly sampled 2 large grassland replicate plots under each of the five land use trajectories. The plots measured 15 × 15 m and within each we