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Soil exo-enzyme activities across Europe—The influence of climate, land-use and soil properties

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ABSTRACT

The assessment and monitoring requirements of soil quality have been debated for many years at European and Global scales. To monitor the activity and diversity of microbial communities a number of methods have been applied including the activity of extracellular soil enzymes. Here is the activity of eight hydrolytic extracellular enzymes on 79 sites across Europe measured, the sites are from five different climatic zones with three different land-uses, and they vary in physicochemical characteristics. The results show that the activity of the enzymes primarily depends on soil organic matter and to a lesser extent on pH, while the activities were not related to climate or land-use. Sites were selected to represent a broad spectrum of key soil properties across three different land-uses and five different climatic biogeographical zones across Europe, so the overall conclusion is based on the analysis of soils representing soil characteristics across Europe.

1. Introduction

The assessment and monitoring requirements of soil quality have been debated for many years at European and Global scales (Doran and Zeiss, 2000; Van Bruggen and Semenov, 2000; Schloter et al., 2003). At the level of the European Union such discussions resulted in a proposal for a Soil Framework Directive (COM/2006/0232). In this proposal the loss of soil biodiversity is recognised as being of great importance as it is essential for ecosystem functioning and it is highlighted that it contributes to primary productivity, decomposition of organic matter, nutrient cycling, water infiltration, creation of soil aggregates, stability of soil structures, dynamics of the soil food web and suppression of pests. The microbial community is considered the main driver of most of these soil functions (Bååth and Anderson, 2003).

To measure the activity and diversity of the microbial community a number of methods can be applied, to cite a few (i) catabolic activity investigated by Biolog-plates (Rutgers et al., 2016), (ii) respiration of different substrates as investigated by the MicroResp method (Campbell et al., 2003; Creamer et al., 2016) and (iii) activity of extracellular soil enzymes. Soil enzymes originate from a variety of organisms, notably fungi and bacteria. Hydrolytic extracellular enzymes are of importance for the decomposition of many labile organic substrates, thus they have pivotal importance for biogeochemical cycling. Their activity reflects the functional diversity and activity of the microorganisms involved in decomposition processes (Sinsabaugh et al., 2008) which are essential processes for soil functioning and soil ecosystem services. The activity of hydrolytic exo-enzymes extracted from soils are often quantified by the use of fluorescent model substrates (German et al., 2011). They often include; (i) β-1,4-glucosidase and cellobiohydrolase which are enzymes which contributes to the degradation of cellulose; the principal function of β-1,4-glucosidase is hydrolysis of cellobiose to glucose, while cellobiohydrolase hydrolyses cellobiose dimers from the non-reducing ends of cellulose molecules; (ii) α-1,4-glucosidase which contributes to the degradation of starch, specifically it hydrolyses terminal non-reducing 1-4 linked alpha-glucose residues to release glucose molecules; (iii) β-*N*-acetyl-glucosaminidase which plays a role in the degradation of chitin analogous to the β-1,4-glucosidase in cellulose degradation, (iv) β-1,4-xylosidase which contributes to the degradation of the hemicellulose xylan by removing successive D-xylose residues from its non-reducing end; (v) aminopeptidases, such as alanine-aminopeptidase and leucin-aminopeptidase, which hydrolyses hydrophobic amino acids from the N terminus of polypeptides. Other classes of aminopeptidases exist, however analysis of soil samples generally show the greatest activities towards the substrates presented in this paper, so they are broadly

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used as an indicator of the peptidase potential (Stursova et al., 2006); (vi) phosphatases which hydrolyse phosphomonoesters releasing phosphate and (vii) arylsulphatases which hydrolyse esterbonds of aryl-sulphate-esters releasing sulphate, and are considered a valid measure for sulphur mineralisation in soils (Castellano and Dick, 1991).

The aim of this paper is to assess the potential activity of the above mentioned eight hydrolytic extracellular enzymes on 79 sites from five different climatic zones with three different land-uses of varying physicochemical characteristics across Europe. The specific objective was to determine the range of potential activity of eight hydrolytic extracellular enzymes of European soils, how these vary according to the climatic zones and land-use and how they are influenced by soil properties and microbial respiratory activity.

2. Materials and methods

Soil samples were collected from a transect across Europe (Stone et al., 2016). The transect sites were selected within the European Union and included all major biogeographical (BG) zones, three land-uses (grassland, forestry, tillage) and a spectrum of soil physical and chemical properties that varied in organic carbon, pH and texture. Full details of the development of the site selection model and sampling can be found in Stone et al. (2016). In brief, soil was sampled from each site following a pre-agreed standard operating procedure, guaranteeing that all sites were sampled in exactly the same fashion. Soil was taken from the top 5 cm of the profile by the use of plastic cores. The cores were sent to a central handling station in Ireland, stored and transported at 4 °C, employing the use of polystyrene boxes and freezer blocks to maintain temperature. Upon immediate receipt of the samples, soil was removed from the cores and hand mixed. The mixed soils was sieved to 2 mm and a sample sent to Denmark, still at 4 °C, at which temperature they also were stored until analysis within a few days. In the present study 79 of the 81 sites in the transect were analysed, the sites not included were Portugal 1 and Slovenia 2.

Environmental variables included in the analysis were: organic carbon content, total N, cation exchange capacity, base saturation, pH, texture and water holding capacity as Stone et al. (2016).

Climate data were obtained for each site using the WorldClim global climate dataset (Hijmans et al., 2005). Specifically, the Bioclim variables summarising monthly precipitation and temperature into 19 meaningful biological variables were used. The latitude and longitude for each site were used to extract values for all Bioclim variables with a 30-arc second resolution. Bioclim files were downloaded, and data were extracted using the Raster package in R.

Extracellular enzyme activities (EEA) in the soils were determined on 8 different fluorogenic model substrates related to the hydrolysis of *O*-glycosyl linkages of five di- and poly-saccharides including starch, cellulose, hemicellulose and chitin, ester linkages of organic phosphates and sulfates and peptide linkages of proteins: 4-methylumbelliferyl (MUF) α-D-glucoside, MUF-β-D-glucopyranoside; MUF-β-D-cellobioside, MUF-β-D-xylopyranoside, MUF-*N*-acetyl-β-D-glucoseamide, MUF-sulphate, MUF-phosphate and L-alanine-7-amido-methylcoumarin (AMC). All substrates were provided by Sigma–Aldrich, Denmark. The assay was performed in microtiter plates essentially as described by Johansen et al. (2005). The enzymes were extracted from the soil by placing 10.0 g samples diluted in 100 ml sterile Milli-Q water in an ultrasonic water-bath (Branson 5210, Branson Ultrasonics, UK) for five minutes followed by five minutes of multi-wrist shaking (Model 3589-1, Lab-line instruments, Claus Damm, Denmark) at speed 5. After centrifugation for 10 min at 100 × *g* at 4 °C 200 μl of the supernatant was added to individual wells of a black microtiter plate (Thermo Scientific, Denmark) along with MOPS buffer (pH 7.4) and the MUF- and AMC-substrates (final concentration 50 μM). The fluorescence derived from the liberated substrates was measured during 90 min at intervals of 10 min at 30 °C by a Chamaeleon multilable detection platform (Hidex OY, Finland) with automated shaking before each measurement. The EEAs were estimated by means of linear regression analysis of fluorescence intensity related to time and transformed to μM h^{-1} g dry $soil^{-1}$ by relating the fluorescence intensity to standard calibration curves of MUF or AMC for each soil sample and calculated for each time point; the first time point after 10 min was omitted from the analysis. All soils were analysed in triplicate.

Data of basal soil respiration were extracted from the MicroResp assay without addition of carbon substrate as reported by Creamer et al. (2016).

Data analysis was performed by XLSTAT 2015 statistical software for Excel (Addinsoft, US). CANOCO for windows 4.5 was used to perform canonical correspondence analysis, Monte Carlo Permutation test using 499 permutations was applied to assess the statistical significance of the environmental variables.

3. Results

EEA for eight different hydrolytic enzymes were quantified for 79 soils across Europe. The soils were sampled across five biogeographical zones (Alpine, Atlantic, Boreal, Continental and Mediterranean) representing three land-use categories (arable, forestry and grassland) with a broad spectrum of soil properties varying considerable in organic carbon and nitrogen content, pH and texture (Stone et al., 2016). The results are summarized in Table 1. The mean EEA across all 79 soils vary between 1.18 μM MUF h^{-1} g dry $soil^{-1}$ for arylsulfatase and 58.21 μM MUF h^{-1} g dry soil for the phosphomonoesterase; for the alanine-aminopeptidase the mean activity is 58.21 μM AMC h^{-1} g dry soil. For the rest the mean activities for α-glucosidase < cellobiosidase < β-xylosidase < chitinase < β-glucosidase. The variability of each enzyme activity, is presented as the coefficient of variation, which is lowest for the alanine-aminopeptidase and highest for the chitinase, which are considerably higher than found for the other enzyme activities. Analysis of all eight enzymes reveals that they significantly ($p < 0.0001$) deviate from the null-hypothesis that the different soils do not bring information to the model. Tukey's HSD test applied to all pairwise differences between means for all eight activities, revealed that they could be divided into a varying number of groups with significant differences between the means varying between 8 for β-xylosidase and 26 for alanine-aminopeptidase (Table 1) ($p < 0.05$). All the enzyme activities are statistically significant and positively correlated (Table 2), the most significant correlation exist notably between the enzymes with activity towards carbohydrates except chitinase, while arylsulfatase and alanine-aminopeptidase show the poorest correlation with the

Table 1
Potential activity of eight extracellular soil enzymes across 79 sites in Europe.

Enzymatic activities	Min.	Max.	Mean	Dev.[a]	Coeff.[b]	Groups[c]
Arylsulfatase	0.07	6.37	1.18	0.97	0.83	14
α-Glucosidase	0.42	44.14	4.33	5.25	1.21	12
β-Glucosidase	5.16	408.99	33.51	49.23	1.47	19
Cellobiosidase	0.27	38.51	4.77	5.76	1.21	10
β-Xylosidase	0.70	50.54	6.30	8.39	1.33	8
Chitinase	1.45	453.64	24.03	72.48	3.02	7
Phosphomonoesterase	9.56	425.53	58.21	55.63	0.96	19
Alanin aminopeptidase	4.42	155.38	46.12	32.59	0.71	26

[a] Standard deviation of the mean.
[b] Coefficient of variation (standard deviation/mean).
[c] Number of groups with statistical significant differences between means.