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Effect of different organic acid on their enantiomers on proteolytic property of soil

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ABSTRACT

Proteases constitute the major biochemical machinery of soil in nitrogen mineralization and degradation of biochemical inputs from various resources. It has been widely studied that proteases are stimulated or inhibited by variety of these anthropogenic inputs and their derivatives to the soil which in turn affect various cycles. Especially, amino acids and mineral nitrogen forms are known to inhibit or stimulate soil proteolysis via up-regulation of proteolytic genes and alteration of microbial C-metabolism. In this work we have proved significant ($P<0.05$) stimulation of inhibition of casein-protease activity by non-protein amino acids (ornithine, citruline and β-alanine) being soil and concentration dependent, probably due to their effect on availability of soil nutrients. More research is necessary to understand the effect of naturally occurring amino acids in soil with respect to different type of ecosystems.

Keywords: Proteases, amino acid, forest soils, pollutants in soil.

INTRODUCTION

Soil acts as receptor, processor and responder to all classes of anthropogenic inputs, which keeps changing with technological advances. Soil biochemical machinery (viz. proteases) reacts differently to industrial nanotechnological waste [1], transgenic inputs [2], LMW organic compounds [3], antibiotics [4] and pollutants [5]. Proteases constitute major class of enzymes in soil for nitrogen (N) mineralization [6] and decomposers of different materials [7]. Aliphatic organic acids play a role in complexation and increasing availability of nutrients and heavy metals in soils [8, 9].

Different methods have been reported to assay protease activity. They involve incubation of soil samples with a non-specific substrate in buffer with or without toluene; incubated at fixed temperature for a definite period of time [10, 11, and 12]. It can also be pre-incubation of soil using different proteins, with consequent measurement of protease activity against Na-caseinate [11]. In addition to non-specific substrates (Na-caseinate), other low molecular weight substrates, including peptides derivatives are used [13]. In some methods both potential and native (sometimes termed as actual proteolysis) protease activities are measured [6, 17]. Actual protease activity is measured under field temperatures with the use of Na-citrate buffer to adjust pH to real soil pH and the application of toluene as a microbial uptake inhibitor without the addition of any exogenous substances. Rejsek et al. [12] measured potential and

native protease activity with pH close to soil pH. Wanek et al. [14] developed a method based on a ^{15}N isotope dilution technique. The assay involves labelling of amino acids occurring in free status in litter and the measurement of ^{15}N: ^{14}N ratios in the individual amino acids by GC-MS over time.

MATERIALS AND METHODS

Soil samples were collected from the Ah horizon of (N 49°30', E 18°32', 825-860 m a.s.l., Gleyic Luvisol, site "Bily Kriz" in Moravian-Silesian Beskids Mountains) and the Oe horizon,(Haplic and Entic Podzols) located approx. 900 m from the experimental meadow. Other samples were collected in old-aged mixed stand of deciduous trees prevalence (N 49°19´, E 16°40´, Rendzic Humic Leptosol) from the Oe, Ahk and Bwk horizons, (N 49°17´, E 16°38´, Haplic Cambisol) from the Ah and Bw horizons sites in Training Forest Enterprise Masaryk Forest Křtiny. Subsequently soils were sieved through 5 mm and stored at 4 ºC till the analyses (see Table 1 for the properties of soils).

Table 1. Selected physical and chemical properties of tested soils.

Plot	Fig. abbr.	C_{ox} (%)	N_t (%)	C/N	pH/H_2O	pH/$CaCl_2$[1]	Clay (%)	Silt (%)	Sand (%)
Meadow (Ah horizon)	1	4.8	0.29	16.4	4.1	3.3	18.6	26.0	55.4
Spruce stand (dense, Oe horizon)	2	23.1	1.01	22.8	4.1	3.4	-	-	-
Spruce stand (thinned, Oe horizon)	3	27.3	1.21	22.5	4.1	3.4	-	-	-
Deciduous forest (old age, Oe horizon)	4	26.2	2.00	13.1	7.7	7.2	-	-	-
Deciduous forest (old age, Ahk horizon)	5	11.1	0.97	11.4	7.5	7.1	12.5	65.0	22.5
Deciduous forest (old age, Bwk horizon)	6	2.2	0.21	10.5	7.1	6.6	23.2	60.3	16.5
Deciduous forest (middle age, Ah horizon)	7	4.3	0.29	14.8	5.1	3.8	6.8	50.0	43.2
Deciduous forest (middle age, Bw horizon)	8	1.1	0.07	15.7	4.7	3.7	26.5	36.0	37.4

[1] $pH/_{0,01\ M}\ CaCl_2$

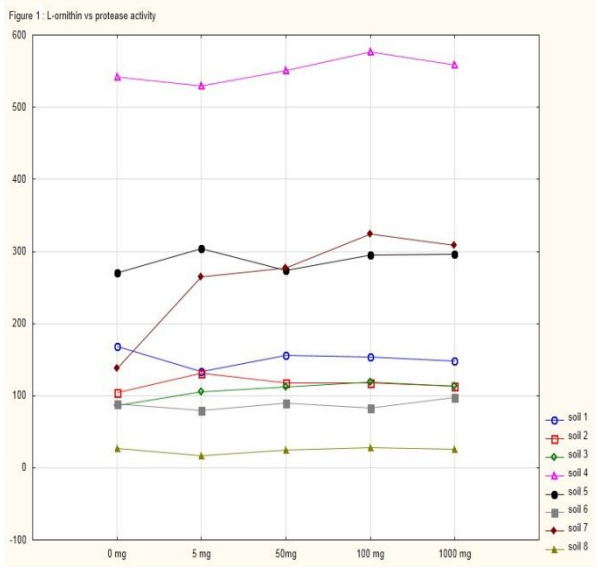
1g of wet soil was treated by L-ornithine, L-citruline, L-β-alanine in concentrations of 0, 5, 50, 100 and 1000 µg g^{-1}. Casein-protease activity was measured according to method described in work of Rejsek et al. [12]. Treated soils were incubated with 0.05 M Tris-HCl buffer (pH 8.5) and 1 % casein (sodium salt, C-8654, Sigma) solution (pH 6.7) at 50 ºC for a period of 2 h. Control samples were incubated by the same way as described above, except that incubations were performed without 1 % casein which was supplied into reaction mixture at the end of incubation. The reaction was stopped by 17.5 % trichloroacetic acid, after centrifugation, 1 ml of supernatant was mixed with 3.7 % Na_2CO_3, 0.06 % $CuSO_4$ and Folin-Ciocalteau reagent (diluted 1:3). Concentration of aromatic acids released by casein-cleavage and expressed by L-tyrosine equivalents was measured colorimetrically at 578 nm.

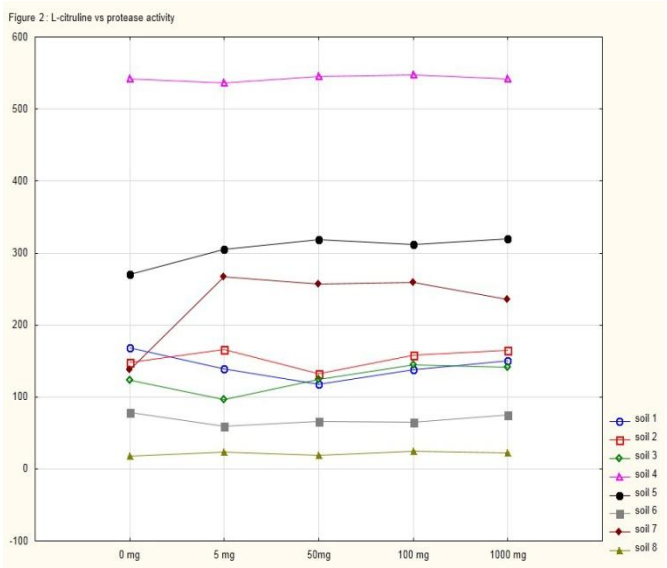
Statistical analysis

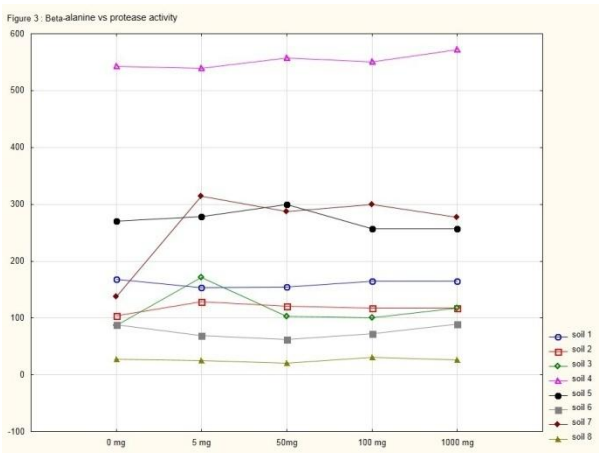
Statistical analysis was performed using (Statistica 9.0) and the values were compared using kruskal Wallis test. A P-value of ≤ 0.05 indicates statistical significance.

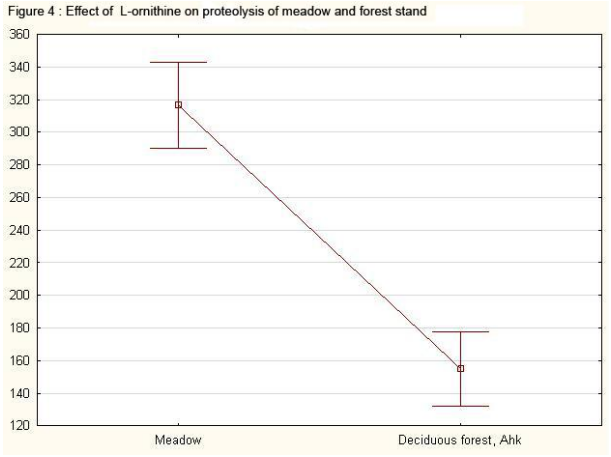
RESULTS AND DISCUSSION

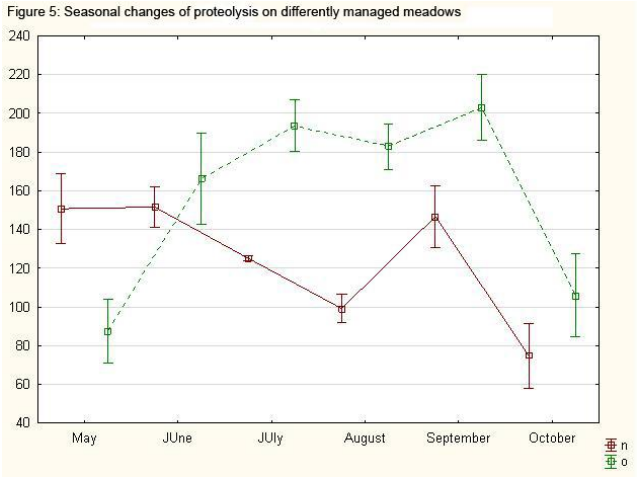
Results of this work indicate majority of soils single peak of protease activity was observed (Fig. 1-3). In few cases zero concentration was found to be showing maximum activity. This can be explained as addition of amino acids caused inhibition of proteases and curve reflects gradual inhibition with increase in concentration. In another cases after a short decrease sudden increase was observed which might be due to effect of amino acids on enzyme inhibitors [16] (Fig. 1-3). β-Alanine one secondary peak along with a primary peak was also observed, which can be single case of concentration dependent stimulation (Fig. 3). In soils from Bw horizons (Fig. 1-3) effect of addition of amino acids was observed to be least in magnitude, which may be due to change in soil properties with depth. Especially texture can play a significant role. Generally, fine-textured soil, with sied soil, with significantly higher total C and N, are known to pose significantly greater protease activity, when compared with coarse-textured soils; however, many aspects of soil protease activity and its limitation by protein availability [15], stimulation by nitrogen deficiency [16, 17], inhibition by N deficiency [18, 19, 20] and control by soil pH [21] remain unclear. In the works we have also compared the effect of 5 mg g^{-1} soil of L-ornithine on proteolysis of meadow and forest stand in the same horizon (A) (Fig. 4) where meadow Ah horizon proteolysis was less inhibited, probably due to qualitatively different proteolytic pool action to non-specific substrate such as casein, and high proportion of plant root proteases [22]. Further, seasonal changes of proteolysis in course of vegetation season on differently managed meadows of the same soil we measured showing high seasonal variability (Fig. 5).











APPLICATIONS

The information collected can be useful in predicting the fate of various biochemical compounds in the soil and their possible impacts on soil proteases. This data can be additionally useful for the assessment of effect of organic acids and their analogues on N mineralization.

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