SHORT COMMUNICATION

Impact of Land-use Change on Proteolytic Activity of Mountain Meadows

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Abstract: Casein-protease activity assessed at 50°C and with adjustment of optimum pH conditions (PA), and casein-protease activity near soil pH and at field soil temperature (LPA) were studied one vegetation period in mountain meadow soils covered with moderately mown vegetation, and over which vegetation had been abandoned for thirteen years. PA peaked in the first part of the vegetation season whereas LPA increased throughout the season; in addition, LPA was not linearly related to temperature (r = 0.127 resp. 0.312; $P > 0.05$). The combined effect of field soil temperature and pH decreased a casein-protease activity by > 98.4%. A management of meadows had no significant ($P > 0.05$) effect on PA and LPA.

Keywords: mountain meadows; management; casein-protease; soil; temperature

Socioeconomic changes have strongly influenced land use in mountainous areas of Europe. Formerly managed meadows and pastures of the Alps and mountainous areas of Central Europe have been abandoned and are now being recolonised by shrub and tree species (the CARBOMONT project "Effects of land-use changes on sources, sinks and fluxes of carbon in European mountain areas", 5FP EU). These changes are accompanied by an alteration of plant-available nitrogen released in soil (e.g. Formánek *et al.* 2008). Bioavailability of N is, to a certain extent, limited by activity of soil proteases (Asmar *et al.* 1994) which are thought to be of microbial origin (e.g. Tjalsma *et al.* 2004). Apart from other factors, the activity of soil proteases is usually limited by a concentration of proteins, a temperature and a soil pH (Weintraub & Schimel 2005; Rejsek *et al.* 2008).

A protease activity in soil is commonly measured (i) under high temperatures, (ii) with unlimited substrate (e.g. casein), and (iii) with adjustment of pH (e.g. Dilly *et al.* 2007). Furthermore, e.g. Weintraub and Schimel (2005) describe assessments of "native" protease rate, i.e. without an addition of exogenous substrate.

The presented study focused on an estimation of the effect of abandonment of mountain meadows on (i) proteolytic activity (PA) at high temperature

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Table 1. PA and LPA in the mountain meadow soil with moderately mown vegetation, and vegetation abandoned for thirteen years (mean ± SD; n = 2–6); the temperatures used for measurement of LPA were as follows: 12.5°C (May), 16.4°C (July), 14.9°C (August) and 14.4°C (September); the values in the table are reported in μg L-tyr equiv/g DM/h; no significant (P > 0.05) differences between the meadows were found

Casein-protease	Treatment	May	June	July	August	September
PA	moderately mown	220.61 ± 14.09	195.41 ± 2.35	215.53 ± 36.19	137.93 ± 42.62	120.51 ± 49.33
	abandoned	201.90 ± 10.14	298.74 ± 7.02	263.76 ± 27.68	169.20 ± 14.07	207.33 ± 18.03
LPA	moderately mown	1.11 ± 0.29	ND	1.22 ± 0.56	1.26 ± 0.09	1.40 ± 0.16
	abandoned	1.18 ± 0.13	ND	1.51 ± 0.36	1.67 ± 0.25	1.94 ± 0.62

ND – no determination; PA – proteolytic activity at high temperature and optimum pH; LPA – proteolytic activity limited by soil pH and temperature

and with adjustment of optimum pH, (ii) proteolytic activity limited by soil pH and temperature (LPA), and (iii) ratio of LPA to PA.

MATERIALS AND METHODS

Experiments were performed on 1 ha meadow located in the Moravian-Silesian Beskids Mountains (near research station "Bílý Kříž" in the northeast of the Czech Republic, N 49°30'17", E 18°32'28") on a slope with an elevation of 825–860 m. a.s.l. The climate is subcontinental, mean annual air temperature is 4.9°C and average precipitation is 1100 mm. Traditional mowing ceased thirteen years ago on half of the meadow (abandoned meadow), whilst the remaining half has been permanently moderately mown (once a season). The moderately mown meadow plant community belongs to the *Nardo-Callunetea* class and mowing treatment was applied there on 3 August 2006. The vegetation of the abandoned meadow belongs to the *Molinio-Arrhenatheretea* class (Zelena in Formánek *et al.* 2008).

The soil of both meadows is classified as Gleyic Luvisol (ISSS Working Group WRB 2006). Three mixed samples (1 mixed sample = 5 random subsamples) were taken from each of the meadows in the depth of 3–13 cm around the 20th of every month throughout the period of May-September 2006. After storage in a refrigerator and sieving through a 5 mm mesh the samples were analysed.

PA was measured using modification of the procedure described in Nannipieri *et al.* (1979) when 1 g of wet soil was incubated with 2 ml 0.05M Tris-HCl buffer (pH 8.56) and 2 ml 1% casein at 50°C for 2 h. Incubation at 50°C was selected due to its frequent use (e.g. Dilly *et al.* 2007) and pH of the reaction mixture was optimum. LPA was measured by incubation of 1 g of wet soil with 2 ml demineralized water and 2 ml 1% casein for 72 h (Rejsek *et al.* 2008). Each incubation temperature was equivalent to averaged soil temperature at 5 cm depth of usually 4 days just before the day of sampling. PA and LPA were measured without an inhibitor of microbial growth.

Statistical analysis was performed by either One-Way Anova plus Fisher LSD test or Nonparametric Kruskal-Wallis ANOVAs plus Dannet's t-test. Regression analysis was carried out by standard procedure.

RESULTS AND DISCUSSION

PA on the mown meadow was higher in the first three sampling occasions (May–July) and in August or September decreased by around 40%. On the abandoned meadow, the PA was the highest one in June and July. LPA increased through the vegetation season (Table 1). No significance of a linear relationship between LPA and temperature on any of the meadows (r = 0.127 for the mown meadow, resp. 0.312 for the abandoned meadow;

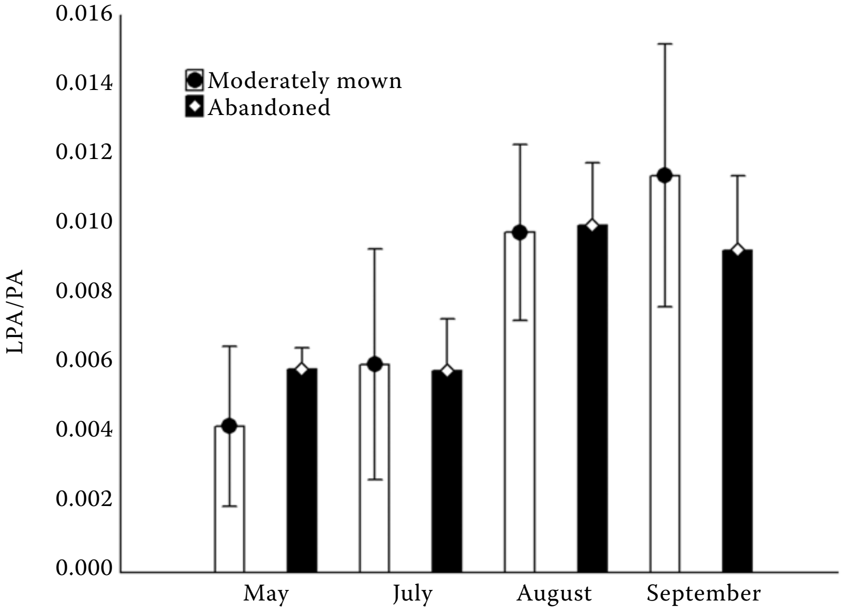

Figure 1. LPA/PA ratio on moderately mown and abandoned mountain meadows (mean ± SD; n = 4–6); no significant ($P > 0.05$) differences between the meadows were found

$P > 0.05$) was found when seasonal fluctuations of soil pH were not taken into account. The ratio LPA/PA in soils of both meadows was < 0.016 and was higher in the second part of the studied period (Figure 1). Values reported for LPA/PA are in agreement with those obtained using the same procedure for forest or vegetable garden soil (Rejsek *et al.* 2008). The ratio LPA/PA in this study is approximately 10 times lower than reported by Nunan *et al.* (2000); nevertheless, in their case LPA was limited only by temperature without influence of soil pH. As reported by Rejsek *et al.* (2008), substitution of 0.05M Tris-HCl buffer (pH 8.52) by demineralized water, accompanied by decreased pH of reaction mixture of soil with casein, can decrease casein-protease activity to almost 50%. In accordance with observations of Nunan *et al.* (2000) we also found no significant relationship between PA and LPA. Low LPA/PA in our study supports the hypothesis that freshly secreted proteolytic enzymes in field conditions can have a significant role, whereas enzymes adsorbed to soil colloids are of lower significance (Nunan *et al.* 2000) and the potential for microbial growth was not verified. The combined effect of field soil temperature and pH decreased casein-protease activity by > 98.4% against casein-protease at 50°C and optimum pH. Previous study results indicated that eleven-year abandonment of the formerly moderately mown mountain meadow is accompanied by the deceleration of N-cycling. Significantly ($P < 0.05$) lower was net microbial release of total bio-available N, and no significant ($P > 0.05$) differences were found in concentrations and net microbial release/immobilization of the individual bio-available amino acids (Formánek *et al.* 2008). This work has proved that thirteen-year abandonment of the same meadow did not lead to significant ($P > 0.05$) effect on the casein-protease activity in the soil of the same depth.

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