Nitrate reductase activity in coexisting high-mountain plants from Central Europe

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Keywords: nitrogen, alpine ans subalpine plants, nitrate reductase activity, plant ecophysiology

Abstract
The overall aim of the study was to conduct comparative characterization of nitrate reductase (NR) activity in high-mountain plant species from Central Europe on the example of Karkonosze mountains. NR is an enzyme involved in primary nitrogen metabolism in plant cell. We measured NRA in typical vascular plant species from subalpine and alpine belts of the Karkonosze in the context of taxonomic position, and growth form. The measured differences reflected taxonomic position mainly, but partially also ecological preferences.

INTRODUCTION

Inorganic soil nitrogen forms are one of the main growth limiting factor for terrestrial pants (Vitousek and Howarth 1991). Nitrate is considered as the one of most important soil nitrogen forms for terrestrial plants. Nitrate reductase (NR) is an enzyme involved in primary nitrate metabolism in plant cell and is the one of most intensively studied enzymes (Campbell 1988, Filippou et al. 2014, Chmura et al. 2016). Due to its function, the NR activity (NRA) can be an useful tool for plant nitrate assimilation preferences assessment. However our knowledge about NRA in high-mountain vascular plants is still very poor. Well example of typical, Central Europe high-mountain conditions are these located above tree-line in Karkonosze, therefore it is a good place to test the nutritional preferences of high-mountain plants typical for this part of the World.

MATERIALS AND METHODS

Twenty six plant species of the families *Asteraceae, Ericaceae, Poaceae, Polygonaceae, Salicaceae, Pinaceae, Ranunculaceae, Woodsiaceae, Cyperaceae, Juncaceae, Rosaceae* and *Urticaceae* representing twelve different growth forms (deciduous dwarf shrubs, deciduous shrubs, deciduous tree, evergreen dwarf shrub, evergreen shrub, evergreen tree, forb, fern, grass, nitrophilous forb, rush and sedge) were investigated in seven habitats of subalpine and alpine belts of the Karkonosze, with respect to leaf NRA and mineral soil nitrogen forms. NRA was measured by an in vivo assay for the first time directly in the field. We used the method of NRA measurement with our own modifications (Rajsz et al. 2017). Soil nitrogen forms concentration was measured with use of flow injection analyzer (MLE Germany). For statistical analyzes we used Statistica package (StatSoft inc. 2014) and Primer 7 statistical software (Primer-E, Plymouth UK) (Anderson et al. 2008).

RESULTS AND DISCUSSION

We observed considerable differences in NR activity among species, families and growth forms (Table 1). The differences reflected mainly the taxonomical position and partially ecological preferences. PERMANOVA analysis confirmed that variance component showed the enzyme activities were mostly explained by plant species and growth form, and to a lesser extent the habitat type. Overall, the high-mountain species from their native habitats were characterized by very low and low abilities for nitrate metabolism. However nitrophilous forbs shoved very high NRA compared to other groups. We also compared NRA values with actual Ellenberg`s N indicator numbers for the studied plants. Using a constructed regression equation, we corrected N values for some species and calculated them for the first time for the others.

Growth form	Median	Minimum	Maximum
NFb	$13{,}55^{d}$	8.39	22,46
Fb	1.64^{c}	0.18	20.80
G	1.58^{b}	0.61	4.07
S	0.83^{ab}	0.11	1.91
R	1.40^{abc}	1.09	1.78
Fn	0.95^{abc}	0.82	1.35
Dt	0.41^{abc}	0.27	0.71
Eds	0.08^{a}	0.00	0.18
Dds	0.00^{a}	0.00	0.13
Es	0.06^{a}	0.03	0.21
Ds	0.08^{ab}	0.06	0.09
Et	0.13^{ab}	0.10	0.24

Table 1. Nitrate reductase activity (NRA) [$\mu M\ NO_2^-\ g^{-1}\ DW\ h^{-1}$] in different plant growth forms. NFb – nitrophilous forbs, Fb – forbs, G – grasses, S – sedges, R – rushes, Fn – ferns, Dt – deciduous trees, Eds – evergreen dwarf shrubs, Dds – deciduous dwarf shrubs, Es – evergreen shrubs, Ds – deciduous shrubs, Et – evergreen trees. Statistically significant differences among growth forms (K-W test (Siegel-Castellan)) are marked by an index (a,b,c,d).

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