A comparison of nitrate reductase activities in leaves of various grassland species

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Abstract

The actual nitrate reductase activity (NRA) in leaves of 19 different grassland species within the same habitat was measured in order to detect differences in their ability to utilise nitrate. This facilitates the classification of grassland species in respect of nitrate nutrition and the creation of NO_3-N indicator values. High levels of NRA in leaves were observed in *Rumex obtusifolius, Heracleum sphondylium* ssp. *sphondylium, Lamium album, Dactylis glomerata* and *Anthriscus sylvestris*. These grassland species are able to utilise large amounts of NO_3-N. Medium levels of NRA in leaves were observed in *Taraxacum officinale* agg., *Pimpinella major* ssp. *major, Trisetum flavescens, Rumex acetosa, Elymus repens, Achillea millefolium* agg., *Chaerophyllum hirsutum, Trifolium repens, Plantago lanceolata* and *Aegopodium podagraria.* Very low levels of NRA in leaves were found in *Crepis biennis, Trifolium pratense* ssp. *pratense, Ranunculus acris* ssp. *acris* and *Ranunculus repens*. These grassland species have a restricted ability to utilise NO_3-N in their leaves. Results are discussed in respect of individual species abundance in grasslands.

Keywords: nitrate reductase activity, utilisation of nitrate, NO_3-N indicator values

Introduction

In well-drained fertilised grassland soils NO_3-N is the most important source of inorganic N (Whitehead, 1995). Therefore grassland species take up most of their nitrogen in the form of nitrate. Before NO_3-N can be utilised by plants it must be reduced to NH_3. The reduction of nitrate to ammonia is mediated by two enzymes, nitrate reductase and nitrite reductase. Both enzymes are substrate-induced, but the more important is nitrate reductase, since it controls the level of nitrite, the substrate for nitrite reductase (Marschner, 1998). Nitrate reductase activity (NRA) can give an indication of the potential of a species to utilise nitrate. NRA is therefore a bioindicator for the nitrate nutrition of plants (Gebauer *et al.*, 1988).
The aim of this study was to compare actual NRA measured in the leaves of 19 different grassland species within the same habitat in order to detect differences in their ability to utilise nitrate. This facilitates the classification of grassland species in respect of nitrate nutrition and the creation of NO_3-N indicator values.

Materials and methods

The investigation area was located in Irdning (Styria, Austria) at an altitude of 700 m. The climate is relatively cool and damp, with a mean annual temperature of 7.4 °C and annual precipitation of 1056 mm, of which 65 % falls during the growing season (April - September). The mean monthly temperature varies from –3.4 °C in January to 16.6 °C in July. The soil is a deep, well-drained Cambisol with a loamy sand texture and medium nutrient supply. Selected chemical properties of the soil are presented in table 1.
Leaves of healthy plants were collected only between 7 and 8 a.m., immediately before harvest in a meadow (*Trisetetum flavescentis* s.l.) managed by three cuts followed by autumn grazing. Nine collections per plant species were made at nearly the same stage of plant development. The meadow was fertilised regularly with slurry and mineral fertiliser. Only leaves were used for the determination of NRA, because leaves are the most important sites

for nitrate reduction (Oaks, 1992). For example, in *Rumex obtusifolius* the NRA in roots has been measured as only 1.6 % of that in the leaves (Bohner *et al.*, in prep.). Actual NRA was assayed *in vivo* by the method described by Srinivasan and Naik (1982). Analyses were carried out immediately after collecting the leaves. Data (arithmetic mean of nine individual values per plant species) were treated by cluster analysis (SPSS 10.1). There are no clear rules in cluster analysis for either the number of objects or for the number of variables (Backhaus *et al.*, 1989). A classification is neither 'correct' nor 'incorrect', but 'useful' or 'useless'. A classification is 'useful', if the clusters can be interpreted well (Kaufmann and Pape, 1984).

Table 1. Soil chemical properties of the Cambisol (0-10 cm soil depth)

$CaCl_2$	$\mu S\ cm^{-1}$	%	%		$mg\ kg^{-1}\ 7d^{-1}$	mg kg^{-1}			$mEq\ 100\ g^{-1}$	% base
						CAL	H_2O	CAL		
pH	EC	C_{org}	N_{tot}	C_{org}/N_{tot}	N_{pot}	P	P	K	CEC_{eff}	saturation
6.3	254	3.8	0.4	9.5	268	56	6.0	151	20.7	99

EC = electrical conductivity; N_{pot} = potentially mineralisable N; CAL = Ca-acetate-lactate solution; P_{H2O} = water soluble P; CEC_{eff} = effective cation exchange capacity ($BaCl_2$-extract).

Results and discussion

Table 2 shows the NRA of 19 different grassland species.

Table 2. Mean nitrate reductase activity in different grassland species, variation coefficient (V %) and NO_3-N indicator values (nomenclature of grassland species from Adler *et al.*, 1994).

NO_3-N indicator value	Grassland species	$\mu mol\ NO_2^-\ h^{-1}g^{-1}$ (fresh weight)	V (%)
1	*Rumex obtusifolius*	3.81	39
2	*Heracleum sphondylium ssp. sphondylium*	2.55	46
	Lamium album	2.04	40
	Dactylis glomerata	1.98	60
	Anthriscus sylvestris	1.83	45
3	*Taraxacum officinale agg.*	1.42	32
	Pimpinella major ssp. major	1.36	58
	Trisetum flavescens	1.29	68
	Rumex acetosa	1.17	92
	Elymus repens	1.14	73
4	*Achillea millefolium agg.*	0.94	25
	Chaerophyllum hirsutum	0.79	63
	Trifolium repens	0.64	78
	Plantago lanceolata	0.62	92
	Aegopodium podagraria	0.54	56
5	*Crepis biennis*	0.22	34
	Trifolium pratense ssp. pratense	0.17	42
	Ranunculus acris ssp. acris	0.10	61
	Ranunculus repens	0.07	47

Five NO_3-N indicator values could be created by cluster analysis (Ward method). The variation in NRA measured within each grassland species was large. It must be taken into consideration that NRA is influenced by many site factors, such as temperature, light intensity, water supply and NO_3-N availability in the soil (Runge, 1983). Nevertheless, the grassland species examined showed either permanently high, medium or low levels of NRA within the same habitat; and the ranking order was similar in the different assays. Therefore a classification of grassland species on the basis of NRA seems to be valid. The grassland species examined can be divided into five groups representing different NO_3-N indicator values. The highest mean level of NRA in leaves was observed in *Rumex obtusifolius*. *Rumex obtusifolius* is a nitrophilic species with the ability to utilise large amounts of NO_3^-. *Rumex obtusifolius* is restricted to nutrient-rich grassland soils of high nitrate and potassium availability (Bohner *et al.*, in prep.). *Heracleum sphondylium* ssp. *sphondylium*, *Lamium album*, *Dactylis glomerata* and *Anthriscus sylvestris* also had comparatively high mean levels of NRA in their leaves. These grassland species are able to utilise large amounts of NO_3^- from the soil and they possess a high capacity for NO_3^- reduction in their leaves. Therefore they are abundant in grassland soils of high nitrate availability. In contrast, very low levels of NRA have been found consistently in leaves of *Crepis biennis, Trifolium pratense* ssp. *pratense*, *Ranunculus acris* ssp. *acris* and *Ranunculus repens*. These grassland species evidently have a restricted ability to utilise NO_3^- in their leaves. Low leaf NRA may indicate either predominantly root nitrate reduction, or an ammonium-based nutrition, or may result from N_2 fixation. *Fabaceae*, such as *Trifolium pratense* ssp. *pratense*, take up only small amounts of soil NO_3-N, because of their symbiosis with N_2 fixing bacteria. Therefore they have a comparatively low mean level of NRA in their leaves. However, other species, such as *Trifolium repens,* use appreciable amounts of NO_3-N from the soil in addition to fixing N_2. *Ranunculus acris* ssp. *acris* and *Ranunculus repens* consistently exhibited a very low level of NRA in their leaves. *Ranunculus repens* is an indicator of compacted, periodically wet topsoils and is abundant in fertilised wet meadows and pastures. Both *Ranunculaceae* profit from a high available K^+/NO_3^- ratio in the soil. The same seems to be the case in *Aegopodium podagraria*. *Aegopodium podagraria* is abundant on sandy or silty nutrient-rich grassland soils at cooler sites. *Crepis biennis* and *Plantago lanceolata* are other grassland species with comparatively low potential to utilise NO_3^- in their leaves. They are often abundant in meadows with low proportion of grasses, presumably due to a lack of plant-available NO_3-N.

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