Nitrate reductase activities in plants from different ecological and taxonomic groups grown in Japan

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Title

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Abstract

Plants generally use soil inorganic nitrogen, ammonium (NH_4^+-N), and nitrate (NO_3^--N) as sources of nitrogen, an essential nutrient. The assimilation processes after uptake differ considerably from ammonium to nitrate. Nitrate must be reduced to ammonium in plant tissue before it is synthesized to amino acids, while ammonium is directly and immediately synthesized to amino acids after its uptake. Nitrate reductase is an enzyme that catalyzes the first and rate-limiting step of nitrate assimilation, reducing nitrate to nitrite. It is a substrate-inducible enzyme, and the capacity to induce nitrate reductase varies greatly among plant species. *In vivo* nitrate reductase activity (NRA) is generally measured as a nitrite production rate during incubation using fine cut pieces of plant tissue, and it is applicable as an indicator on plant nitrate use. Here we present *in vivo* NRA of leaves from a total of 108 species including arboreal trees, small trees, shrubs, herbs, a vine and a moss. For 75 of the species, NRA in fine roots was also determined. At least 20 species in sampled plants were imported and planted for scientific or industrial purposes, but most sampled species were native to Japan. Several inventory studies of plant NRA have been conducted, mainly in Europe, and they provided information on the species-specific capacity of nitrate use by plants in Europe. However, to our best knowledge, there has been hitherto no published inventory of the NRA of plants in Japan where many endemic species are distributed. Our dataset contains plant NRA with species, family name, life form, leaf lifespan (evergreen or deciduous), growth stage, the season of sample collection, growth conditions (natural or cultivated) and other treatments/conditions when applicable. The data provided by this study may contribute to future works that require information regarding the plant species characteristics for nitrate use capacity or nitrate preference.

Keywords

Angiosperm; bryophyte; gymnosperm; Japan; nitrate; nitrate assimilation; nitrate reductase activity; plants; soil nitrogen

METADATA

1. TITLE

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2. IDENTIFIER

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4. GEOGRAPHICAL COVERAGE

A. Geographical description and Position:

Samples were collected from six locations in Japan, including a growth chamber, a greenhouse, an arboretum, and two plantation sites, in addition to the collection of natural vegetation. Table 1 shows the positions, environmental factors, and descriptions of the sampling sites.

Table 1 Description for sampling sites.

Site	Location		Elevation (m a.s.l.)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Vegetation type	Dominant species
	LAT	LON					
Ashiu	35°18′ N	135°43′ E	about 600-700	12.2	2548	Cool temperate deciduous forest	*Fagus crenata*, *Quercus crispula*
Daisen	35°21′ N	133°32′ E	about1000	10.5	2736	Cool temperate deciduous forest	*Fagus crenata*
Isaki	35°12′ N	136°05′ E	about 200	15.1	1475	Warm temperate coniferous plantation	*Chamaecyparis obtusa*, *Camellia japonica*
Kamigamo	35°04′ N	135°46′ E	about 200	15	1584	Warm temperate deciduous forest/plantation	*Quercus serrata*, *Quercus glauca*
Kitashirakawa	35°02' N	135°47'E	about 100	15.1	1465	Arboretum	-
Tottori	35°32' N	134°13'E	0 - 100	-	-	Green house	-
Tottori	35°32' N	134°13'E	0 - 100	-	-	Growth chamber	-
Tottori	35°32' N	134°13'E	0 - 100	14.9	1914	Coastal sand dunes	*Elaeagnus umbellata*, *Vitex rotundifolia*, *Artemisia capillaris*

5. TEMPORAL COVERAGE

Aug, 1997 - August, 2017

6. METHODS

A. Sampling sites

Samples were collected from the six locations shown in Table 1. The following are additional descriptions for each site.

In Ashiu, the dominant inorganic N form on the upper slope was NH_4^+-N, while it was NO_3^--N on the lower slope of this site (Tateno and Takeda 2003). In total, 16 species were sampled, and samples of *Fagus crenata* were collected from both the upper slope and lower slope.

In Daisen, fertilization was conducted from 1987 to 2002, with an annual supply of 5,250 kg yr^{-1} ha (N:P:K = 20:10:10). Samples were collected from the fertilization plot and the control plot in this site.

Isaki was located on a peninsula in Lake Biwa and had been colonized by great cormorants (*Phalacrocorax carbo*) since 1980-90s (Kameda 2012), and the cormorant-derived N influenced N dynamics (Hobara et al. 2005). Three sampling locations were selected to represent different levels of cormorant colonization effects at this site; a currently occupied area, a previously occupied but now abandoned area, and a control area never colonized by cormorants. In the currently occupied area and the previously occupied area, both the N mineralization rate and the nitrification rate were high, and the ratio of nitrification to N mineralization was close to 100 %, suggesting high NO_3^--N availability in these areas (Hobara et al. 2001).

In Kamigamo, a very low nitrification rate in comparison with the N mineralization rate was reported for both the organic layer and the mineral soil layer

(Tokuchi et al. 2002) Accordingly, the dominant inorganic N form in the soil was NH_4^+-N. Samples from 11 species were collected at this site.

Kitashirakawa is an arboretum of the Field Science Education and Research Center, Kyoto University, where about 500 species are planted in a 1.3 ha area. Forty-eight species were sampled from this site, including coniferous and broad-leaved species of trees, shrubs, and herbaceous species, including native and imported species.

In Tottori, samplings were conducted under two artificial conditions (greenhouse and growth chamber) and a natural condition (coastal sand dunes).

The greenhouse was located on the campus of the Arid Land Research Center, Tottori University. Imported arid land plant species and domestic plant species were grown in the greenhouse to supply experiments, and 28 species were sampled. They were planted in locally available sand and regularly watered. Three levels of fertilization were applied to four *Salix* species; non-fertilized (0 N-kg ha^{-1}), fertilized (7.4 N-kg ha^{-1}) and intensively fertilized (29.4 N-kg ha^{-1}). Yamamoto et al. (2003) described in detail the influence of fertilization on the four Salix species. Eight Fagaceae species grown in the greenhouse were regularly fertilized with 1000-fold diluted Hyponex solution (Hyponex, Japan).

The growth chamber was located on the campus of the Arid Land Research Center, Tottori University. The chamber conditions were set to a temperature of 18 C, humidity at 90 %, illuminance of 0 lux during the dark period, while in the light period the temperature was set at 25 °C, the humidity at 60 %, and illuminance 80,000 lux. During the light treatment, 11 hours of darkness were alternated with 13 hours of light.

Coastal sand dunes were located immediately north of the Arid Land Research Center, Tottori University, facing the Sea of Japan. The dunes were surrounded by a *Pinus thunbergii* plantation mixed with *Robinia pseudoacasia* established as a windbreak to protect agricultural and residential areas nearby. Since the establishment of the windbreak

plantation, coastal plant species such as *Elaeagnus umbellata*, *Vitex rotundifolia,* and *Artemisia capillaris* began invading the dunes. Soil nitrate pool sizes were spatially and temporally heterogeneous, and minimum and maximum nitrate pool sizes were 0.9 and 240 mg N m^{-2}, respectively (Lina A. Koyama unpublished data). Samples were collected from 11 species at this site.

B. Sample collection

To avoid the effect of diurnal changes in leaf NRA, samples were collected from 10:00 to 14:00 on sunny days except for growth chamber samples, and were kept on ice until laboratory analysis. Growth chamber samples were collected 5 hours after initiation of the last light period. In the dark treatment, the illuminance was kept to 0 lux after the last dark period for 5 hours, and then the samples were collected.

C. Nitrate reductase activity assay

The *in vivo* NRA assay was performed based on a modified version of the Jaworski method (Jaworski 1971; Thomas and Hilker 2000; Koyama and Tokuchi 2003). Root samples were washed with tap water, followed by deionized water, to remove soil. About 100 mg (fresh weight) of leaf laminae, needles, or fine roots were cut into small fragments (2.5-mm-diameter disks or about 4-mm^2 segments of leaves, and about 2-mm-long needles or roots) and transferred to test tubes. The incubation buffer (5 mL) was added to the needles and roots, and the tube contents were vacuum infiltrated. The composition of the incubation buffer was as follows: 0.1 mol L^{-1} KNO_3, 0.1 mol L^{-1} KH_2PO_4, and 1.5 % 1-propanol; the pH was adjusted to ca. 7.5 using a NaOH solution. The samples were incubated for 1 h in darkness, and the NO_2^--N concentration in the incubation buffer was measured as the end-point. Before the measurement, enzyme activity was terminated by

placing the sample vials in hot water (>80 °C). The concentration of NO_2^--N in the incubation buffer was measured colorimetrically following diazotization (Keeney and Nelson, 1982). The confounding effects of plant pigments were corrected by subtracting the absorbance of controls to which N-naphthylethylene diamine dihydrochloride was not added (Gebauer et al. 1998). A fraction of each leaf sample was oven-dried at 105 °C and then weighed to calculate the activity per unit dry weight.

7. ACCESSIBILITY

A. License

This data set is provided under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0: https://creativecommons.org/licenses/by-nc/4.0/).

B. Location of storage

http://db.cger.nies.go.jp/JaLTER/ER_DataPapers/archives/2019/ERDP-2019-06

8. DATA STRUCTURE

A. Data table

Table 2 List of data tables.

Data file name	Description
Data.csv	Dataset of plant nitrate reductase activity (NRA) with species taxonomic and growth conditions descriptions

B. Format type

The data files are presented as ASCII text and comma-delimited (csv) files.

C. Header information

Headers corresponding to variable names (see section 9.D) are included in the first row of the data file.

D. Variable definitions

The variables are listed according to their order of appearance in the data file. The variable names are headers included as the first row in the data file.

Table 3 List of variables in the dataset and their descriptions.

Data file name	Variable name	Variable definition
Data.csv	ID	Identification number of each sample
	Scientific name	Identification of taxa (Species)
	Family name	Identification of taxa (Family)
	Life form	Tree*/Small tree*/Shrub*/Herb/Vine *: Trees are 10 m~, Small trees are 5 – 10 m, Shrubs are ~5 m at maturity.
	Leaf lifespan	Tree, Small tree or Shrub: Evergreen/Deciduous Herb: Perennial/Annual
	Leaf NRA	Leaf NRA (μmol g dry wt^{-1} h^{-1})
	Root NRA	Root NRA (μmol g dry wt^{-1} h^{-1})
	Year	The year in which the sample was collected
	Month	The month in which the sample was collected representing the season
	Site	Sample collection site: Ashiu/Daisen/Isaki/Kamigamo/Kitashirakawa/Tottori

	Growth stage	Growth stage of the individual sample: Mature/Young tree*/Seedling *: a young tree is defined as a tree that did not reach the canopy height.
	Growth condition	Growth conditions of the individual samples: Arboretum/Greenhouse/Growth chamber/Natural/Plantation
	Treatment / Condition	Particular treatments or conditions for the individual samples: Ashiu: Upper slope/Lower slope Daisen: Non-fertilized/Fertilized Isaki: Currently colonized by great cormorants /Previously colonized and abandoned by great cormorants /Never colonized by great cormorants Greenhouse: Non-fertilized/Fertilized/Intensively fertilized Growth chamber: Light/Dark See Table 1 and 6.A Sampling sites for details.
	Remarks	Additional information

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