Title

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

Authors

Lina A. Koyama[1,2], Masakazu Terai[1,3], Naoko Tokuchi[4]

Affiliations

1: Laboratory of Forest Ecology, Graduate School of Agriculture, Kyoto University

2: Laboratory of Biosphere Informatics, Department of Social Informatics, Graduate School of Informatics, Kyoto University

3: FUJICCO Co., Ltd.

4: Field Science Education and Research Center, Kyoto University

Correspondence

Lina A. Koyama

Laboratory of Biosphere Informatics, Department of Social Informatics, Graduate School of Informatics, Kyoto University

Kyoto 606-8501, Japan

Phone: +81-75-753-3297

E-mail: linak@bre.soc.i.kyoto-u.ac.jp

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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3. CONTRIBUTOR

A. Dataset owner

Name: Lina A. Koyama

Affiliation: Laboratory of Biosphere Informatics, Department of Social Informatics,

Graduate School of Informatics, Kyoto University

Address: Kyoto 606-8501 Japan

Tel: +81-75-753-3297

Fax: +81-75-753-3133

E-mail: linak@bre.soc.kyoto-u.ac.jp

B. Contact person

Name: Lina A. Koyama

Affiliation: Laboratory of Biosphere Informatics, Department of Social Informatics,

Graduate School of Informatics, Kyoto University

Address: Kyoto 606-8501 Japan

Tel: +81-75-753-3297

Fax: +81-75-753-3133

E-mail: linak@bre.soc.kyoto-u.ac.jp

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.

1 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*

2

3   5. TEMPORAL COVERAGE

4   Aug, 1997 - August, 2017

5

6   6. METHODS

7   A. Sampling sites

8   Samples were collected from the six locations shown in Table 1. The following are

9   additional descriptions for each site.

10   In Ashiu, the dominant inorganic N form on the upper slope was NH_4^+-N, while it

11   was NO_3^--N on the lower slope of this site (Tateno and Takeda 2003). In total, 16 species

12   were sampled, and samples of *Fagus crenata* were collected from both the upper slope and

13   lower slope.

14   In Daisen, fertilization was conducted from 1987 to 2002, with an annual supply

15   of 5,250 kg yr^{-1} ha (N:P:K = 20:10:10). Samples were collected from the fertilization plot

16   and the control plot in this site.

17   Isaki was located on a peninsula in Lake Biwa and had been colonized by great

18   cormorants (*Phalacrocorax carbo*) since 1980-90s (Kameda 2012), and the cormorant-

19   derived N influenced N dynamics (Hobara et al. 2005). Three sampling locations were

20   selected to represent different levels of cormorant colonization effects at this site; a

21   currently occupied area, a previously occupied but now abandoned area, and a control area

22   never colonized by cormorants. In the currently occupied area and the previously occupied

23   area, both the N mineralization rate and the nitrification rate were high, and the ratio of

24   nitrification to N mineralization was close to 100 %, suggesting high NO_3^--N availability

25   in these areas (Hobara et al. 2001).

26   In Kamigamo, a very low nitrification rate in comparison with the N

27   mineralization rate was reported for both the organic layer and the mineral soil layer

28 (Tokuchi et al. 2002) Accordingly, the dominant inorganic N form in the soil was NH_4^+-N.

29 Samples from 11 species were collected at this site.

30   Kitashirakawa is an arboretum of the Field Science Education and Research

31 Center, Kyoto University, where about 500 species are planted in a 1.3 ha area. Forty-eight

32 species were sampled from this site, including coniferous and broad-leaved species of

33 trees, shrubs, and herbaceous species, including native and imported species.

34   In Tottori, samplings were conducted under two artificial conditions (greenhouse

35 and growth chamber) and a natural condition (coastal sand dunes).

36 The greenhouse was located on the campus of the Arid Land Research Center, Tottori

37 University. Imported arid land plant species and domestic plant species were grown in the

38 greenhouse to supply experiments, and 28 species were sampled. They were planted in

39 locally available sand and regularly watered. Three levels of fertilization were applied to

40 four *Salix* species; non-fertilized (0 N-kg ha^{-1}), fertilized (7.4 N-kg ha^{-1}) and intensively

41 fertilized (29.4 N-kg ha^{-1}). Yamamoto et al. (2003) described in detail the influence of

42 fertilization on the four Salix species. Eight Fagaceae species grown in the greenhouse

43 were regularly fertilized with 1000-fold diluted Hyponex solution (Hyponex, Japan).

44   The growth chamber was located on the campus of the Arid Land Research

45 Center, Tottori University. The chamber conditions were set to a temperature of 18 C,

46 humidity at 90 %, illuminance of 0 lux during the dark period, while in the light period the

47 temperature was set at 25 °C, the humidity at 60 %, and illuminance 80,000 lux. During

48 the light treatment, 11 hours of darkness were alternated with 13 hours of light.

49   Coastal sand dunes were located immediately north of the Arid Land Research

50 Center, Tottori University, facing the Sea of Japan. The dunes were surrounded by a *Pinus*

51 *thunbergii* plantation mixed with *Robinia pseudoacasia* established as a windbreak to

52 protect agricultural and residential areas nearby. Since the establishment of the windbreak

53   plantation, coastal plant species such as *Elaeagnus umbellata*, *Vitex rotundifolia,* and

54   *Artemisia capillaris* began invading the dunes. Soil nitrate pool sizes were spatially and

55   temporally heterogeneous, and minimum and maximum nitrate pool sizes were 0.9 and

56   240 mg N m^{-2}, respectively (Lina A. Koyama unpublished data). Samples were collected

57   from 11 species at this site.

58

59   B. Sample collection

60   To avoid the effect of diurnal changes in leaf NRA, samples were collected from 10:00 to

61   14:00 on sunny days except for growth chamber samples, and were kept on ice until

62   laboratory analysis. Growth chamber samples were collected 5 hours after initiation of the

63   last light period. In the dark treatment, the illuminance was kept to 0 lux after the last dark

64   period for 5 hours, and then the samples were collected.

65

66   C. Nitrate reductase activity assay

67   The *in vivo* NRA assay was performed based on a modified version of the Jaworski method

68   (Jaworski 1971; Thomas and Hilker 2000; Koyama and Tokuchi 2003). Root samples were

69   washed with tap water, followed by deionized water, to remove soil. About 100 mg (fresh

70   weight) of leaf laminae, needles, or fine roots were cut into small fragments

71   (2.5-mm-diameter disks or about 4-mm^2 segments of leaves, and about 2-mm-long needles

72   or roots) and transferred to test tubes. The incubation buffer (5 mL) was added to the

73   needles and roots, and the tube contents were vacuum infiltrated. The composition of the

74   incubation buffer was as follows: 0.1 mol L^{-1} KNO$_3$, 0.1 mol L^{-1} KH$_2$PO$_4$, and 1.5 %

75   1-propanol; the pH was adjusted to ca. 7.5 using a NaOH solution. The samples were

76   incubated for 1 h in darkness, and the NO$_2^-$-N concentration in the incubation buffer was

77   measured as the end-point. Before the measurement, enzyme activity was terminated by

78 placing the sample vials in hot water (>80 °C). The concentration of NO_2^--N in the

79 incubation buffer was measured colorimetrically following diazotization (Keeney and

80 Nelson, 1982). The confounding effects of plant pigments were corrected by subtracting

81 the absorbance of controls to which N-naphthylethylene diamine dihydrochloride was not

82 added (Gebauer et al. 1998). A fraction of each leaf sample was oven-dried at 105 °C and

83 then weighed to calculate the activity per unit dry weight.

84

85 7. ACCESSIBILITY

86   A. License

87 This data set is provided under a Creative Commons Attribution-NonCommercial 4.0

88 International (CC BY-NC 4.0: https://creativecommons.org/licenses/by-nc/4.0/).

89

90   B. Location of storage

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

92

93 8. DATA STRUCTURE

94   A. Data table

95 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

96

97   B. Format type

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

99

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

109

9. Acknowledgments

10. References

Gebauer G, Hahn G, Rodenkirchen H, Zuleger M (1998) Effects of acid irrigation and liming on nitrate reduction and nitrate content of *Picea abies* (L.) Karst. and *Oxalis acetosella* L. Plant and Soil 199: 59-70. DOI: 10.1023/A:1004263223917

Hobara S, Koba K, Osono T, Tokuchi N, Ishida A, Kameda K (2005) Nitrogen and phosphorus enrichment and balance in forests colonized by cormorants:

123   Implications of the influence of soil adsorption. Plant and Soil 268: 89-101. DOI:

124   10.1007/s11104-004-0231-6

125 Hobara S, Osono T, Koba K, Tokuchi N, Fujiwara S, Kameda K (2001) Forest Floor

126   Quality and N Transformations in a Temperate Forest Affected by Avian-Derived

127   N Deposition. Water, Air, and Soil Pollution 130: 679-684. DOI:

128   10.1023/a:1013869115132

129 Jaworski E (1971) Nitrate reductase assay in intact plant tissues. Biochemical and

130   Biophysical Research Communications 43: 1274-1279.

131 Kameda K (2012) Population Increase of the Great Cormorant *Phalacrocorax carbo* and

132   Measures to Reduce its Damage to the Fisheries and Forests of Lake Biwa. In: H

133   Kawanabe, M Nishino, M Maehata (eds) Lake Biwa: Interactions between Nature

134   and People. Springer, Dordrecht, pp. 491-496

135 Keeney DR, Nelson DW (1982) Nitrogen - Inorganic forms. In: AL Page, RH Miller, DR

136   Keeney (eds) Methods of Soil Analysis Part 2. ASA and SSSA, Madison, WI, pp.

137   643-698

138 Koyama L, Tokuchi N (2003) Effects of NO3- availability on NO3- use in seedlings of

139   three woody shrub species. Tree Physiology 23: 281-288.

140 Tateno R, Takeda H (2003) Forest structure and tree species distribution in relation to

141   topography-mediated heterogeneity of soil nitrogen and light at the forest floor.

142   Ecological Research 18: 559-571.

143 Thomas F, Hilker C (2000) Nitrate reduction in leaves and roots of young pedunculate

144   oaks (*Quercus robur*) growing on different nitrate concentrations. Environmental

145   and Experimental Botany 43: 19-32.

146 Tokuchi N, Fujimaki R, Terai M, Shiroshita Y, Kuroki K (2002) Soil nitrogen dynamics of

147   temperate conifer forest in central Japan: The case study of Japanese cypress forest

148   at the Kamigamo Experimental Forest (In Japanese with English abstract). Forest

149   Research, Kyoto 74: 47-52.

150 Yamamoto M, Tamai S, Tokuchi N, Yamanaka N (2003) Effects of fertilization on growth

151   of cuttings in Salix species (in Japanese with English abstract). Journal of the

152   Japanese Society of Revegetation Technology 29: 95-100.

153