HOKKAIDO UNIVERSITY

Title	Differences in acquisition of organic nitrogen in soils between bok choy and tomato
Author(s)	Watanabe, Toshihiro; Enomoto, Takumi; Sakurai, Michihiko; Okamoto, Miwa; Shimano, Takuro; Osaki, Mitsuru
Citation	Journal of Plant Nutrition, 35(10), 1468-1476 https://doi.org/10.1080/01904167.2012.689907
Issue Date	2012-06-26
Doc URL	http://hdl.handle.net/2115/52932
Rights	This is an electronic version of an article published in Journal of Plant Nutrition, 35(10), 1468-1476, Jun-2012. Journal of Plant Nutrition is available online at: http://www.informaworld.com/openurl?genre=article&issn=0190-4167&volume=35&issue=10&spage=1468
Type	article (author version)
File Information	JPN35-10_1468-1476.pdf

Running head: Difference in acquisition of organic nitrogen

Title: Differences in acquisition of organic nitrogen in soils between bok choy and tomato

Authors: Toshihiro Watanabe[1], Takumi Enomoto[1], Michihiko Sakurai[2], Miwa Okamoto[3], Takuro Shinano[3], Mitsuru Osaki[1]

Affiliations:

1 Graduate School of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kitaku, Sapporo 0608589, Japan

2 Hokkaido Central Agricultural Experiment Station, Naganuma 0691395, Japan

3 National Agricultural Research Center for Hokkaido Region, Sapporo 0628555, Japan

Corresponding author: T. Watanabe

Graduate School of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kitaku, Sapporo 0608589, Japan

Tel.: +81 706 2498   Fax: +81 706 3845

Email: nabe@chem.agr.hokudai.ac.jp

ABSTRACT

Seedlings of bok choy and tomato were grown in soils with different N sources [no N (−N), ammonium sulfate (AS), and cattle farmyard manure (CM)]. Comparison between soils treated with −N and CM indicated that the growth and N accumulation in bok choy were significantly enhanced by CM treatment, whereas no difference was found in tomato. In the rhizosphere soils, the highest protease activity was detected in CM treatment irrespective of species. Correlation analysis between rhizospheric protease activity and total N accumulation of plant treated with –N and CM showed a significant positive correlation only for bok choy. The determination of amino acid absorption rate in excised roots indicated that glycine was taken up at a significantly higher rate in bok choy than tomato. This study suggested that at least two possible factors affected the acquisition of organic N: rhizospheric protease activity and ability to absorb amino acids in roots.

INTRODUCTION

Effective utilization of organic matter is strongly desired to promote sustainable agriculture. Although it has been well documented that growth responses to organic matter differ among plant species (Matsumoto et al., 1999; Okamoto et al., 2003), factors affecting these characteristics have not yet been elucidated. Major part of organic nitrogen (N) in soil organic matter is a proteaceous compound (Kumar et al., 2006). In soil, proteins are degraded by proteases, and the released amino acids and peptides are mineralized to ammonium (NH_4) and nitrate (NO_3), which can be utilized by plant roots. A protease-mediated process is considered to be the rate-limiting step in degradation of organic N in soils. The protease activity in soils is considered to be derived from soil microorganisms (Sakurai et al., 2007). The *apr*, *npr*, and *sub* genes encode alkaline metalloprotease, neutral metalloprotease, and serine protease, respectively. These are the primary microbial extracellular proteases in soils (Sakurai et al., 2007; Fuka et al., 2008). Fuka et al. (2008) quantified *sub* and *npr* genes in soils using real-time PCR, and found a positive relationship between the *sub* and *npr* coding genes and potential protease activity in sandy soils. Although it has been suggested that plant roots secrete proteases (Godlewskia and Adamczyk, 2007; Adamczyk et al., 2008), their degree of contribution is unclear.

Amino acids and peptides are released in the soil solution through proteolysis. It has been shown that many plant species can absorb not only inorganic N but also amino acids and low

molecular weight peptides as significant sources of N for their growth (Paungfoo-Lonhienne et al., 2008; Streeter et al., 2000). It is considered that amino acids are an important source of N for plants growing in cold regions where organic N mineralization is limited (Kielland 1994). Furthermore, plants growing in temperate or tropical regions can absorb amino acids as a source of N (Watanabe et al., 2006; Weigelt et al., 2005).

In this study, we propose two hypotheses accounting for the factors responsible for differences in plant response to organic N. The first hypothesis is that higher protease activity in the rhizosphere contributes to the ability of plants to utilize organic N in soils. Plant species having a greater efficacy in utilizing organic N may render their rhizospheric environment more suitable for protease-producing microorganisms or they may secrete proteases from their roots, resulting in an enhancement of proteolysis in the rhizosphere. The second hypothesis is that differences in the ability to absorb amino acids released by proteolysis are responsible for the different characteristics of organic N utilization. Plant species having greater efficacy in absorbing amino acids are considered to possess superior characteristics when competing with rhizosphere microorganisms for N acquisition. In this study, plant utilization of organic N is comprehensively studied by testing these hypotheses.

MATERIALS AND METHODS

Pot experiment

Each pot contained 300 mL of vermiculite mixed with field soils, as a source of microorganism inocula (vermiculite:field soil = 4:1, v/v). Field soils were collected from Hokkaido Central Agricultural Experiment Station, Naganuma, Japan. Over the past 10 years, 25,000 kg ha^{-1} year^{-1} of manure, 2000 kg ha^{-1} year^{-1} of rice bran, and 1070 kg ha^{-1} year^{-1} of fish meal have been applied to the collected soils. The chemical properties of the field soils are shown in Table 1. Different sources of N [no N (−N), ammonium sulfate (AS), and cattle farmyard manure (CM)] were applied to each pot for N treatment (70 mg N pot^{-1} in AS and CM treatments). CM contained 26 mg N g^{-1}, 6.6 mg P g^{-1}, and 8.3 mg K g^{-1} on a dry weight basis. The concentration of inorganic N (NH$_4$–N and NO$_3$–N) in CM was 0.89 mg g^{-1}, indicating that most of the N in CM is in the organic form. To each pot, 93 mg P (as superphosphate), 117 mg K (as potassium sulfate), and 200 mg calcium carbonate (to adjust the soil pH to 6) were applied. The amounts of P and K derived from CM were subtracted from that supplied by chemical fertilizers in CM treatment. After fertilization, all the pots were incubated in a greenhouse at Hokkaido University for 2 weeks, with occasional watering. Seeds of bok choy (*Brassica rapa* L. var. chinensis) and tomato (*Solanum lycopersicum* L.) were surface sterilized with sodium hypochlorite for 10 min, washed with deionized water, and sowed in the pots. After germination, seedlings were thinned to two per

5

pot and grown in a greenhouse (13-h photoperiod and a mean day/night temperature of 20–25°C). The soils were irrigated with deionized water everyday. The experiment was performed with four replicates.

After treatment for 28 days, seedlings and soils (rhizosphere and unplanted bulk soils) were sampled. In each pot the plant shoots were cut off and non-rhizosphere soils were gently removed from the roots. Then, the roots were shaken vigorously in a plastic bag to separate the rhizosphere soil, i.e., soil not tightly adhering to the roots. The roots and shoots were washed with deionized water, lyophilized, weighed, and ground for analysis. Soils were passed through a 2 mm sieve and stored at either 4°C for determination of protease activity or −20°C for chemical analysis. Plant N concentrations were determined using the indophenol-blue method after Kjeldahl digestion. Protease activity in soils was determined according to Ladd and Bulter (1972), using benzyloxycarbonyl phenylalanyl leucine as a substrate. The NH_4–N and NO_3–N concentrations in the soils were determined by auto analyzer (AACS-3, Bran+Luebbe, Norderstedt, Germany) after extraction with 2 M KCl.

Determination of amino acid uptake rate

Surface-sterilized seeds of bok choy and tomato were sown in perlite. Ten days after germination, seedlings were transferred to 4 L containers filled with low N nutrient solution

containing 0.2 mM N (NH_4NO_3), 32 µM P ($NaH_2PO_4 \cdot 2H_2O$), 0.77 mM K (K_2SO_4:KCl = 1:1),

1.25 mM Ca ($CaCl_2 \cdot 2H_2O$), 0.82 mM Mg ($MgSO_4 \cdot 7H_2O$), 35.8 µM Fe ($FeSO_4 \cdot 7H_2O$), 9.1

µM Mn ($MnSO_4 \cdot 4H_2O$), 46.3 µM B (H_3BO_3), 3.1 µM Zn ($ZnSO_4 \cdot 7H_2O$), 0.16 µM Cu

($CuSO_4 \cdot 5H_2O$), and 0.05 µM Mo ((NH_4)$_6Mo_7O_{24} \cdot 4H_2O$); total SO_4 = 1.06 mM. The pH of

the solution was adjusted to 5.2 daily, and solutions were renewed every week. At the end of

the 3-week preculture with low N nutrient solution, the seedlings were transferred to nutrient

solutions without N for N-deficient treatment. Although it was reported that pretreatment

with amino acid increased amino acid uptake rates in plants (Mori et al., 1979), we used roots

with N deficiency for determining amino acid uptake to avoid N contamination. After a 3-day

−N pretreatment, the roots were washed with deionized water and submerged in 0.2 mM

$CaCl_2$ (pH 5.2) for the determination of amino acid uptake rates. The excised roots (young

growth, 100–200 mg fresh weight) of bok choy and tomato were weighed and incubated in

10 mL of 0.2 mM $CaCl_2$ solution (pH 5.2) containing different amino acids [glycine (Gly)

and glutamic acid (Glu)] for 2 h at either 25°C or 4°C. The solution was occasionally stirred.

N concentration in the solution before and after incubation was determined, and the decrease

in concentration was calculated. Active uptake rate for each amino acid was estimated by

subtracting the rate at 4°C (passive uptake) from that at 25°C. The experiment was performed

with four replicates. It was confirmed that mineralization of amino acids was negligible

during incubation (Watanabe et al., 2006).

RESULTS AND DISCUSSION

Plant growth in both species was highest in soils treated with AS (Figure 1). In tomato, no

significant difference in growth was found between –N and CM treatments. However, the

growth of bok choy was significantly enhanced in soils treated with CM compared with soils

treated with –N. Furthermore, N accumulation paralleled growth in the plants (Figure 1).

These results suggest that bok choy can utilize organic N in soils more efficiently than

tomato.

As described in Introduction, protease activity in rhizosphere soils is one of the key factors

contributing to organic N acquisition in plants. In rhizosphere soils, the highest activity was

detected in CM treatment irrespective of plant species (Table 2), which suggests that organic

matter application enhanced activity. It has been reported that application of organic matter

often stimulates the activity of various enzymes in soils (Crecchio et al., 2001; Madejon et al.,

2001; Kandeler et al., 1999), including proteases (Sakurai et al., 2007). Kielland et al. (2007)

showed that soils with a high protein concentration possessed high soil protease activity.

Although the rhizosphere effect (Badalucco et al., 1996) was observed for protease activity

when comparing activity between rhizosphere and unplanted soils in CM treatment, there

was no difference in this effect between the two species (Table 2). Following this, the correlation between rhizospheric protease activity and plant total N accumulation was determined using individual replicates in both species grown with –N and CM treatments (Figure 2). A significant positive correlation was found only in bok choy ($r^2 = 0.89$, $P < 0.01$). These results suggest that rhizospheric protease activity is a potential factor contributing to organic N acquisition in plants, however, other factors are likely to be involved.

Inorganic N (NO_3–N + NH_4–N) concentration in rhizosphere soils treated with CM did not show any significant difference from those treated with –N (Figure 3), suggesting that available N released by proteolysis was absorbed by microorganisms and/or plant roots relatively quickly. It is well known that plant roots compete with soil microorganisms for N acquisition in the rhizosphere. It can be an advantage to take up amino acid N more efficiently to defeat rhizosphere microorganisms in N acquisition. Therefore, the ability of plants to absorb amino acid N was examined using excised roots of each plant species grown in hydroponic culture. As expected, Gly, which is one of the major free amino acids in soils (Streeter et al., 2000), was taken up at a significantly higher rate in excised roots of bok choy than tomato (Table 3). This result strongly supports the hypothesis that differences in the ability to absorb amino acids are responsible for the different characteristics of organic N utilization in plant growth in soils. Recently, it has been indicated that several

9

amino acid transporters are involved in organic N acquisition in plants. Hirner et al. (2006)

reported that screening of knockout and RNAi mutant lines of six amino acid transporters

resulted in the identification of lysine histidine transporter 1 as being involved in amino acid

uptake in *Arabidopsis*. Furthermore, amino acid permeases 1 and 5 were shown to

contribute to amino acid uptake in roots of *Arabidopsis* (Lee et al., 2007; Svennerstam et al.,

2008).

Several research groups have reported that plant roots can absorb high molecular weight N

compounds such as protein as a source of N. Matsumoto et al. (2000a,b) reported that plant

roots can absorb phosphate buffer-extractable organic N (PEON) directly. PEON has been

described as a protein-like N compound with a uniform molecular weight of 8000–9000.

They analyzed xylem sap and PEON using a size exclusion column and found a peak with

similar retention time to that of PEON in the xylem sap of bok choy grown in a soil culture,

but not of that grown in an inorganic solution culture (Matsumoto et al., 2000b). They

therefore, concluded that plant roots can absorb PEON directly. In contrast, Miyazawa et al.

(2008) grew leaf lettuce and bok choy with different sources of N; NO_3–N, amino acid, and

PEON, under aseptic conditions. They showed that the growth of leaf lettuce and bok choy

was promoted only by NO_3–N and/or amino acid, and that PEON application had no

significant effect. This indicates that these plant species cannot utilize PEON directly.

However, direct utilization of protein as a source of N in plants should be studied more extensively because endocytosis or protease released from root cells may be involved (Paungfoo-Lonhienne et al., 2008).

In conclusion, this study strongly suggests that organic N acquisition in plants depends on the following two factors: protease activity in the rhizosphere and the ability to absorb amino acids. It would be interesting to characterize the microbiota in the rhizosphere from the view point of protease production and/or competition in amino acid acquisition.

REFERENCES

Adamczyk, B., M. Godlewski, J. Zimny, and A. Zimny. 2008. Wheat (*Triticum aestivum*) seedlings secrete proteases from the roots and, after protein addition, grow well on medium without inorganic nitrogen. *Plant Biology* 10: 718-724.

Badalucco, L., P.J. Kuikman, and P. Nannipieri. 1996. Protease and deaminase activities in wheat rhizosphere and their relation to bacterial and protozoan populations. *Biology and Fertility of Soils* 23: 99-104.

Crecchio, C., M. Curci, R. Mininni, P. Ricciuti, and P. Ruggiero. 2001. Short-term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. *Biology and Fertility of Soils* 34: 311-318.

Fuka, M.M., M. Engel, A. Gattinger, U. Bausenwein, M. Sommer, J.C. Munch, and M.

Schloter. 2008. Factors influencing variability of proteolytic genes and activities in arable

soils. Soil *Biology and Biochemistry* 40: 1646-1653.

Godlewskia, and M., B. Adamczyk. 2007. The ability of plants to secrete proteases by roots.

Plant Physiology and Biochemistry 45: 657-664.

Hirner, A., F. Ladwig, H. Stransky, S. Okumoto, M. Keinath, A. Harms, W.B. Frommer, and

W. Koch. 2006. Arabidopsis LHT1 is a high-affinity transporter for cellular amino acid

uptake in both root epidermis and leaf mesophyll. *Plant Cell* 18: 1931-1946.

Kandeler, E., M. Stemmer, and E.-M. Klimanek. 1999. Response of soil microbial biomass,

urease and xylanase within particle size fractions to long-term soil management. *Soil Biology

and Biochemistry* 31: 261-273.

Kielland, K.. 1994. Amino acid absorption by arctic plants: implications for plant nutrition

and nitrogen cycling. *Ecology* 75: 2373-2383.

Kielland, K., J. McFarland, R. Ruess, and K. Olson. 2007. Rapid cycling of organic nitrogen

in taiga forest ecosystems. *Ecosystems* 10: 360-368.

Kumar, K., C. J. Rosen, and M. P. Russelle. 2006. Enhanced protease inhibitor expression in

plant residues slows nitrogen mineralization. *Agronomy Journal* 98: 514-521.

Ladd J.N., and J.H.A. Bulter. 1972. Short-term assays of soil proteolytic enzyme activities

using proteins and dipeptide derivatives as substrates. *Soil Biology and Biochemistry* 4: 19-30.

Lee, Y., J. Foster, J. Chen, L. Voll, A. Weber, and M. Tegeder. 2007. AAP1 transports uncharged amino acids into roots of Arabidopsis. *Plant Journal* 50: 305-319.

Madejon, E., P. Burgos, R. Lopez, and F. Cabrera. 2001. Soil enzymatic response to addition of heavy metals with organic residues. *Biology and Fertility of Soils* 34: 144-150.

Matsumoto, S., N. Ae, and M. Yamagata. 1999. Nitrogen uptake response of vegetable crops to organic materials. *Soil Science and Plant Nutrition* 45: 269-278.

Matsumoto, S., N. Ae, and M. Yamagata. 2000a. Extraction of mineralizable organic nitrogen from soils by a neutral phosphate buffer solution. *Soil Biology and Biochemistry* 32: 1293-1299.

Matsumoto, S., N. Ae, and M. Yamagata. 2000b. Possible direct uptake of organic nitrogen from soil by chingensai (*Brassica campestris* L.) and carrot (*Daucus carota* L.). *Soil Biology and Biochemistry* 32: 1301-1310.

Miyazawa, K., T. Murayama, and M. Takeda. 2008. Can plants absorb and utilize phosphate buffer extractable soil organic nitrogen without its prior mineralization? *Soil Science and Plant Nutrition* 54: 247-252.

Mori, S., Y. Nishimura, and H. Uchino. 1979. Nitrogen absorption by plant root from the

culture medium where organic and inorganic nitrogen coexist. I. Effect of pretreatment

nitrogens on the absorption of treatment nitrogen. *Soil Science and Plant Nutrition* 25: 39-50.

Okamoto, M., K. Okada, T. Watanabe, and N. Ae. 2003. Growth responses of cereal crops to

organic nitrogen in the field. *Soil Science and Plant Nutrition* 49: 445-452.

Paungfoo-Lonhienne, C., T.G.A. Lonhienne, D. Rentsch, N. Robinson, M. Christie, R.I.

Webb, H.K. Gamage, B.J. Carroll, P.M. Schenk, and S. Schmidt. 2008. Plants can use

protein as a nitrogen source without assistance from other organisms. *Proceedings of the*

National Academy of Sciences of the United States of America 105: 4524-4529.

Sakurai, M., K. Suzuki, M. Onodera, T. Shinano, and M. Osaki. 2007. Analysis of bacterial

communities in soil by PCR-DGGE targeting protease genes. *Soil Biology and Biochemistry*

39: 2777-2784.

Streeter, T.C., R. Bol, and R.D. Bardgett. 2000. Amino acids as a nitrogen source in

temperate upland grasslands: the use of dual labelled (^{13}C, ^{15}N) glycine to test for direct

uptake by dominant grasses. *Rapid Communications in Mass Spectrometry* 14: 1351-1355.

Svennerstam, H., U Ganeteg, and T. Näsholm. 2008. Root uptake of cationic amino acids by

Arabidopsis depends on functional expression of amino acid permease 5. *New Phytologist*

180: 620-630.

Watanabe, T., M. Okamoto, S. Misawa, M. Urayama, and M. Osaki. 2006. Different

characteristics of nitrogen utilization between lupin and soybean: can lupin utilize organic

nitrogen in soils? *Canadian Journal of Botany* 84: 20-27.

Weigelt, A., R. Bol, and R.D. Bardgett. 2005. Preferential uptake of soil nitrogen forms by

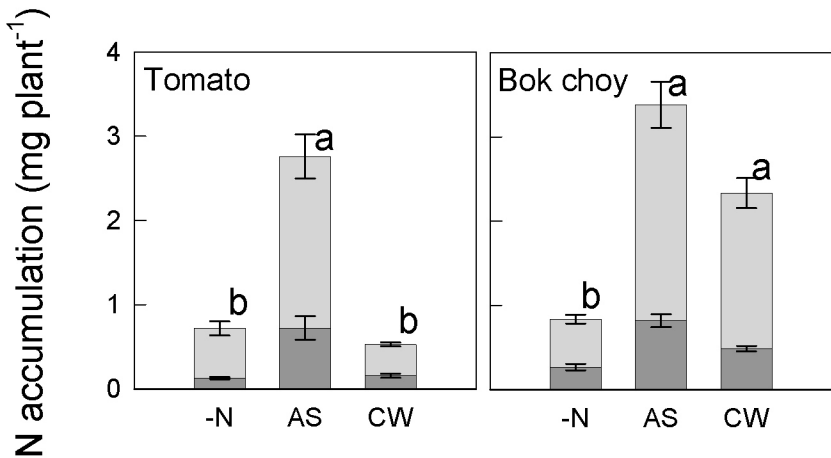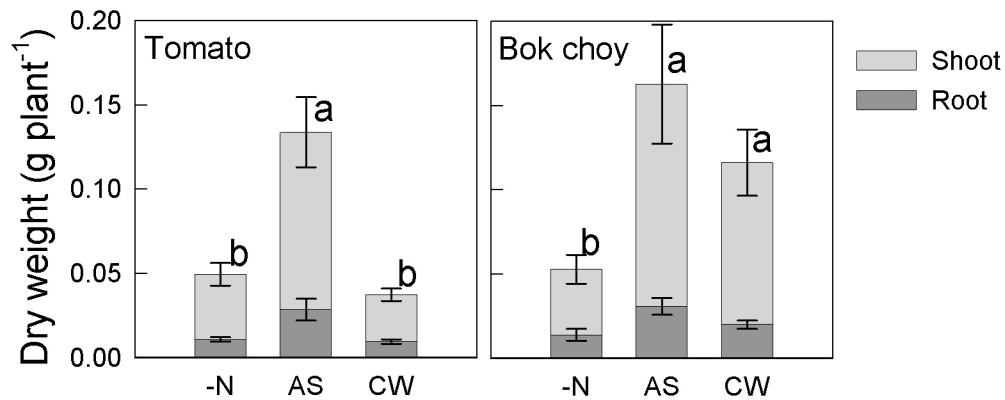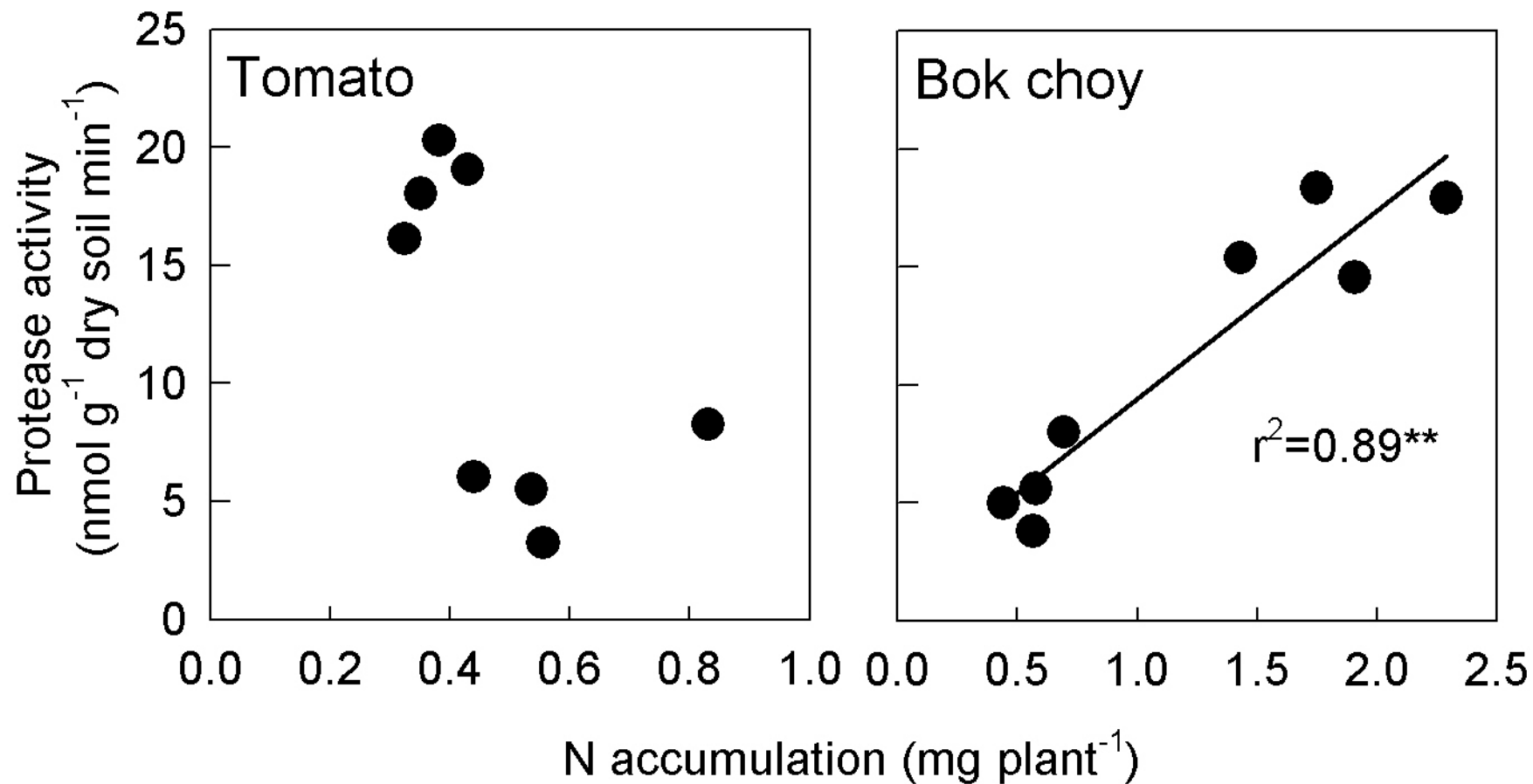grassland plant species. *Oecologia* 142: 627-635.

Figure legends

Figure 1. Dry weight and N accumulation of tomato and bok choy seedlings following treatment. –N, no N application; AS, ammonium sulfate; CM, cattle farmyard manure. Different letters indicate statistically significant differences at $P < 0.05$ in total dry weight or total N accumulation.

Figure 2. Relationship between N accumulation and rhizospheric protease activity in tomato and bok choy seedlings grown with –N (no N application) and CM (cattle farmyard manure) treatments. ** significant correlation at $P < 0.01$.

Figure 3. Inorganic N (NO_3-N and NH_4-N) concentrations in rhizosphere soils. –N, no N application; AS, ammonium sulfate; CM, cattle farmyard manure. Different letters indicate statistically significant differences at $P < 0.05$ in total inorganic N.
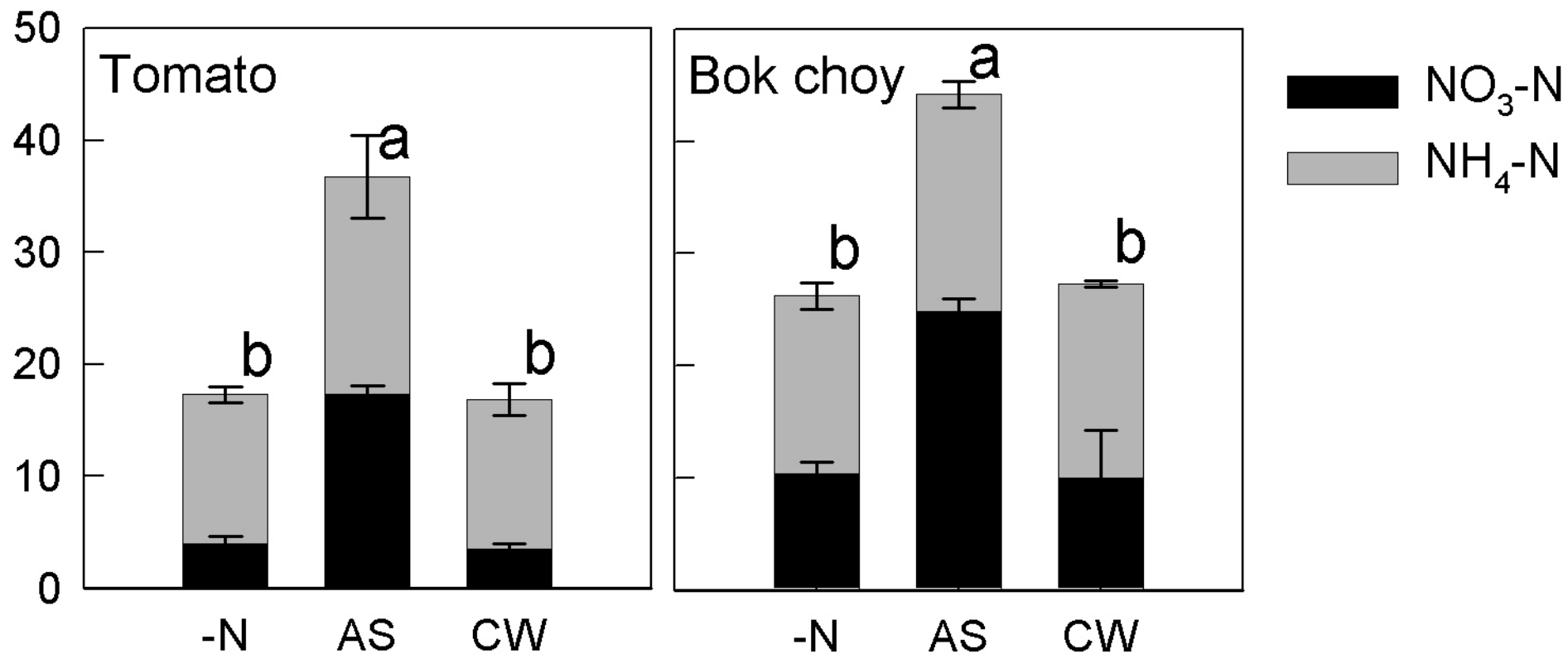
Dry weight (g plant^{-1})

Tomato

Bok choy

Shoot
Root

N accumulation (mg plant^{-1})

Tomato

Bok choy

Tomato

Bok choy

$r^2=0.89$**

Protease activity (nmol g^{-1} dry soil min^{-1})

N accumulation (mg plant^{-1})

Table 1. Chemical properties of field soils used in this study

pH (H_2O)	Total C (g kg^{-1})	Total N (g kg^{-1})	Inorganic N (mg kg^{-1})	Truog-P (mg kg^{-1})	Exchangeable K (cmol(+) kg^{-1})
6.37±0.13	13.9±0.6	1.52±0.09	31.4±1.4	218.5±3.1	100.3±3.9

Table 2. Protease activity (nmol g^{-1} dry soil min^{-1}) in rhizosphere and unplanted bulk soils

	Tomato	Bok choy	Unplanted soil
−N	5.73±1.88	5.57±2.40	8.95±0.79
AS	n.d.	8.03±1.62	n.d.
CM	18.3±1.64	16.54±1.17	13.45±2.88

Table 3. Net uptake rate of glycine and glutamic acid by excised roots of tomato and bok choy (μmol g^{-1} root FW 2h^{-1})

	Gly	Glu
Tomato	4.16±0.61	4.30±0.89
Bok choy	11.1±3.52	3.89±2.03