Front. Agric. China 2009, 3(2): 122–129
DOI 10.1007/s11703-009-0041-y

RESEARCH ARTICLE

Yulin LIAO*, Xiangmin RONG*, Shengxian ZHENG*, Qiang LIU*, Meirong FAN*, Jianwei PENG Guixian XIE

Influences of nitrogen fertilizer application rates on radish yield, nutrition quality, and nitrogen recovery efficiency

Abstract Radishes (*Raphanus sativus* L.) were grown in plastic pots in a screenhouse to investigate the influences of nitrogen fertilizer application rates (NFAR) on yield, nitrate content, nitrate reductase activity (NR), nutrition quality, and nitrogen recovery efficiency (NRE) at commercial mature stage. Five N-rate treatments, 0.644, 0.819, 0.995, 1.170, and 1.346 $g \cdot pot^{-1}$, were set up in the screenhouse pot experiments, and nitrogen fertilizer (unlabeled N and ^{15}N-labeled fertilizer) was applied as basal dressing and topdressing, respectively. The results indicated that the fresh and dry weight yields of radish increased with the increase of NFAR at the range of 0.099 to 0.180 $g\ N \cdot kg^{-1}$ soil, decreased at 0.207 $g\ N \cdot kg^{-1}$ soil, and accordingly there was a significant quadratic relationship between the fresh and dry weight yields of radish and the NFAR. At the high addition of urea-N fertilizer, the nitrate content accumulated in the fleshy roots and leaves due to the decline in NR activity. From 0.644 to 0.819 $g\ N \cdot pot^{-1}$ NR increased most rapidly, the highest NR activity occurred at 0.819 $g\ N \cdot pot^{-1}$, and the lowest NR activity happened at 1.346 $g\ N \cdot pot^{-1}$. Soluble sugar and ascorbic acid initially increased to the highest value and then decreased, and, contrarily, crude fiber rapidly decreased with the increase of NFAR. Total N uptake (TNU), N derived from fertilizer (N_{dff}), and N derived from soil (N_{dfs}) in radish increased, except that N_{dfs} relatively and slightly decreased at the rate of 0.207 $g\ N \cdot kg^{-1}$soil. The ratio of N_{dff} to TNU increased, but the ratio of N_{dfs} to TNU as well as NRE of N fertilizer decreased with the increase of NFAR. Therefore, the appropriate NFAR should be preferably recommended for improving the yields and nutrition qualities of radish and NRE of N fertilizer.

Keywords ^{15}N-labeled nitrogen fertilizer, radish, yields, nutrition quality, nitrogen recovery efficiency

1 Introduction

Nitrogen (N) affects all levels of plant function, from metabolism to resource allocation, growth, and development (Crawford, 1995; Stitt and Krapp, 1999). In modern agricultural production where crops rely on fertilizers to meet their demand for N, more and more N fertilizer is applied in the field to obtain high yield of crop. As a result, inadequate practices inevitably cause environmental problems (Ji et al., 2006), mainly linked to nitrate (NO_3^-) loss in the environment. Presently, with the increasing input of chemical fertilizers, N fertilizer use, therefore, is a major issue as it can result in ground and surface water pollution. Common practices such as high rates of N fertilizer application combined with heavy irrigation are presumed to cause ground and surface water pollution through NO_3^- leaching and soil erosion (Gastal and Lemaire, 2002; Wang et al., 2002).

Most previous studies have shown that excessive application of N fertilizer not only resulted in the lower yield of crops, but also negatively affected the quality of agricultural produce (Hu et al., 1992; Chen et al., 2004). For instance, excessive application of N fertilizer in agricultural production may lead to considerable uptake of NO_3^-, and NO_3^- absorbed by plants, rapidly causing high NO_3^- accumulation in plants, especially in most

Received December 3, 2008; accepted December 30, 2008

Yulin LIAO, Xiangmin RONG (✉), Qiang LIU, Meirong FAN, Jianwei PENG, Guixian XIE
College of Resources and Environment, Hunan Agricultural University, Changsha 410128, China
E-mail: rongxm2005@126.com

Yulin LIAO, Shengxian ZHENG (✉)
Soil and Fertilizer Institute of Hunan Province, Changsha 410125, China
E-mail: sxzheng@ipni.ac.cn

Meirong FAN
Changsha Environmental Protection Colleges, Changsha 410004, China

* These authors contributed equally to this work

vegetable crops. However, NO_3^- has proved to be involved in the occurrence of methaemoglobinemia and possibly in gastric cancer as well as other diseases (Ikemoto et al., 2002; Ishiwata et al., 2002). Consequently, NO_3^- accumulation in plants is a major concern, as a general problem in most crops (Cárdenas-Navarro et al., 1999).

The reduction of NO_3^- consists of two enzymatic steps, including the reduction of NO_3^- to nitrite (NO_2^-) by NO_3^- reductase (NR), and the reduction of NO_2^- to NH_4^+ by NO_2^- reductase. Hence, the excessive accumulation of NO_3^- may result in an imbalance between the uptake and the translocation of NO_3^- to NH_4^+ and subsequently in rapid soil acidification (Wang and Li, 1996). Nevertheless, from another point of view, NO_3^- is highly accumulated possibly due to over-fertilization. It has been found that soil-N and fertilizer-N affected the NO_3^- accumulation in vegetable crops (Hu et al., 1992; Chen et al., 2004).

Radish is one of the most important root vegetables widely cultivated throughout China, and is a nutritionally well-balanced vegetable in existence. However, farmers generally prefer excessive application of N fertilizer during vegetable production to obtain high yields. Unfortunately, the high rate of N fertilizer application sometimes increases the radish yields, and simultaneously may lead to groundwater pollution, decrease N recovery efficiency (NRE), and poorly improve the quality. For minimizing the application of N fertilizer, decreasing production costs, and diminishing underground water contamination, it is necessary to elucidate the response of radish to N application rates (NFAR).

Urea-N fertilizer is widely applied in radish fertilization in southern China. Therefore, the ^{15}N-labeled urea was designed in five N regimes, which were used in our pot experiment with soil culture, closer to the natural environment and agricultural production. The objectives of our study were to determine the effects of different rates of urea-N fertilization on yields and nutrient contents (crude fiber, carbohydrate, ascorbic acid, etc.) of radish, analyze the relationship between NR activity and NO_3^- accumulation at different rates of urea-N fertilizer application, and estimate nitrogen use efficiency of chemical N fertilizer in radish at the different rates of urea-N fertilizer application by the ^{15}N isotope techniques.

2 Materials and methods

2.1 Radish variety and ^{15}N- labeled nitrogen fertilizer

Radish seeds were purchased from Sichuan Zhongdu Seed Co., Ltd. The radish variety was Xinchun. The ^{15}N-labeled urea (5.35% atom excess) was produced by Shanghai Chemical Institute, China. The N content of urea used in this experiment was 46%.

2.2 Soil used for the experiment

The soil (0–20 cm) was taken from upland fields at the farm of Hunan Agricultural University, Changsha, China, and was typical of a reddish yellow clayey earth derived from Quaternary red clay, with pH (H_2O) of 6.4, total organic C of 25.12 $g\cdot kg^{-1}$, total N of 1.56 $g\cdot kg^{-1}$, available N of 121.36 $mg\cdot kg^{-1}$, available P of 15.48 $mg\cdot kg^{-1}$, and available K of 195.76 $mg\cdot kg^{-1}$. The soil type at this site was Ari-Udic Ferrosols. Soil pH was determined using 1∶2 paste (10 g soil to 20 mL water). Soil organic C was determined using a wet oxidation method with $K_2Cr_2O_7$ and concentrated H_2SO_4 (Lu, 2000). Total soil N was determined using the method described by Lu (2000). Soil available P was determined using the Olsen-P method, based on the extraction of air-dry soil with 0.5 $mol\cdot L^{-1}$ $NaHCO_3$ at pH 8.5. Soil available K was measured using the NH_4OAc-extraction method and a flame photometer (Lu, 2000).

2.3 Pot experiment

The pot experiment was conducted in a screenhouse. Pots (20 cm in diameter, 30 cm in depth) with closed bottoms were used to prevent N loss through leaching. The 6.5-kg soil (air-dried and sieved to pass through a 0.5-mm sieve, and mixed thoroughly) was loaded into pots. NFAR for the five N treatments consisted of 0.099, 0.126, 0.153, 0.180, and 0.207 $g\ N\cdot kg^{-1}$ soil (which corresponded to 0.644, 0.819, 0.995, 1.170, and 1.346 $g\ N\cdot pot^{-1}$), respectively. The rate of 0.180 $g\ N\cdot kg^{-1}$ soil was approximately equal to the application rate of 270 $kg\ N\cdot hm^{-2}$ which was the recommended N application rate to radish growers. There were 20 pots per treatment, in 15 of which unlabeled urea was applied, for determining the fresh weight and dry weight yields, and the nutrition quality of the radish. ^{15}N-labeled urea was applied in the other five pots, which was used for calculating NRE.

In this experiment, the rate of 0.180 $g\ N\cdot kg^{-1}$ soil was used as reference treatment, and thus, on the base of 0.180 $g\ N\cdot kg^{-1}$ soil, 55%, 75%, 85%, and 115% of 0.180 $g\ N\cdot kg^{-1}$ soil, namely 0.099, 0.126, 0.153, and 0.207 $g\ N\cdot kg^{-1}$ soil, were applied to estimate the optimal rates of N fertilizer application for the radish. The application amounts of potassium and phosphate fertilizer were 0.10 $g\ K\cdot kg^{-1}$ soil and 0.043 $g\ P\cdot kg^{-1}$ soil in all pots, respectively. Nitrogen fertilizer was applied in four splits: 60% of the total N application rates per treatment as basal dressing, 10% as topdressing on October 1, 10% as topdressing on November 11, and 20% as topdressing on November 21. Phosphate fertilizer was only applied as basal dressing. Potassium fertilizer was applied in three splits: 60% as basal dressing, 10% as topdressing on November 11, and 30% as topdressing on November 21. To allow aeration, a PVC tube was installed in each pot.

During the radish growth period, deionized water was added to the top of the pots, and soil water content was maintained at about 0.25 $m^3 \cdot m^{-1}$ (35 kPa) using time domain reflectometry measurements (Trace, Soil moisture Equipment Crop., USA).

2.4 Plant sampling and analysis

Four radish seeds were sown and thinned to one plant per pot soon after emergence. The plants were grown for 79 days in the screenhouse condition. The plant sampling was conducted 79 days after sowing when it was at the commercial mature stage of the radish. All the fleshy root and leaf samples were taken at the mature stage. The samplings were rinsed repeatedly in deionized water after disinfection with non-detergent at 1%, and then blotted on filter paper.

At sampling, fresh fleshy root and leaf samplings were used for the analysis of nitrate contents, ascorbic acids, and NR. The subsamples of root and leaf were dried in a forced-air oven at 65°C for 24 h, ground in a mill, and then placed in plastic bags until analysis for soluble sugar and crude fiber. Fresh weight (FW) and dry weight (DW) of fleshy roots and leaves were recorded and expressed as $g \cdot pot^{-1}$.

Nitrate (NO_3^--N) content was determined colorimetrically, as described by Wang and Li (1996). NR activity was determined by a slightly modified method as described by Aslam et al. (2001) and Basra et al. (2002). In brief, NR activity was assayed using NADH or NADPH as electron donor. The assay mixture [50 mmol potassium phosphate buffer (pH 7.5), 20 mmol KNO_3], was added to 0.49 mL leaf blade extract in a final volume of 2.0 mL. The reaction was started by adding 0.01 mL NADH or NADPH (1 $mmol \cdot L^{-1}$) with gentle mixture. After incubation at 30°C for 15 min, the reaction was terminated by adding 1∶1 mixture of 1% (w/v) sulphanilamide in 1.5 $mol \cdot L^{-1}$ HCl and 0.2% (w/v) N-naphtylethylenediamine dihydrochloride. After color development for 15 min, the nitrite formed was determined spectrophotometrically by measuring A_{540} (Basra et al., 2002). NR activity was expressed as mg $NO_2^- \cdot g^{-1}$ $FW \cdot h^{-1}$.

Soluble sugar was determined according to the method described by Lowell et al. (1989). Ascorbic acid was determined by the 2, 6-dichlorophenol-indophenol method (Lu, 2000). Crude fiber was determined according to the method of Lu (2000). The above analyzed parameters were calculated on a dry weight basis.

Root and leaf samples from ^{15}N-labeled and unlabeled nitrogen fertilizer pots from each treatment were dried at 60°C to a constant weight. Ground samples were analyzed for total N with a modified Kjeldahl method in which NO_3 was reduced by salicylic acid (Lu, 2000). Total N uptake was calculated as the product of total N concentration in plant parts and dry matter weight. Atom % of ^{15}N in plant samples from ^{15}N pots and unlabeled N pots was determined by spectrometric analysis with a MAT–251 isotope ratio mass spectrometer at the Institute of Agro-Food Science and Technology, CAAS, China.

2.5 Fertilizer nitrogen recovery efficiency

Total N uptake by roots plus leaves per gram of applied N (NRE) was calculated based on ^{15}N (NRE_{dff}) uptake by the crop and by the non-isotopic difference method (NRE_{dff}). In the isotope method, NRE was estimated from the ^{15}N enrichment measurement based on the amount of fertilizer N applied (N_F) and the total N uptake by the crop (TNU), both expressed in $g\ N \cdot pot^{-1}$. N_{dff}TNU was the amount of N derived from the applied fertilizer in total N uptake by the crop, calculated by formula (1):

$$NRE_{isot}(\%)=(N_{dff}TNU/N_F)\times 100, \qquad (1)$$

N_{dff} (the amount of N derived from the applied fertilizer) was calculated by formula (2):

$$N_{dff}=[(\text{atom}\%^{15}\text{N excess in plant tissue})/(\text{atom}\%^{15}\text{N excess in fertilizer})]\times \text{TNU}, \qquad (2)$$

using the ^{15}N abundance in the unfertilized radish plants as the background. The isotope method involves the assumption that the biological interchange of labeled N with unlabeled N is negligible.

The calculation of N derived from soil (N_{dfs}) was based on total N uptake by crop (TNU) and the amount of N derived from the applied fertilizer (N_{dff}), calculated by formula (3):

$$N_{dfs}=TNU-N_{dff.} \qquad (3)$$

2.6 Statistical analysis

Data from the study were analyzed with the ANOVA procedures for statistical analysis (SPSS, for MS Windows release 14.0). Mean separation was accomplished by calculating the least significant difference, with the *LSD* multiple comparison tests ($\alpha=0.05$) for each data set where the initial *F*-test was significant. Correlation coefficients and regression analyses were determined with the SPSS correlation and regression analyses procedure.

3 Results

3.1 Biomass and yield

NFAR had a significant influence on the yields of radish (Fig. 1(a)). The results showed that the fresh and dry weight yields of radish were increased with the increase of NFAR at a range of 0.099 g to 0.180 g $N \cdot kg^{-1}$ soil, but decreased at 0.207 g $N \cdot kg^{-1}$ soil, and thus there were

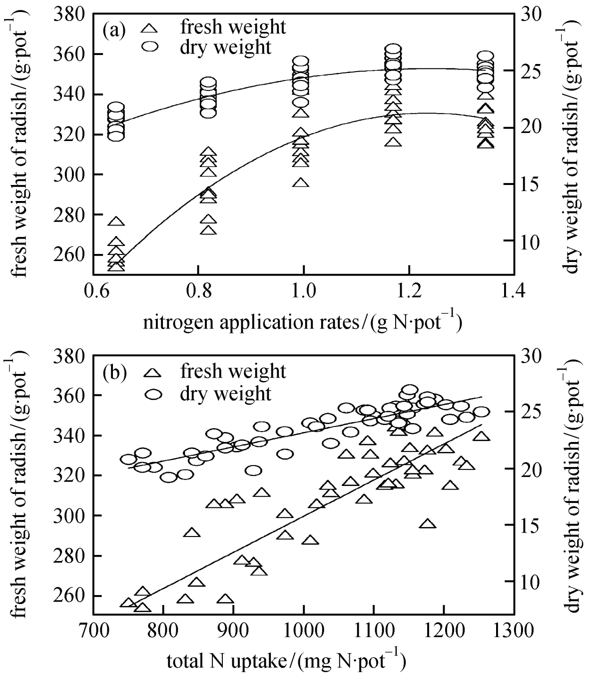

Fig. 1 The correlations of nitrogen fertilizer application rates (a) and total N uptake (b) with fresh weight yield (open triangles) and dry weight yield (open circles) of radish

Note: The lines through the points are least-squares regressions. The regression functions are (a) $y = 2.55 + 531.26x–215.08x^2$ ($R^2 = 0.87$, $P < 0.01$) and $y = 4.07 + 34.01x–13.69x^2$ ($R^2 = 0.82$, $P < 0.01$) for fresh weight and dry weight; (b) $y = 119.24 + 0.24x$ ($R^2 = 0.73$, $P < 0.01$) and $y = 10.66 + 0.0125x$ ($R^2 = 0.77$, $P < 0.01$) for fresh weight and dry weight.

significant quadratic relationships between the NFAR (g N·pot^{-1}) and the fresh weight yields ($R^2 = 0.87$, $P < 0.01$) and the dry weight yields ($R^2 = 0.82$, $P < 0.01$). On the other hand, total N uptake (TNU) in radish positively affected the fresh and dry weight yields of radish (Fig. 1(b)). Correlation coefficients and regression analyses suggested that TNU was positively and linearly correlated with the fresh weight yields ($R^2 = 0.73$, $P < 0.01$) and the dry weight yields ($R^2 = 0.77$, $P < 0.01$).

3.2 Nitrate concentration and nitrate reductase activity

NFAR had a significant influence on NO_3^- content in both fleshy roots and leaf blades (Fig. 2). NO_3^- content decreased most rapidly from 0.644 to 0.995 g N·pot^{-1}, but the increase in NO_3^- content was observed at more than 0.995 g N·pot^{-1}. In addition, the NO_3^- content was generally higher in fleshy roots than in leaves. On the other hand, NR activity increased at the low rate of nitrogen fertilizer application, reaching the maximum value of 10.9 mg NO_2^-·g^{-1} FW·h^{-1} in leaf blades of radish at the rate of 0.819 g N·pot^{-1}, but then the considerable decline in NR activity was observed at the high rate of nitrogen fertilizer application ranging from 0.995 to 1.346 g N·pot^{-1} (Fig. 3). Furthermore, the decline in NR activity presumably affected NO_3^- accumulation in radish (Fig. 4); a linear and negative relationship between the content of NO_3^- and the activity of NR was observed in leaf blades of radish ($R^2 = 0.54$, $P < 0.01$).

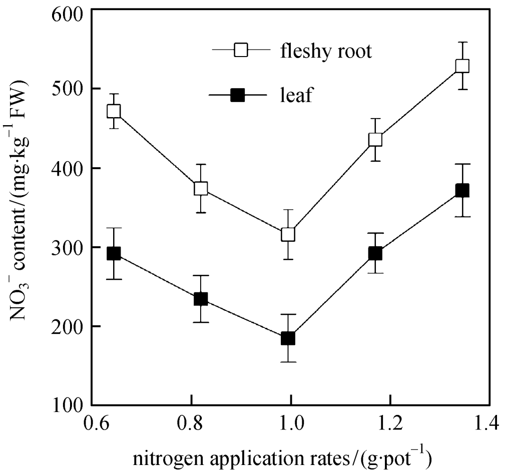

Fig. 2 Influences of nitrogen fertilizer application rates on the contents of NO_3^- in the fleshy roots (open squares) and the leaves (filled squares) of radish

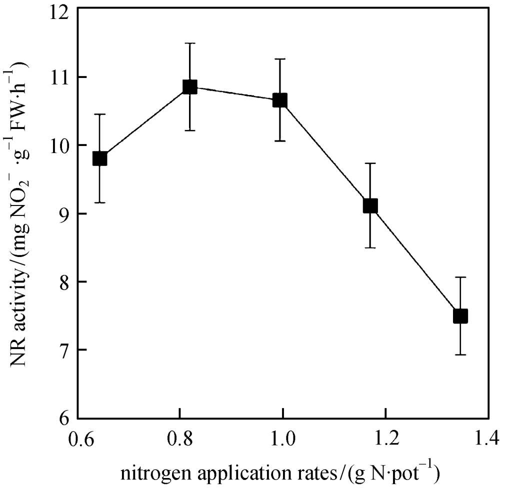

Fig. 3 Influences of nitrogen fertilizer application rates on NR activity in the leaf blades of radish

Note: Data are shown in mean±*SD*, $n = 5$.

3.3 Nutrition quality

NFAR had a significant influence on the soluble sugar and ascorbic acid both in the fleshy root and the leaf of the

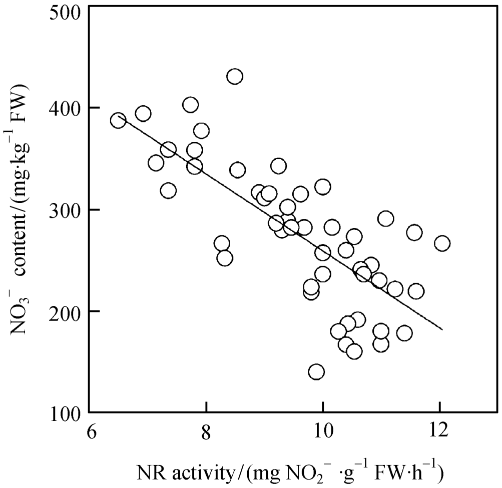

Fig. 4 The relationship between the NO_3^- contents and the NR activity in the leaf of radish

Note: The lines through the points are least-squares regressions. The regression function is $y = 637.08 - 37.75x$ ($R^2 = 0.54$, $P < 0.01$).

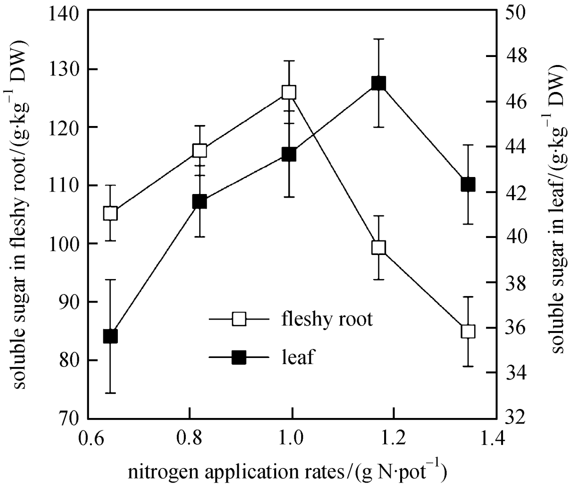

Fig. 5 Influences of nitrogen fertilizer application rates on the contents of soluble sugar in the fleshy roots (open squares) and the leaves (filled squares) of radish

Note: Data are shown in mean±*SD*, *n* = 5.

radish (Figs. 5 and 6). The soluble sugar content reached the maximum value of 126.0 g·kg^{-1} DW in fleshy roots at the rate of 0.995 g N·pot^{-1}, and 46.8 g·kg^{-1} DW in leaves at the rate of 1.17 g N·pot^{-1}. Similarly, ascorbic acid reached the maximum value of 49.9 mg·kg^{-1} DW in fleshy roots at the rate of 0.819 g N·pot^{-1}, and 306.5 mg·kg^{-1} DW in leaves at the rate of 0.995 g N·pot^{-1}. In contrast, crude fiber drastically decreased with the increase of NFAR (Fig. 7), declining from 132.1 to 92.0 g·kg^{-1} DW in fleshy roots, and from 149.1 to 105.0 g·kg^{-1} DW in leaves. Furthermore, there were negative and linear correlations of the content of crude fiber with the total N uptake in the fleshy roots ($R^2 = 0.65$, $P < 0.01$) and the leaves ($R^2 = 0.21$, $P < 0.01$) of radish (Fig. 8).

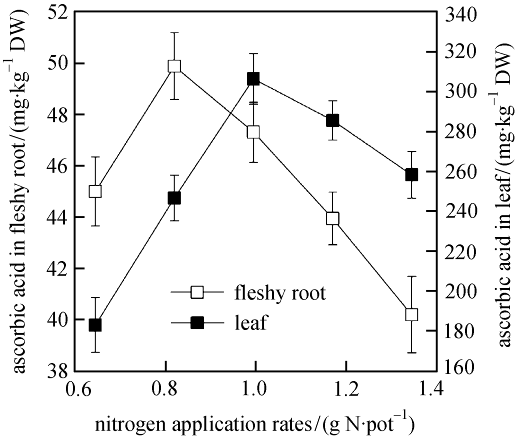

Fig. 6 Influences of nitrogen fertilizer application rates on the contents of ascorbic acid in the fleshy roots (open squares) and the leaves (filled squares) of radish

Note: Data are shown in mean±*SD*, *n* = 5.

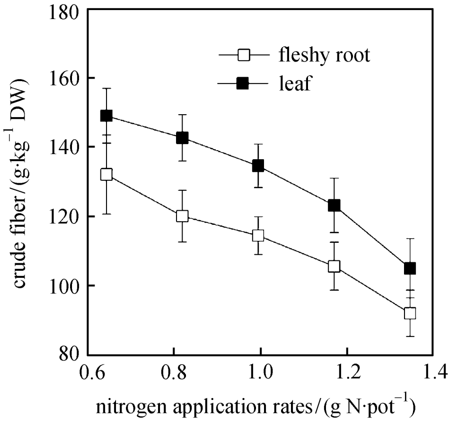

Fig. 7 Influences of nitrogen fertilizer application rates on the contents of crude fiber in the fleshy roots (open squares) and the leaves (filled squares) of radish

Note: Data are shown in mean±*SD*, *n* = 5.

3.4 Nitrogen recovery efficiency

TNU and N_{dff} in radish increased from 824.3 to 1178.7 mg per plant and from 246.3 to 430.5 mg per plant with the increase of NFAR, but N_{dfs} increased to the maximum value of 731.8 mg per plant at the rate of 1.17 g N·pot^{-1}, and slightly declined to 707.4 mg per plant at the rate of 1.346 g N·pot^{-1} (Fig. 9). On the other hand, the ratio of N_{dfs} to TNU gradually decreased, and the ratio of N_{dff} to TNU progressively increased (Fig. 10). In addition, NRE of fertilizer N declined from 38% to 32% at the rates of N fertilizer application ranging from 0.644 to 1.346 g N·pot^{-1} (Fig. 11).

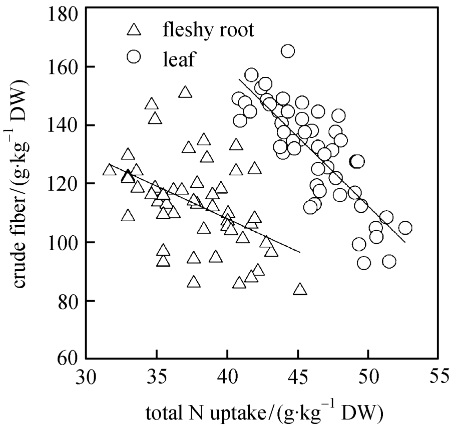

Fig. 8 The correlations of crude fiber content with total N content in the fleshy roots (open triangles) and the leaves (open circles) of radish

Note: The lines through the points are least-squares regressions. The regression functions are $y = 348.63 - 4.73x$ ($R^2 = 0.65$, $P < 0.01$) and $y = 196.89 - 2.22x$ ($R^2 = 0.21$, $P < 0.01$) for the fleshy roots and the leaves.

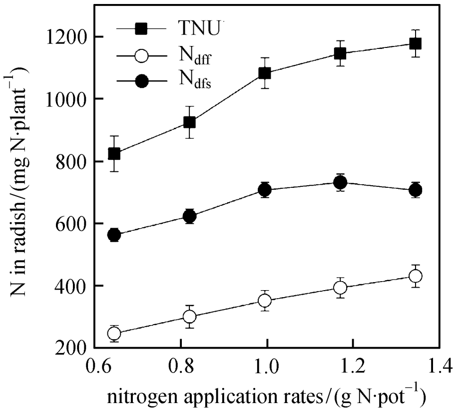

Fig. 9 Influences of N fertilizer application rates on total N uptake (TNU, filled squares), N derived from fertilizer (N_{dff}, open circles), and N derived from soil (N_{dfs}, filled circles) of radish

Note: Data are shown in mean±*SD*, $n = 5$.

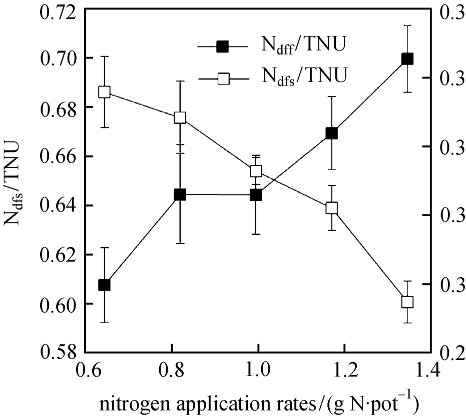

Fig. 10 Influences of nitrogen fertilizer application rates on the ratios of N derived from fertilizer (N_{dff}, filled squares) and N derived from soil (N_{dfs}, open squares) to total N uptake (TNU) in radish

Note: Data are shown in mean±*SD*, $n = 5$.

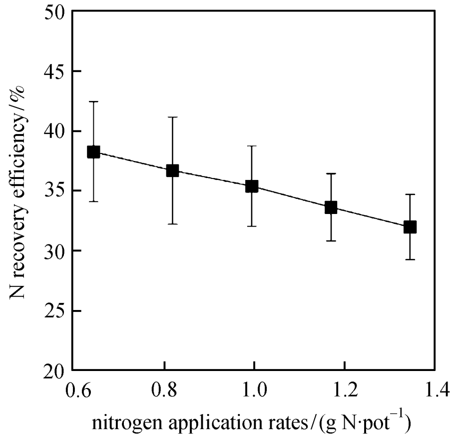

Fig. 11 Influences of nitrogen fertilizer application rates on N recovery efficiency of fertilizer N in radish

Note: Data are shown in mean±*SD*, $n = 5$.

4 Discussion

There were significantly positive and linear relationships between total N uptake and the fresh weight and the dry weight of radish in our experiments (Fig. 1 (b)). Elia et al. (1999) reported that by increasing N level, the yields of spinach increased, and the highest yield of spinach by different N sources application was achieved. However, the results demonstrated that there are quadratic relationships between the NFAR (g N·pot^{-1}) and the fresh weight and dry weight of radish (Fig. 1 (a)), indicating that the yields of radish will not decrease until reaching the maximum yield, but will decrease with the increase of NFAR. The negative influences of the high NFAR on the yields of radish may be due to the accumulation of the ions NO_3^-. The increased NO_3^- accumulation above the optimum substantially suppressed the apparent and potential photosynthesis, reduced the starch accumulation and plant productivity, and modified the pathways of N assimilation in leaves (Ai et al., 2002). In the present experiment, the increase in NO_3^- accumulation in radish at the high NFAR (Fig. 2) could affect the process cited by Ai et al. (2002), principally N assimilation, and therefore explain the reduction of yields. It also agreed with our results that the high accumulation of NO_3^-, due to the high NFAR, reduced productivity in the plants (Sánchez et al., 2004; Chen et al., 2004). Moreover, recent studies indicate that high NO_3^- accumulation results in NO_2^- production which is converted into nitric oxide (NO) in plants, while, in turn, NO and O_2^- can be rapidly catalyzed by NR into peroxynitrite, which is highly toxic to plants (Durner and Klessig, 1999). Consequently, the high NO_3^- accumulation in plants resulting from high nitrogen fertilizer supply was not only harmful to human health but also detrimental to plant growth (Ikemoto et al., 2002; Ishiwata et al., 2002). Hence, the reduced yields of radish after reaching the highest yield might be caused by excessive accumulation of NO_3^- due to the high NFAR.

The high NFAR would lead to the increment in NO_3^- in radish (Fig. 2). It is consistent with some previous reports. For instance, Elia et al. (1999) found that the high NFAR increased the NO_3^- content in spinach. Nevertheless, in our experiment, the content of NO_3^- initially declined to the lowest value, and then increased (Fig. 2); on the contrary, NR activity firstly increased to the highest value, and then decreased (Fig. 3) with the increasing addition of urea-N fertilizer. Such variation in NO_3^- content might be attributed to the changes in NR activity in response to the varying NFAR. NR activity was higher, suggesting that more NO_3^- might be reduced to NO_2^-, and consequently the accumulation of NO_3^- decreased. Therefore, the negative and linear correlation of NR activity with the NO_3^- content was observed in the leaves of radish (Fig. 4). It agrees with the previously reported result, that the higher NR activity was, the more NO_3^- nitrate might be reduced, and thereby, there was a negative relationship between NR activity and NO_3^- concentration (Hu et al., 1992). Indeed, most studies showed that, with NR being a substrate-induced enzyme, the higher the substrate-nitrate concentration was in plants, the higher the NR activity might be, so there was a positive correlation between them (Zheng et al., 1995). Also, some investigations demonstrate that a very small amount of NO_3^- is sufficient for induction (Samuelson et al., 1995; Scheible et al., 1997; Matt et al., 2001), specifically, NR activity is not induced by NO_3^- when NO_3^- concentration is higher than a certain level (Joseph and Michael, 1979). In addition, it has been found that the activities of NR and NiR might have been inhibited by the presence of high NH_4^+ concentrations (Lam et al., 1996; Ivashkina and Sokolov, 1997). In our experiment, NH_4^+ was analyzed, but it may be speculated that NH_4^+ would increase with the increase of NFAR, because the capacities for incorporation of NH_4^+ into organic compounds were limited. Therefore, the decline in NR activity observed in the present study may be presumably attributed to the increased NH_4^+ due to the increasing NFAR, and thereby the increased NO_3^- might be a consequence of the decline in NR activity.

The contents of soluble sugar and ascorbic acid in radish increased at the low NFAR, and then decreased at the high NFAR (Figs. 5 and 6). The accumulation of carbohydrates in plant species is derived from photosynthesis, which correlates strongly with leaf N concentration. The increments in N content may enhance photosynthesis, and consequently increase accumulation of carbohydrates; contrarily, excessive N in leaves decreases photosynthesis and carbohydrates (Foyer et al., 1998). Therefore, the decreased ascorbic acid and soluble sugar were observed in radish at the high NFAR, and the increased ascorbic acid and soluble sugar were found at the low NFAR. It is consistent with the reported result that low N availability induced carbohydrate accumulation in leaves (Noguchi and Terashima, 2006). The N assimilation in the leaves of higher plants requires both energy and C skeletons. The increments in N content indicate that much more photosynthates have to be invested to N assimilations, and thereby the crude fiber content in radish decreased with the increase of NFAR (Fig. 7). Texture is an important factor determining the sensory quality of vegetables, and fibrousness is undesirable (Shou et al., 2007). The crude fiber development in plant tissues is influenced by a variety of factors. In our experiment, there is a significant negative relationship between N content and crude fiber content in fleshy roots and leaves (Fig. 8). Hence, the appropriate rates of chemical N fertilizer application may improve the quality of vegetable species.

The TNU, N_{dff}, and N_{dfs} in radish increased with the increase of NFAR, but then N_{dfs} relatively slightly decreased at the high NFAR (Fig. 9). It has been quite well established that fertilizer N applied to crops interacts with native soil N, resulting in an increase in uptake of the latter (Zheng et al., 1994). The enhanced soil N availability was termed added N interaction (ANI). In our experiment, ANI markedly occurred in radish at the low NFAR. Nevertheless, at the high NFAR, N_{dfs} in radish slightly decreased, and ANI relatively declined. In general, the increased N_{dfs} in radish with the increase of NFAR was presumably attributable to ANI. On the other hand, the ratio of N_{dff} to TNU increased, but the ratio of N_{dfs} to TNU decreased (Fig. 10) and meanwhile, NRE of fertilizer N gradually declined with the progressive increase of NFAR (Fig. 11). The addition of labeled-N fertilizer results in an increase in plant uptake of unlabelled N (native soil N), indicating the possible decline in the uptake of fertilizer nitrogen applied to soil, and thereby leading to the decrease in NRE of fertilizer N for radish. An increment in the availability of soil N due to fertilizer application has been reported by other researchers (Zheng et al., 1995). In our experiment, TNU and N_{dff} in radish increased, but the ratio of N_{dfs} to TNU as well as NRE of fertilizer N rapidly declined with the increase of NFAR. Therefore, the appropriate NFAR should be applied to radish for enhancing NRE of chemical N fertilizer and decreasing the waste of nitrogen fertilizer.

5 Conclusions

The NFAR can influence not only the yields, but also the quality of radish. At the high NFAR, radish can readily accumulate NO_3^- due to the decline in NR activity, and consequently the increased NO_3^- content above the optimum may reduce the yields of radish. In addition, the high NFAR can enhance the availability of soil N, and decrease NRE of fertilizer N.

Acknowledgements This research was funded by the International Plant Nutrition Institute, China (IPNI China Program: Hunan-11) and the Hunan Provincial Key Subject, China (No. 04NK2006). We thank the workers at the farm of Hunan Agricultural University for assistance with the experiments. We also thank our colleagues for providing basic soil data.

References

Ai S Y, Yao J, Huang W, Luo X H, Ke Y S, Ling D Q (2002). Study on the nitrate reduction characteristic of vegetables. Plant Nutrition and Fertilizer Science, 8: 40–43 (in Chinese)

Aslam M, Travis R L, Rains D W (2001). Enhancement of nitrate reductase activity and metabolic nitrate concentration by methionine sulfoximine in barley roots. Plant Science, 161: 133–142

Basra A S, Dhawan A K, Goyal S S (2002). DCMU inhibits *in vivo* nitrate reduction in illuminated barley (C_3) leaves but not in maize (C_4): a new mechanism for the role of light. Planta, 215: 855–861

Cárdenas-Navarro R, Adamowicz S, Robin P (1999). Nitrate accumulation in plants: a role for water. Journal of Experimental Botany, 50: 613–624

Chen B M, Wang Z H, Li S X, Wang G X, Song H X, Wang X N (2004). Effects of nitrate supply on plant, nitrate accumulation, metabolic nitrate concentration and nitrate reductase activity in three leafy vegetables. Plant Science, 167: 635–643

Crawford N M (1995). Nitrate: nutrient and signal for plant growth. Plant Cell, 12: 2383–2349

Durner J, Klessig D F (1999). Nitric oxide as a signal in plants. Current Opinion in Plant Biology, 2: 369–374

Elia A, Santamaria P, Serio F (1999). Nitrogen nutrition, yield and quality of spinach. Journal of the Science of Food and Agriculture, 76 (3): 341–346

Foyer C H, Valadier M H, Migge A, Becker T W (1998). Drought-induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves. Plant Physiology, 117: 283–292

Gastal F, Lemaire G (2002). N uptake and distribution in crops: an agronomical and ecophysiological perspective. Journal of Experimental Botany, 53: 789–799

Hu C X, Deng B E, Liu T C (1992). Effects of nitrogen fertilizer on nitrate accumulation by the Chinese cabbage (*Brassica chinenses*) and tomato (*Lycopersicum esculentum*). Journal of Huazhong Agricultural University, 11: 239–243 (in Chinese)

Ikemoto Y, Teraguchi M, Kogayashi Y (2002). Plasma level of nitrate in congenital heart disease: comparison with healthy children. Pediatric Cardiology, 23: 132–136

Ishiwata H, Yamada T, Yoshiike N, Nishijima M, Kawamoto A, Uyama Y (2002). Daily intake of food additives in Japan in five age groups estimated by the market basket method. European Food Research and Technology, 215: 367–374

Ivashkina N V, Sokolov O A (1997). Regulation of nitrate distribution in maize seedling by nitrate, nitrite, ammonium and glutamate. Plant Science, 123: 29–37

Ji X H, Zheng S X, Lu Y H, Liao Y L (2006). Dynamics of floodwater nitrogen and its runoff loss, urea and controlled release nitrogen fertilizer application regulation in rice. Scientia Agricultura Sinica, 39: 2521–2530 (in Chinese)

Joseph H S, Michael J P (1979). *In vitro* stability of nitrate reductase from wheat leaves. Plant Physiology, 63: 346–353

Lam H M, Coschigano K T, Oliveira I C, Melo-Oliveira R, Coruzzi G M (1996). The molecular-genetics of nitrogen assimilation into amino acids in higher plants. Annual Review of Plant Physiology and Plant Molecular Biology, 47: 569–593

Lowell C A, Tomlinson P T, Koch K E (1989). Sucrose metabolizing enzymes in transport tissue and adjacent sink structures in developing citrus fruit. Plant Physiology, 90: 1394–1402

Lu Y K (2000). Analysis Methods of Soil Agricultural Chemistry. Beijing: China Agricultural Scientech Press (in Chinese)

Matt P, Geiger M, Walch-Liu P, Engels C, Krapp A, Stitt M (2001). The immediate cause of the diurnal changes of nitrogen metabolism in leaves of nitrate-replete tobacco: a major imbalance between the rate of nitrate reduction and the rates of nitrate uptake and ammonium metabolism during the first part of the light period. Plant Cell and Environment, 24: 177–190

Noguchi K, Terashima I (2006). Responses of spinach leaf mitochondria to low N availability. Plant Cell and Environment, 29: 710–719

Samuelson M E, Campbell W H, Larasson C M (1995). The influence of cytokinins in nitrate regulation of nitrate reductase activity and expression in barley. Physiologia Plantarum, 93: 533–539

Sánchez E, Rivero R M, Ruiz J M, Romero L (2004). Changes in biomass, enzymatic activity and protein concentration in roots and leaves of green bean plants (*Phaseolus vulgaris* L. cv. Strike) under high NH_4NO_3 application rates. Scientia Horticulturae, 99: 237–248

Scheible W R, Lauerer M, Schulze E D, Caboche M, Stitt M (1997). Accumulation of nitrate in the shoot acts as signal to regulate shoot-root allocation in tobacco. Plant Journal, 11: 671–691

Shou S Y, Lu G, Huang X Z (2007). Seasonal variation in nutritional components of green asparagus using the mother fern cultivation. Scientia Horticulturae, 112: 251–257

Stitt M, Krapp A (1999). The molecular physiological basis for the interaction between elevated carbon dioxide and nutrients. Plant Cell and Environment, 22: 583–622

Wang Z H, Li S X (1996). Relationships between nitrate contents and water, total N as well as total P in different organs of vegetable plants. Plant Nutrition and Fertilizer Science, 2: 144–152 (in Chinese)

Wang Z H, Zong Z Q, Li S X, Chen B M (2002). Nitrate accumulation in vegetables and its residual in vegetable fields. Environment Science, 23: 79–83 (in Chinese)

Zheng G S, Peng G Y, Zhang Q G (1994). Studies on N-utilization of leaf-vegetables with ^{15}N tracer technique. Acta Agriculturae Universitatis Pekinensis, 20: 257–261 (in Chinese)

Zheng G S, Peng G Y, Zhang Q G (1995). The studies on the nitrate accumulation in celery with ^{15}N tracer techniques. Acta Agriculturae Nucleatea Sinica, 9: 42–46 (in Chinese)