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Modeling Nitrogen Uptake in Eight Common Leafy Vegetables in Red River Delta, Vietnam

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ABSTRACT

Fertilizer originated nitrate excess in vegetables has attracted numerous studies for its effects on food quality. However, the relationship between plant nitrate accumulation and fertilizer-derived nitrogen (FTN) in the soil in continuous research is rarely reported. This study examines the impact of conventional ammonium fertilizer application (50.4 kg/ha) on the constant trend of soil nitrogen and plant nitrate uptake of 8 common leafy vegetables grown in Red Delta River, Vietnam. The vegetables reveal both FTN and plant nitrate took about 17 days to release from topsoil and plant. The trend of FTN is well fitted by a regression model (Decay model, R2=0.945, p<0.001), which shows the nitrogen loss rate of FTN range from 0.120-0.139 g N/day. Meanwhile, the trend of plant nitrate uptake fitted the quadratic equation (R2=0.889, p<0.01). Although the correlation between FTN and plant nitrate is weak, this study finds that autumn crops have a tighter relationship than summer crops (R=0.71 and R=0.46, respectively). It can be concluded that regression models could be suitable methods to observe the behavior of fertilizer nitrogen in soil and vegetable uptake.

INTRODUCTION

Nitrogen is one of the two essential elements of nutrients (together with carbon) in soil, affecting vegetable growth and quality. Application fertilizers of both main styles strongly regulate soil nitrogen content: chemical fertilizer and non-chemical fertilizer (Cheng et al., 2016; Nguyen-Sy, Cheng, et al., 2020; Nguyen-Sy, Tan, et al., 2021; Tang et al., 2022). Together with ammonium, nirate plays a vital role in plant growth. It is well known that ammonium and nitrate are the two main forms of nitrogen that plants can absorb directly from input sources from the soil (Attri, Koga, Okumura, Takeuchi, & Shiratani, 2021). In the recent decades, several reports mentioned about chemical fertilizer overuse in many big fertilizer consumer countries like India (Randive, Raut, & Jawadand, 2021), China (Ren et al., 2021; Wan & Long, 2022; Wu et al., 2021), and also worldwide (Liu & Wu, 2022; Randive, Raut, & Jawadand, 2021; Wan & Long, 2022; Wang, Zhu, Zhang, & Wang, 2018). However, the overdose of ammonium has negatively impacts on plants and the environment. It is reported that some 50% of chemical fertilizer application in agriculture lost in agricultural use (Savci, 2012a; 2012b; Wang, Zhu, Zhang, & Wang, 2018). Plants absorb nitrogen from the soil in nitrate form before converting it into protein and other substances. The impact of organic fertilizers on vegetable growth and nitrate uptake has attracted research in the last few decades (Maynard, Barker, Minotti, & Peck, 1976) until recently (Bandian, Nemati, & Moghaddam, 2018; Kyriacou, Soteriou, Colla, & Rouphael, 2019; Wang & Li, 2004; Zandvakili et al., 2019). Noroozlo, Souri, & Delshad (2019) report that the leaf N concentration increases by organic fertilization in sweet basil.

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The application of ammonium fertilizer is also addressed to have a substantial effect on nitrate uptake in spinach, a common vegetable (Bandian, Nemati, & Moghaddam, 2018; Wang & Li, 2004). The nitrogen uptake excess in vegetables has been shown clearly in numerous previous studies (Duan, Fan, Zhang, & Xiong, 2019; Qin et al., 2019; Taneja, Labhasetwar, & Nagarnaik, 2019; Wang & Li, 2004). Recent research on nitrate uptake in vegetable crops showed that more attention should be paid, especially in rural areas (Muhaidat, Al-Qudah, Al-Taani, & AlJammal, 2019; Taneja, Labhasetwar, & Nagarnaik, 2019). The soil nutrients regulate the vegetable nitrate intake, fertilizer, crop cultivars, and environment (Abdo et al., 2020; Barker, Meagy, Eaton, Jahanzad, & Bryson, 2017; Kyriacou, Soteriou, Colla, & Rouphael, 2019).

Red River Delta is one of two central agricultural practice regions in Vietnam, besides the Mekong River Delta. The region is about 17.000 km^2 and plays a vital role in producing vegetables. The risk of vegetable nitrate uptake in this region is still a severe problem. Despite several research studies focused on the impact of fertilizer on vegetable nitrate uptake, there is no report on modeling the trend of fertilizer nitrogen content in soil and vegetable nitrate uptake. Therefore, this study attempts to obtain a regression model to predict the behaviors of fertilizer nitrogen in soil regarding the plant nitrate uptake in a continuous observation of common vegetables in Vietnam.

MATERIALS AND METHODS

Site Description

In June and October 2019, the samples collected for this study were from Co Dien village, Hanoi, northern Vietnam, situated along the Red River Delta (21°11' N, 105°79'E). The region features a warm, humid subtropical climate, with the mean annual temperature at the Hanoi Meteorological Station is 23.6°C, and the mean annual precipitation is 1800mm. These are vegetables produced specially targeted for Hanoi and sub-regions.

Soil and plant samples were collected from eight vegetables produced in fields, grown with the common vegetables produced in this region. The initial soil properties had a pH of 5.2, organic carbon of 11.1 g/kg, total nitrogen of 1.49 g/kg, and CEC of 0.71 meq/g soil. The soil texture was defined as alluvial soil (45.6% sand, 39.9% clay, 14.5% silt). Eight vegetable types were divided into different plots—the plot size with a width of 1.5 m and a length of 18 m. Five summer vegetables i.e., Amaranthus, *Basella alba* L., Corchorus, *Ipomoea aquatica*, *Sauropus androgynus* (abbreviated as Ama, Bas, Cor, Ipo, and Sau, respectively) were grown in late May. Plant samples were collected in June, while three primary autumn vegetables i.e., *Brassica juncea*, Mustard Green, *Brassica integrifolia* (abbreviated as BrJ, Mus, and BrI, respectively) and soil samples were collected in October. This study collected soil samples with three replicates down to 20 cm because all plots' roots mostly reached this depth. The soil and plant statement before ammonium fertilizer addition is described in Table 1. The ammonium fertilizer was applied at a rate of 50.4 kg/ha. Fertilizer was spread immediately after transplanting, and on days 3, 5, 7, 9, 11, 13, 15, 17, soil and plant samples were collected for measures of total soil nitrogen and plant nitrate uptake.

Nitrate and Total Nitrogen Measurement

The collected samples of vegetables were analyzed for NO_3^- following the standard protocols described by Singh (1988). In short, a sample of fresh tissue was taken and crushed thoroughly. Fifty milliliters of 2.0% acetic acid were added. The powder mixture was added to the extract; after 10 minutes, a pinkish purple color was developed and measured in a colorimeter (JENWAY 6305) at 540 nm. Additionally, total soil nitrogen was measured by Kjeldahl procedures (Bremner & Mulvaney, 1982).

Calculation and Data Analysis

After fertilizer addition, the soil samples of all plots were taken every two days. It was considered that during the experiment survey (17 days), the changes in initial soil total nitrogen were negligible; therefore, the soil fertilizer nitrogen was calculated as:

$FTN = TN - TN_o$ .... 1)

where FTN is fertilizer-derived nitrogen content remaining in topsoil, TN is the total soil nitrogen content, and TN_o is the original complete soil nitrogen content determined before fertilizer addition (described as TN in Table 1).

SPSS version 20 software was used to generate the N-fertilizer remaining time curve by Exponential Decay, Single,2 Parameter model as described by the following equation:

f = a*exp(-bt) .... 2)

where a is the N-fertilizer lost potential (g N/kg), b is the N-fertilizer loss rate constant (per day), and t is time (day).

A quadratic equation modeled the nitrate accumulation in the plant:

$F = at^2 + bt + c$ .... 3)

where a, b, c were constants, t was the time (day).

Correlation coefficients were used to assess the significance of pairwise relationships between the main parameters at three-level *P*=0.05, 0.01, and 0.001.

RESULTS AND DISCUSSION

Changes in Soil Total Nitrogen

After three days of adding fertilizer, soil TN reached an average of 10.60 g/kg, about 6.03 times higher than the original soil. However, after that, all treatments resulted in a continuous decrease. On day 17, the soil N meets with the original soil TN (Fig. 1). FTN is derived in two pathways: the first one is absorbed by the plants, and the second is runoff through the ground. All changes in the vegetables are similar to each other and followed the regression model for exponential decay (R^2>0.96) (Table 2).

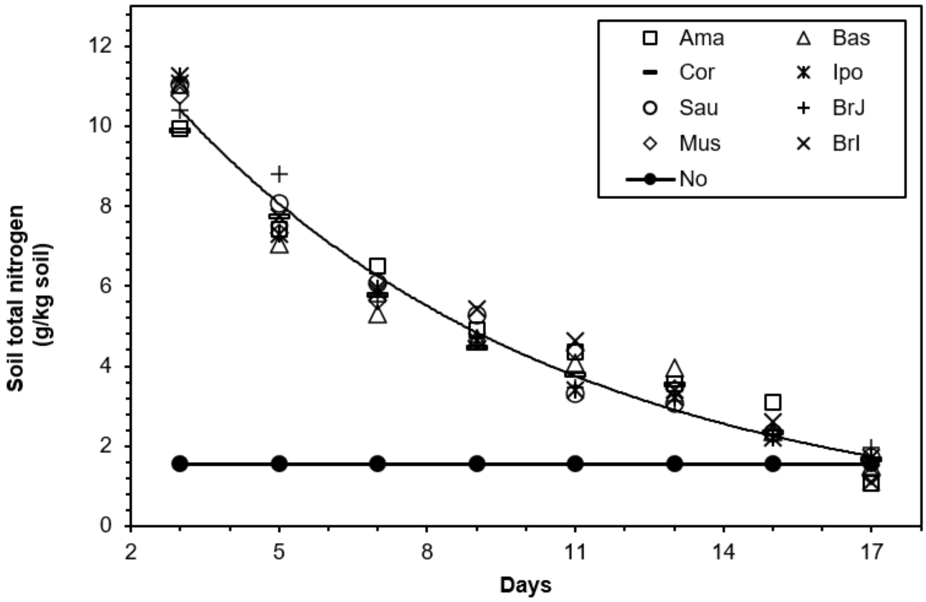

Fig. 1. Changes in Soil Total Nitrogen after 17 days addition of fertilizer

Table 1. Soil and plant properties in the experiment before fertilizer addition

Vegetable types	Abbreviation	Culture cycle	Soil total nitrogen (TN)	Plant nirate content (NO_3^--N)
		Days	g N/kg soil	g N/kg plant
Summer Season (in May)				
Amaranthus	Ama	30-35	1.73	36.8
Basella alba L.	Bas	30-35	1.71	49.4
Corchorus	Cor	30-35	1.75	47.4
Ipomoea aquatica	Ipo	35-40	1.80	54.7
Sauropus androgynus	Sau	35-40	1.75	52.8
Autumn Season (in October)				
Brassica juncea	BrJ	30-35	1.55	48.2
Mustard Green	Mus	40-45	1.57	56.9
Brassica integrifolia	BrI	30-35	1.50	46.3

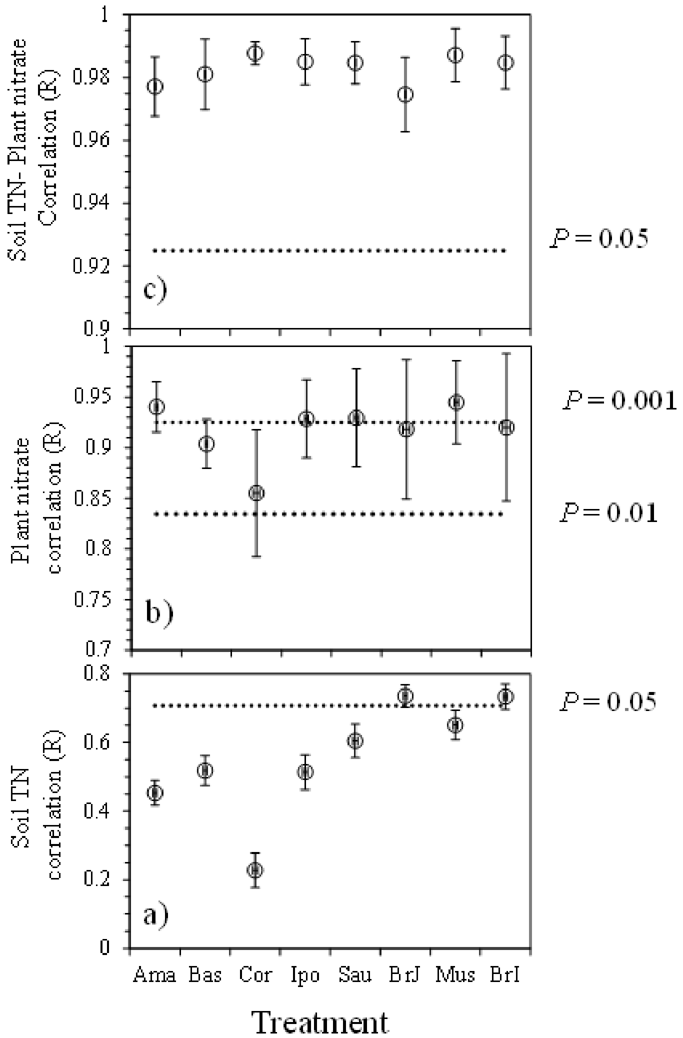

Fig. 2. The average correlation each treatment to others for N –fertilizer absorbed in soil a), NO_3^- accumulated in plant b), and between them c). The dot line (…) presents for the significant correlation at different level *P*=0.05, 0.01 and 0.001. The bar was the standard deviation.

Table 2. The trend of soil total nitrogen

Vegetable types	a (constant)	B (g N/kg soil/day)	R^2
Ama	15.83	0.130	0.875
Bas	14.47	0.122	0.936
Cor	13.99	0.120	0.980
Ipo	14.81	0.126	0.986
Sau	15.68	0.129	0.989
BrJ	14.33	0.120	0.983
Mus	15.77	0.133	0.944
BrI	17.42	0.139	0.916
Average	15.29	0.127	0.951

Remarks: All parameters obtained with the equation: f = a*exp(-bt)

The slow release of nitrogen in soil organic matter is comparable to inorganic fertilizers since organic nitrogen typically does not provide nitrogen in a readily accessible form (Herencia, García-Galavís, Dorado, & Maqueda, 2011). In a short period, changes in soil total nitrogen could be negligible. For the changes in total nitrogen in the soil, after 17 days of fertilizer application, the total nitrogen equals the original soil's total nitrogen. This study proposes that the fertilizer-derived nitrogen is almost released from the soil in all plots (Fig. 1).

The average air temperature recorded in May was 28.9°C, and in October was 26.8°C from the Hanoi meteorological station. This temperature is favorable for most vegetables to uptake nitrate (Bose & Srivastava, 2001). Hence the effects of temperatures would be eliminated among eight plant species. Although the released rates varied among plots, it is found that Bas and Ipo have the highest rates, while the lowest are Ama and Sau. They are all summer vegetables, and this study cannot explain the actual reasons for this soil behavior (Fig. 2).

Changes in Vegetable Nitrate Accumulation

The changes in vegetable nitrate accumulation are shown in Fig. 3 and Table 3. The trend of nitrate is divided into two periods: the initial period is from the beginning to day 9, and the second period is the remaining time. It is clear that plant nitrate concentration increased quickly after fertilizer addition and reached maximum on day nine. After three days, the nitrate concentration of most of the vegetables came to over 500 mg N/kg plant, and this stayed until day 13 after fertilizer application. It is estimated about 17 days for nitrate to be released in all the vegetables down to the original before fertilizer application. Similar to soil TN changes, trend and duration for changes in plant nitrate are identical in all plot treatments. The trend correlation of each treatment to others is also presented in Fig. 2b. Similar to nitrogen trends, plant nitrate in each plot correlates with the others ($p<0.01$). However, the relationship was weaker ($R_{Nitrate}=0.92$ compared with $R_{Nitrogen}=0.983$ on the average of 8 treatments). Additionally, Cor was the anomaly for nitrate accumulation correlation with other plants, with the lowest correlation (R= 0.855).

After fertilizer addition, the increase rates of eight vegetables are different in the first three days, and then they tend to get close to each other at the maximum values. All the vegetables are leafy vegetables that can accumulate high nitrate compared to other vegetables (Colla, Kim, Kyriacou, & Rouphael, 2018; Pate, 1980). The three autumn vegetables belong to brassica species, which have a large leaf area compared to the others. These may lead to the initial uptake of nitrate. However, the nitrate accumulation potential between the two groups is not significantly different (Table 3). Therefore, our finding proves that the leafy vegetables enhanced the nitrate uptake rate, but not for the potential uptake content. The potential nitrate uptakes in eight vegetables are from 569-622 mg/kg fresh weight, which is not very high compared to the safety standard in Vietnam (500 mg/kg). It is similar to the typical leafy plant nitrate absorbance reported elsewhere (Parks, Huett, Campbell, & Spohr, 2008). Although the mechanism is unclear, one possible explanation: in autumn, the temperature is lower than in summer, making it quickly transform ammonia nitrogen into available nitrate for vegetables.

Plant nitrate uptake behavior differed when divided into two phases. In the first phase, when enough amount of available nitrogen exists in the soil, plant roots absorb them very quickly in the first five days. Since the original plant nitrate was low, it resulted in a demand for supplemental nitrogen for growth. It is clear that nitrate demand in eight vegetables is likely similar but with different absorbed rates. To determine the other changing trends among eight vegetables, the correlation of each plot must remain at seven plots. The relationship among treatments in fertilizer nitrogen content is high and stable ($P<0.01$), meanwhile, the correlation in nitrate uptake is less stable and sensitive (Fig. 2). It is not surprising that the nitrate derived in plants is primarily determined by its different species.

Relationship Between Total Soil Nitrogen and Plant Nitrate

The trend correlation of each treatment to others is also presented in Fig. 2a. It features a high correlation among treatments ($P<0.001$) for all treatments. It confirms that nitrogen lost in soil was not managed by the grown vegetables but by the soil property and environmental conditions. The correlation between soil fertilizer and nitrogen showed in Fig. 2c. As an observation, there are only two treatments BrJ and BrI which showed a significant level ($P<0.05$), while the others did not establish any vital relationship. The Cor has the lowest correlative relationship with other treatments. All autumn vegetables have a higher correlation than summer vegetables (R=0.706 compared to R=0.460).

The complex relationship is shown in Fig. 4. The treatments are in two groups regarding the two trends of plant nitrate tendency. The first period is 3-9 days, and the second period is 11-17 days after fertilizer addition. In short, plant nitrate is in a high relationship with soil fertilizer nitrogen (R=0.784) which is applied to the first period (3-9 days after fertilizer addition) (Fig. 4a). Meanwhile, appearance with a negative relationship was observed in the second period (R=0.467).

The correlation between fertilizer nitrogen and nitrate uptake is weak, but the relationships are significant (P=0.05) (Fig. 3b and 3c) for the three brassica vegetables. It reconfirms that these three vegetables are more sensitive to fertilizer than the others. Cor is anomalous compared to other vegetables, showing the lowest correlation with others. Since the trend of nitrate is derived in two ways, which increase at the beginning and decrease in the later phase, it resulted in similar behaviors in terms of relationship with fertilizer nitrogen (Fig. 4).

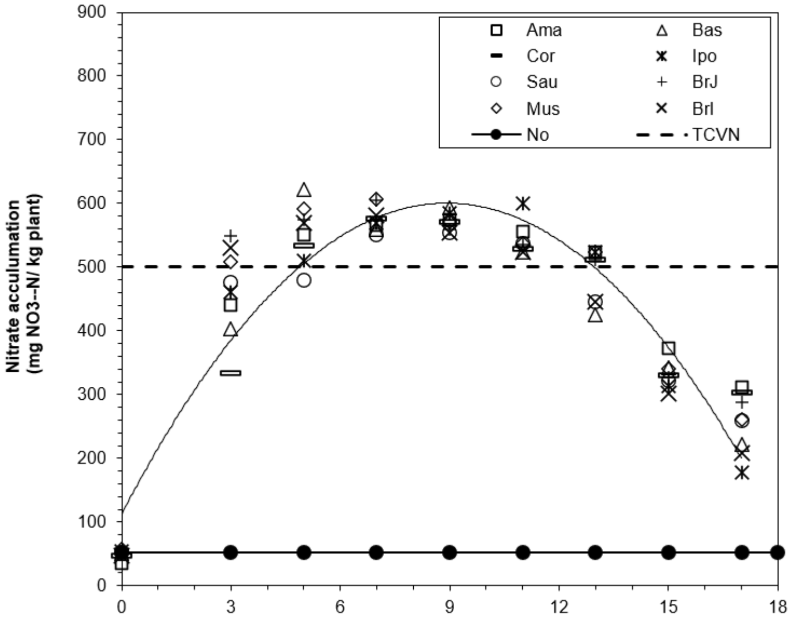

Fig. 3. Nitrate accumulation in plant after 17 days fertilizer application. No was the initial nitrate content in plant, TCVN is the highest allowed of nitrate content in Vietnam.

Table 3. Plant nitrate uptake of treatment block in the experiment

Treatment	Equation model	Potential plant nitrate accumulation (mg/kg)	Correlation
Ama	$y = -5.84t^2 + 109.0t + 96.8$	605	$R^2 = 0.929$
Bas	$y = -6.39t^2 + 112.34t + 104.4$	598	$R^2 = 0.910$
Cor	$y = -5.83t^2 + 109.19t + 71.01$	583	$R^2 = 0.940$
Ipo	$y = -6.81t^2 + 119.88t + 88.4$	616	$R^2 = 0.963$
Sau	$y = -5.63t^2 + 100.8t + 117.7$	569	$R^2 = 0.905$
BrJ	$y = -6.00t^2 + 105.97t + 148.1$	616	$R^2 = 0.833$
Mus	$y = -6.24t^2 + 110.03t + 136.6$	622	$R^2 = 0.891$
BrI	$y = -6.31t^2 + 107.73t + 138.6$	598	$R^2 = 0.876$
Average	$y = -6.13t^2 + 109.37t + 112.7$	600	$R^2 = 0.888$

Remarks: All parameters obtained with the quandratic equation: $y = at^2 + bt + c$

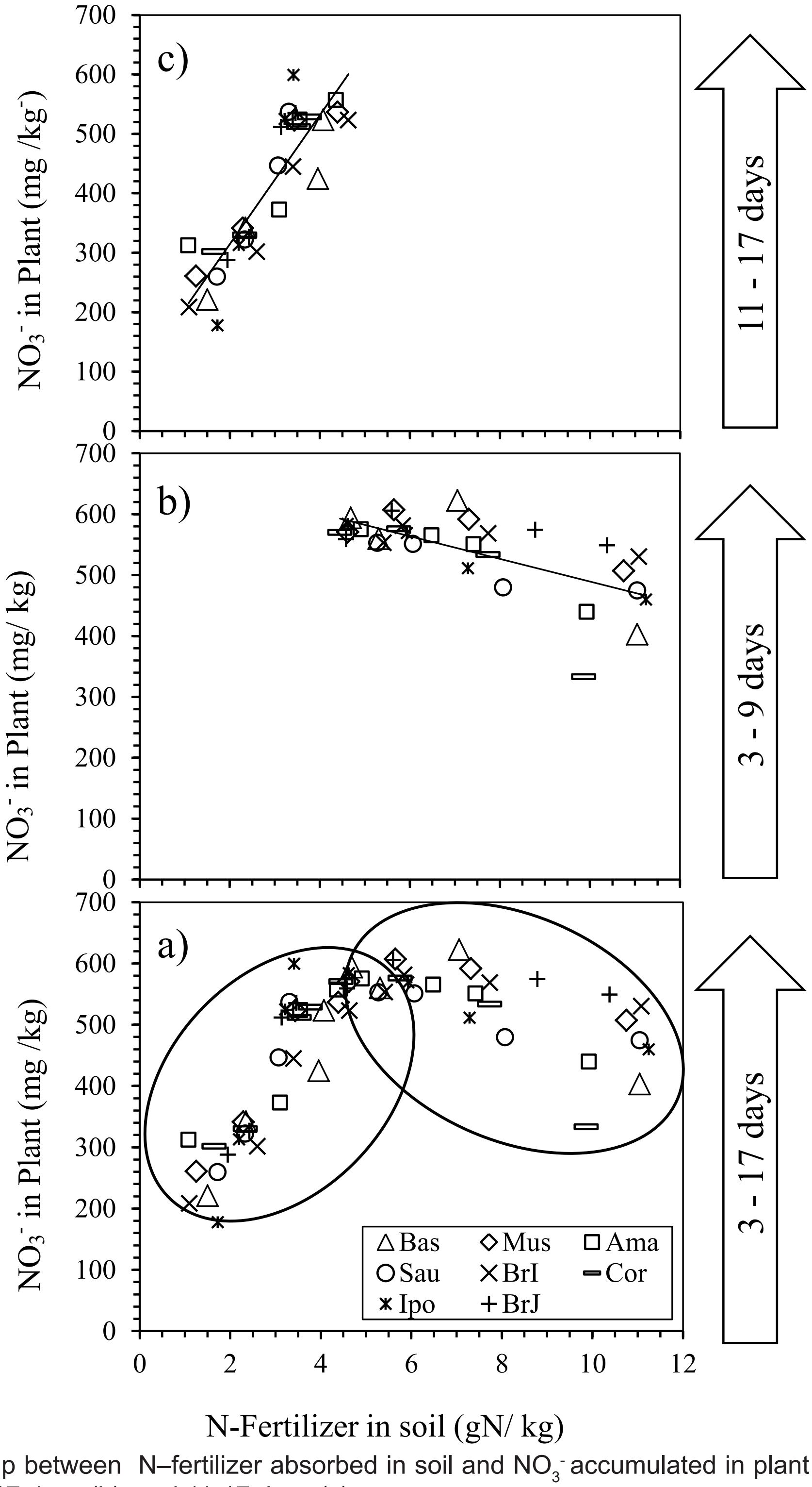

Fig. 4. Relationship between N–fertilizer absorbed in soil and NO_3^- accumulated in plant at three periods 3-17 days (a), 11-17 days (b), and 11-17 days (c)

The Fit of Fertilizer Nitrogen Content and Plant Nitrate Uptake to the Prediction Models

As mentioned above, the soil fertilizer nitrogen loss is suitable for decay regression as f = a*exp(-bt). The very high correlation between measured and predicted models (R^2>0.945) confirms that this model would be suitable for modeling the trend of nitrogen lost from fertilizer intakes. The movement is divided into two phases for the plant nitrate uptake behavior described above. The previous research by Cheng et al. (2016) shows the behavior of nitrogen mineralization in soil via anaerobic incubation is also well modeled by the Rise to the maximum regression, which is similar to the plant nitrate uptake at the first nine days in this study. Despite much research on the behavior of nitrate uptake in plants, there is no report to attempt to build up the behavior equation. The lack of continuous data on soil nitrogen content and plant nitrate uptake may be the reason.

Limitations of the Study

The study addressed the behavior trends of fertilizer nitrogen and vegetable nitrate uptake, which is in line with the regression model. It is a limitation that this study lacks nitrate uptake on day 1 and day 2. As the changes in nitrate uptake are quick initially, more values would strengthen the research hypothesis model during this period. Future research on a large scale with more species would be necessary to confirm this result. Furthermore, a combined measurement of both total soil nitrogen and soil organic carbon is crucial to understanding the behavior of soil microorganisms through fertilizer application, especially for the labile carbon components.

CONCLUSION

This study accessed the response of 8 common leafy vegetables to nitrogen fertilizer addition in plant nitrate uptake and found that all nitrogen fertilizer was released from the soil after 17 days. Meanwhile, the plant nitrate uptake reached maximum concentration after nine days and started declining to the initial concentration within the same duration. Although both showed a high correlation (*P*<0.01), the trend correlation among treatments of soil fertilizer nitrogen is weaker than those in plant nitrate (($R_{Nitrate}$=0.931and $R_{Nitrogen}$=0.982). This study proved that a linear model could describe fertilizer application and plant absorb nitrogen.

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