Title: N-deficiency damps out circadian rhythmic changes of stem diameter dynamics in tomato plant.

Syunsuke Kanai[1 a], Joseph Adu-Gymfi[2], Kei Lei[1], Junki Ito[3], Katsumi Ohkura[4], Reda E.A. Moghaieb[5], Hany El-Shemy[5], Rashmi Mohapatra[6], Pravat K. Mohapatra[6], Hirofumi Saneoka[1], Kounosuke Fujita[1]

Running title: N deficiency on stem diameter in tomato

[1]Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-hiroshima, 739-8528, Japan.

[2]International Atomic Energy Agency (IAEA), Wagramer Strasse 5, A-1400 Vienna Austria.

[3]Hiroshima Prefectural Agricultural Center, Hara, Hachihonmatsu, Higashi-hiroshima, 739-0151, Japan.

[4]Faculty of Environmental and Information Sciences, Yokkaichi University, 1200 Kayoucho, Yokkaichi, 512-8512, Japan.

[5]Faculty of Agriculture, Cairo University, Cairo University Street, Giza, 12613, Egypt.

[6]School of Life Science, Sambalpur University, Jyoti vihar, Sambalpur 768019, India.

[a]Author for correspondence. E-mail: skanai@hiroshima-u.ac.jp

Tell: +81-(0)82-424-7963, Fax: +81-(0)82-424-7963.

Number of pages of manuscripts: 40, Number of Table: 1, Number of Figure: 11.

Abstract

Tomato (*Lycopersicon esculentum*) plants were grown in hydroponics. At the fruiting stage, N was withdrawn from the growing medium for a period of 19 days and its effects were studied on plant biomass production, photosynthesis, partitioning of ^{13}C and ^{15}N, and changes in the stem and fruit diameters etc, in order to monitor the mechanism of resource management on the plant parts at low N and prevent excess use of the fertilizer. N-deficiency treatment decreased leaf photosynthesis immediately and affected biomass accumulation of tomato. Conversely, N-deficiency increased stem diameter for a period of two weeks before reducing it below the control. During this period, these results suggest that N deficiency more suppress source activity than sink activity. N-deficiency reduced the amplitude of the circadian pattern of daytime shrinkage and nocturnal expansion of the stem diameter by decreasing the magnitude of the former. Circadian pattern of contraction and expansion of diameter was less evident in the fruit. Under N-deficiency, distribution of ^{13}C and ^{15}N decreased and increased to fruits respectively. Restricted partitioning of carbon to fruits could be responsible for accumulation of unused assimilates and consequential osmotic adjustment for maintenance of stem water potential. This effect might have precluded contraction of stem diameter of N-deficient plants until the production of assimilates became limiting on account of depression of leaf photosynthesis.

Key words: Nitrogen, source-sink relationship, stem and fruit diameter, tomato, micro-morphometry

Introduction

Glasshouse tomatoes grown hydroponically, is provided with high levels of inorganic nutrients. Nitrogen fertilizers are most essential for growth and development of tomato plant [1,2] and always given to the plant in excess of requirement. While this practice ensures unhindered growth of the plant, it can exacerbate the release of extra nutrition to the environment. The ecological price for nitrogen waste of aqua culture tomato is very high to pay since large amount of fertilizers are leached from this agrosystem to ground water [3]. Siddiqi et al. [2] reported that reduction of nitrogen to 50% of the normal levels had no adverse effect on fruit yield and quality. The storage capacity of the plant for the element is in excess of that required for growth. Moreover, N in one organ can be reused for the growth another [4]. Therefore, it is important to grow aqua culture tomato at reduced N-concentrations and restrict eutrophying nitrogen waste. But, it is not easy to define the critical N-concentration, which allows maximum growth rate [5] in order to avoid deficiency symptoms. Under the circumstances, a quick and reliable technique is necessary to identify the N-deficiency symptoms and replenish to stock solution in exigency.

Nitrogen is always a part of the carbon compounds in plant cells [6]. Assimilatory processes of

carbon and nitrogen are closely interlinked and their dynamics are of key importance for crop production [7]; deficiency of either of the nutrients can result in marked changes in the assimilation and metabolic activities of the other [8]. Many studies have shown strong positive correlations between nitrogen content and photosynthetic capacities of leaves [9,10]. N-deficiency decreases the amount of thylakoids, carboxylation activity [11], photochemical efficiency [12,13], chlorophyll contents [14] and stomatal conductance of leaves [15] and these effects are ultimately reflected on biomass production. Glasshouse tomato grown in hydroponics is no exception to the adverse effects of N-deficiency on the source activities of the plant as illustrated above [16, 17]. Effect of N-deficiency on sink and source has already well studied, respectively. However, it has not been elucidated that effect of N-deficiency on source organ, sink organ and translocation of photosynthate at the same time in terms of source-sink relationship. Recently, it has been reported that separation of effects of source and sink on biomass production is possible by simultaneous monitoring of the amplitude of rhythmic changes of fruit and stem diameter dynamics and photosynthetic activities of the source leaf [18-20]. There are many reports in the literature that major sink organs like stem and fruits of plants fluctuated diurnally to coincide with the changes in water status of the plant [21-24]. It is possible that sub-optimal nitrogen supply decreases hydraulic conductance of root, therefore growth of sink organs is inhibited by deficient of turgor. [25-28]. The manner in which the nutrient stress is

transduced into a hydraulic response is not known. But, monitoring changes in the amplitude of the circadian rhythm in shrinkage and expansion of the diameter of the plant organ by micromorhometric technique [29] can be a reliable method to measure the changes in water status of the plant resulting from adverse effects of the stress. In the present study, the objective was to identify the process of resource management on the part of the plant at low N by monitoring changes in stem and fruit diameter dynamics as well as primary production.

Materials and methods

Plant material and culture

Tomato (*Lycopersicon esculentum* L.cv. Momotarou) plants were grown in pots (70L) inside the glass house of Graduate School of Biosphere Science, Hiroshima University, Japan. Each pot was filled with nutrient solutions, consisting of N ($Ca(NO_3)_2.4H_2O$) 3.57 mM, P ($NaH_2PO_4.2H_2O$) 0.32 mM, K (K_2SO_4/KCl 1:1) 1.02 mM, Ca ($CaCl_2.H_2O$) 0.75 mM, Mg ($MgSO_4.7H_2O$) 0.82 mM, Fe (Fe^{+3}-EDTA) 0.02 mM, Mn ($MnSO_4.4H_2O$) 3.64 μM, B (H_3BO_3) 0.05 mM, Zn ($ZnSO_4.7H_2O$) 0.15 μM, Cu ($CuSO_4.5H_2O$) 0.16 μM, Mo ($NaMoO_4.2H_2O$) 0.1 μM, Co ($CoSO_4.7H_2O$) 0.17 μM. There were six pots, each having three plants. At the first fruiting stage (65 days old), N was withdrawn from the nutrient medium in three pots and this treatment was continued for 20 days. The plants of the other pots received full nutrition. The plants were grown under natural light

(3200 E m^{-2} s^{-1}). The maximum and minimum temperatures were 32 and 23^{o}C respectively. The relative humidity of the glass house varied between 45 to 65%.

Measurement of biomass production

Plants from both control and N-deficiency treatment were sampled at 1, 9 and 19 days after treatment in three replicates. Each plant was separated into roots, leaves, fruits and stem. The plant organs were dried in an open air draught oven at 70^{o}C for 72 h before the estimation of dry weight. The dry materials were ground to powder with a vibrating sample mill (Model T1-100, Heiko Co Ltd., Fukushima, Japan) and aliquots were taken for analysis of nitrogen.

Leaf blade immediately below the second truss was harvested at 0, 3, 6, 12 and 20 day after treatment at 4 replicates. The leaf blades were lyophilized, and then estimated for dry weight. These blades were ground to powder for measurements of N and sugar concentration in leaf blade to employ examination of relationship between photosynthetic rate and leaf nitrogen and sugar content.

Measurement of photosynthesis, transpiration and stomatal conductance

Photosynthetic rate, transpiration and stomatal conductance of the 1st and 2nd leaves below the fruiting truss were measured with a portable infra red gas analyser (Model L1 6400, Licor Co.

Ltd., Lincoln, Nebraska, USA) as described in our earlier report [19]. The leaf chamber was open type and measurements were taken once at 11.00 am on each day in both control and N-deficiency treatment plants during the period of treatment. The photosynthetically active radiation was above 1000 μmol m^{-2} s^{-1} and observations were recorded after the plant reached a steady photosynthesis state. All measurements were recorded five times and differences between the observations were negligible.

Measurement of stem and fruit diameter

Changes in stem and fruit diameter were continuously recorded in both control and N-deficient plants during the period of treatment with a shrinkage type micro-displacement detector [19]. The sensors were connected to a computerized data acquisition system (NEC, Sanei Kogyo Co. Ltd., Tokyo). The sensors were fastened to the stem or a growing fruit and connected to the power system and data logger. The control run was carried out by placing a glass rod of 12 mm diameter. The fluctuation of the measuring system was within ±2μm. Measurement of stem and fruit diameter were recorded three times and the pattern of response was similar in all stem and fruit.

Measurement of nitrogen

An aliquot of the plant organs and leaf blade were used for N content determination by the

Kjerdahl method [30].

Measurement of sugar concentration

Aliquots of the powdered leaf blade were boiled with 80% (v/v) aqueous ethanol three times for the extraction of sugars. The extracts were pooled in a volumetric flask and the flask was filled up to the mark with distilled water. The sugar content in the ethanol-soluble extract was determined using the anthrone reagent, according to the method of Suwa et al. [31].

$^{13}CO_2$ feeding

$^{13}CO_2$ feedings were given to the leaf immediately above the first fruiting node on days 7 and 17 after N-deficiency treatment in both control and treated conditions. The leaf was enclosed in a transparent plastic bag and 200 mL of gas mixture comprising of 80% nitrogen, 19% oxygen and 350 μ L L^{-1} of $^{13}CO_2$ (99 ^{13}C atom % excess) was introduced from a cylinder. The leaf was allowed to assimilate $^{13}CO_2$ for one hour. The PAR was above 1700 μ mol m^{-2} s^{-1} during the assimilation process. The plants were harvested 48 h after feeding by separating into $^{13}CO_2$ fed leaf, other leaves, fruits, stem and other plant parts. The plant parts were ground to powder for measurement of ^{13}C abundance.

^{15}N-NO_3 feeding

The roots were dipped into a solution of ^{15}N-NO_3 (10 atom %, 100 ppm N) in a 20 L container for 24 h before the initiation of N-deficiency treatment. The plants were harvested 19 days after imposition of N-deficiency treatment. The plants were separated into leaves, stem, fruits and roots. The samples were dried in an oven for 72 h before estimation of dry weight. The materials were ground to powder for determination of ^{15}N abundance.

^{13}C and ^{15}N analyses

The ^{13}C or ^{15}N abundance in the powdered plant sample was determined with a mass spectrometer (model Delta plus, Finnigan Co., San Jose, CA, USA). The ^{13}C atom % excess in the plant sample was calculated as the difference in ^{13}C atom % between the sample and standard pure chemical glycine. The amount of labeled C or N in the plant sample was calculated using the equation mentioned below [19].

$$A = \frac{^{13}C \text{ or } ^{15}N \text{ amount in the plant part}}{100} \times \text{Amount of C or N in the sample}$$

The element analyzer facilitated in the mass spectrometer determined the amount of total C. Similarly; the amount of labeled N in plant sample was calculated. The total N of the plant material was determined by micro-kjeldahl method [30].

Statistical analysis

The experimental data were analysed for the effect of N-deficiency treatment according to the expected mean squares given by [32]. For mean separation, treatment sum squares was partitioned by method of orthogonal contrasts. The coefficients of variation for all response variables were below 10%.

Results

Dry mass accumulation

Increase in dry mass accumulation was slower in the N-deficient plants compared to the control during the 19 d period of treatment (Fig.1). N-deficiency affected fruit and leaf growth more than the other organs during this period. The effect of N-deficiency on root growth was not significant.

Stem and fruit diameter

Diameter of the stem in the control exhibited daytime shrinkage and nighttime expansion and increased temporally up to 19 days after treatment (Fig. 2). The circadian pattern of change in diameter of stem was similar for the plants subjected to N-deficiency. During the first two weeks

of the treatment period, the diameter of the N-deficient plant was larger than the control. But, the stem diameter did not expand in size thereafter in N-deficient plants and became lower than the control plants. The diameter started to decrease early in the morning with sunrise (above 140 E m^{-2} s^{-1}) and the shrinkage was high throughout the day (Fig.3). It recovered in the evening (bellow 420 E m^{-2} s^{-1}) and expanded at night (0 E m^{-2} s^{-1}). During the initial period, N-deficiency reduced daytime shrinkage and encouraged nocturnal expansion of stem diameter more than the control. The amplitude of the rhythm damped out gradually two weeks after the initiation of the treatment; the night-time expansion became very low in the treated plant in contrast to the control by the end of the treatment period (Fig.3).

The diameter of the fruit increased with passage of time in both control and N-deficient plants during the period of treatment (Fig.4). N-deficiency marginally decreased expansion during the first two weeks, but this effect did not continue till the end of the treatment period. The amplitude of the circadian rhythm in contraction and expansion of fruit diameter was not as distinct as that of the stem (Figs.3, 5). In the early part of treatment period (Fig.5A), there was no shifting of the rhythmic phase in the fruit diameter and expansion occurred all throughout the 24-hour daily period in control plants. N-deficiency reduced daytime expansion of fruit diameter and effect lasted till the night. The reduction of fruit diameter size was restored gradually 7 days after

treatment (Fig.5B) due to faster expansion in N-deficient plants and the diameter became larger than the control towards the end of the treatment period (Fig.5C).

Apparent photosynthetic rate, stomatal conductance and transpiration rate

N-deficiency depressed the apparent photosynthetic rate immediately after initiation of the treatment and the impact continued to increase with passage of time (Fig. 6 A). However, the adverse effect of N-deficiency could not be noticed on stomatal conductance and leaf transpiration rate until 8 days after treatment (Fig.6B, C). The stomatal conductance and rate of transpiration continued to decline thereafter till the end of the period.

N concentration

N-deficiency decreased significantly nitrogen concentration of all plant parts in spite of the preferential partitioning (data not shown), which occurred in favour of the fruits and roots.

N concentration of leaf blade was lower in N-deficient plants than in the controls at 3 DAT (control and N-deficiency; 31.4 and 25.5 mg kg^{-1} D.W., respectively) (Fig. 10A). This phenomenon was increased with passage of time, but N concentration of the control was kept almost constant during treatment periods. At 20 DAT, leaf N concentration was lowered from 31.8 mg kg^{-1} D.W. in control to 12.9 mg kg^{-1} D.W. in N-stressed plants.

Leaf sugar concentration

Treatment of N stress increased leaf sugar concentration in comparison to the control after 3 DAT (control: 98, -N: 134 mg kg^{-1} D.W.) (Fig. 10B). The phenomenon was continued throughout the period of experiment.

^{13}C partitioning

^{13}C atom % accumulation was very high in the fed leaf and the next preferred target was the fruit (Table 1). The other leaves, upper stem and roots gained very low percentage of the isotope on day 9. On day 19, the concentration of the isotope did not change in the fed leaf, but receded in the fruits and increased in the other leaves and upper parts of stem. N-deficiency did not influence ^{13}C atom % of the fruits; in case of roots, the treatment improved ^{13}C atom % on day 19. The export rate of ^{13}C into other plant parts from the fed leaf in the control was lower at day 9 after treatment than at day 19 (Fig.7). N-deficiency decreased the export rate of ^{13}C from the fed leaf into other parts on day 9 after treatment and the effect increased with passage of time. In the control, ^{13}C mostly partitioned into the fruits and other plant parts received only a small part of the isotope (Fig.8). N-deficiency had no influence on partitioning of ^{13}C to fruits on day 9 after treatment. But at day 19, it decreased partitioning to fruits and leaves and increased export in

favour of roots and stem

^{15}N partitioning

Compared to ^{13}C, percentage of ^{15}N partitioning into the fruits was low (Fig.9). In the control, ^{15}N partitioning occurred mostly in favor of the leaves and fruits were the next favorite destination. Unlike the situation in carbon partitioning, N-deficiency significantly increased partitioning in favor of the fruits. This was done largely at the cost of the leaves. N-deficiency also increased partitioning into the roots.

Discussion

We have previously evaluated quality of resource management of tomato [19, 20] and Japanese persimmon [18] on the basis their capacity for partitioning of assimilates from source to sink in response to P and K deficiency and salinity stress respectively. Nitrogen is another important growth factor and its influence on source sink relationship of tomato was elucidated in the present endeavor. The source activity of the leaves declined immediately after imposition of the stress (Fig. 6), and diameter of stem in the sub-optimal N treatment expanded more than that of the control (Fig. 2) due to accumulation of a lot of carbohydrate reserves (data not shown)

probavly along the phloem transport pathway. However, there was no reflection on the expansion of the sinks like fruit (Figs.4). The responses of fruit to N-deficiency were similar to that of P-deficiency, but influences on source activity differed considerably between the stresses. Both the stresses affected ^{13}C export from the source leaf to other organs (Fig.7), but the preferential partitioning carbon and nitrogen elements in favour of the fruit ensured partial homeostasis in growth. However, adverse effects of N-deficiency were more steadfast on carbon translocation to the sink organs of tomato compared to P-deficiency; the export of ^{13}C from source leaf (Fig.7) declined severely in N-deficient plant compared to P-deficient plant within the 19 days period of treatment. Unlike phosphorus, nitrogen always moves with carbon compounds in the phloem solutes and deficiency of one element is bound to have a consequential influence on the other. Severe reduction of the nitrogen concentration of the N-deficient plant fruit, stem, root (data not shown) and leaf blade (Fig. 10A) corroborates decrease of ^{13}C export rate from leaves (Fig.7) by diminished utilizable N in leaves (Fig 10A).

Further, De Groot et al. [33] distinguished the contrasting effects of N and P deprivation on leaf photosynthesis in tomato plants; N-stress affects photosynthesis by reduced utilization of assimilates and absorption of light while P-stress produces a decreased rate of CO_2 fixation. Therefore, influence of N-deprivation on leaf photosynthesis could be more instantaneous

compared to that of P-deprivation and our results support this proposition.

Proseus et al. [34] proposed that rapid changes in turgor pressure of plant organs could provide new information on the mechanism of growth. Johnson et al. [23] observed a strong correlation between circadian rhythmic fluctuation in diameter and the water potential of fruit and stem of tomato plants. Low water potential can decrease phloem turgor reducing the driving force for entry of sap to the fruit. In our study, withdrawal of N from the growth medium, encouraged stem diameter expansion more than that of the control during the first two weeks of treatment period before reducing it subsequently. In plants nitrogen is a part of carbon compounds [6]. Carbon assimilates provide the skeleton for synthesis of various amino acids. Excess soluble carbohydrates that cannot be used in amino acid synthesis accumulate in plant parts [6]. It is plausible that abrupt limitation of exogenous N could increase organic acids used in NO_3 reduction and sucrose concentration, all of which may be involved in turgor maintenance of the plant cells [8,35,36]. Accumulation of sucrose, an osmotic solute, can increase the gradient for water flux and maintain cell turgor [37,38]. In our study, N-deficient tomato might have accumulated such carbon osmolytes for retention of high phloem turgor, and the stem diameter increased temporarily as long as the reduced hydraulic conductance of roots [25] did not depress water potential of plant below the control level.

However, a high concentration of sugar could not be sustained for long and the stem diameter decreased below the control level two weeks after imposition of the stress (Fig.2). Photosynthesis of N-deficient leaves decrease on account of end product accumulation [39] and loss of Rubisco activity [10]. In our experiment, apparent photosynthetic rate of leaf increased and decreased with increase of nitrogen (Fig. 10A) and sugar concentrations of the leaves (Fig. 10B), respectively (Fig. 11 A, B). These observations provide support for change in source sink balance among plant organs, which became necessary for reallocation of resources under sub-optimal N [40]. Since photosynthetic assimilate production was low in N-deficient plants (Fig.6A), the stem could not conserve water for osmotic adjustment and the diameter decreased below the control level. N-deficiency reduced stomatal conductance and transpiration rate from 8 DAT (Fig.6B,C)) ; and the effect on stomatal conductance [15, 41] possibly led to a concomitant increase of water potential of the plant organs.

Presence of high concentration of solutes in the phloem might have reduced daytime shrinkage of stem diameter. The resulting decrease of amplitude might have contributed to the damping out of the circadian rhythm of stem diameter dynamics (Fig.2) Similar to the stem, the fruit maintained its water potential and did not exhibit any change of diameter under N-deficiency, although the

diameter declined marginally during the first few days of treatment. Unlike the stem, fruit diameter did not change in the later part of the treatment period, because of the preference received in partitioning of assimilates (Fig.8). Similar preference in partitioning to heterotrophic organs at the cost of photosynthetic organs was noticed under P-deficiency stress [19, 42]. But unlike phosphorus, N-deficiency effects are more explicit on the source activity and also on the sink activity. In our study, N-deprivation did not decrease fruit diameter in spite of reduction in ^{13}C assimilate partitioning (Fig.8) and ^{13}C export rate from leaves (Fig.7). Whereas N-deficiency decrease dray weight of fruits at 19 DAT, but not influence at 9 DAT (Fig. 1). These results suggest that N-deficiency do not affect water content of fruit before 9 DAT and then depress dry weight production of fruit and diameter of fruit is maintained by water content. In fact, water content percentage of fruit became higher than control by N deficiency treatment (data not shown).

Simultaneous monitoring of both source and sink activities has distinguished the effects of the stress on these organs. N-deficiency effects were instantaneous and comparable to that of K-deficiency [20]. However, the mechanisms of these effects are not similar, the former regulates water potential, because unused sugar accumulate in the plant [33], whereas, K becomes a direct

osmolyte for cell water. Conversely, P limitation down regulates carbohydrate synthesis [33] and soluble carbohydrates concentration cannot be a factor for regulation of plant water potential in the deficient plants. The water potential of P-deficient plants might have increased for sometime on account of reduced stomatal conductance [19].

References

[1] J. Le Bot, B. Jeannequin, R. Fabre, Growth and nitrogen status of soil less tomato plants following nitrate withdrawal from the nutrient solution, Ann. bot. 88 (2001), 361-370.

[2] M.Y. Siddiqi, H.J. Kronzucker, D.T. Britto , A.D.M. Glass, Growth of a tomato crop at reduced nutrient concentrations as a strategy to limit eutrophication, J. Plant Nutr. 21 (1998), 1879-1895.

[3] C. Bellert, J. Le Bot, M. Dorais, Lopez J., A.Gosselin, Nitrogen accumulation and growth of fruiting tomato plants in hydroponics. Proceedings IS Water quality and quantity in greenhouse horticulture. Ed. R.M. Carpena. Acta Horticulturae. 458, ISHS. 1998, pp. 293-299.

[4] P. Millard, The accumulation and storage of nitrogen by herbaceous plants, Plant Cell Environ. 11(1988) 1-8.

[5] F. Gastal, G. Lemaire, N uptake and distribution in crops: an agronomical and eco-physiological perspective, J. Exp. Bot. 53 (2002) 789-799.

[6] L. Taiz, E.Zeiger, Plant Physiology, 3rd Edition, Sinauer Associates, Inc., Publishers. Massachusetts 2002.

[7] D.W. Lawlor, Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems, J. Exp. Bot. 53(2002) 773-787.

[8] T.W. Rufty, Jr, S.C. Huber, R.J. Volk, Alterations in leaf carbohydrate metabolism in response to nitrogen stress, Plant Physiol. 88 (1988) 725-730.

[9] J.R. Evans, Nitrogen and photosynthesis in flag leaves of wheat (Triticum aestivum L.), Plant Physiol. 72(1983) 297-302.

[10] J.R. Evans, I. Terashima, The effects of nitrogen nutrition on electron transport components and photosynthesis in spinach, Aust. J. Plant Physiol. 14 (1987) 59-68.

[11] I. Terashima, J.R. Evans, Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach, Plant Cell Physiol. 29 (1988) 143-155.

[12] D.W. Lawlor, F.A. Boyle, A.T. Young, A.J. Keys, A.C. Kendall, Nitrate nutrition and temperature effects on wheat: photosynthesis and photorespiration of leaves, J. Exp. Bot. 38 (1987) 393-408.

[13] C. Lu, J. Zhang, Photosynthetic CO_2 assimilation, chlorophyll fluorescence and

photoinhibition as affected by nitrogen deficiency in maize plants, Plant Sci.. 151 (2000) 135-141.

[14] D. Zhao, K.R. Reddy, V.G. Kakani, V.R. Reddy, Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum, Eur. J. Agron. 22 (2005) 391-403.

[15] M.R. Broadley, A.J. Escobar-Gutierrez, A. Burns, I.G. Burns, Nitrogen-limited growth of lettuce is associated with lower stomatal conductance, New Phytol. 152 (2001) 97-106.

[16] F.S. Chapin III, C.H.S. Walter, D.T. Clarkson, Growth response of barley and tomato to nitrogen stress and its control by abscisic acid, water relations and photosynthesis, Planta. 173 (1988) 352-366.

[17] L. Guidi, G. Lorefice, A. Pardossi, F. Malorgio, F. Tognoni, G.F. Soldatini, Growth and photosynthesis of Lycopersicon esculentum (L.) plants as affected by nitrogen deficiency, Biol. Plantarum 40 (1997) 235-244.

[18] K. Fujita, J. Ito, P.K. Mohapatra, H. Saneoka, K. Lee, H. Kurban, K. Kawai, K. Ohkura, Circadian rhythm of stem and fruit diameter dynamics of Japanese persimmon (Diospyrus kaki thunb.) is affected by deficiency of water in saline environments, Funct Plant Biol. 30 (2003a) 747-754.

[19] K. Fujita, M. Okada, K. Lei, J. Ito, K. Ohkura, J.J. Adu-Gyamfi, P.K. Mohapatra, Effect of

P-deficiency on photoassimilate partitioning and rhythmic changes in fruit and stem diameter of tomato (Lycopersicon esculentum) during fruit growth, J. Exp. Bot. 54 (2003b) 2519-2528.

[20] S. Kanai, K. Ohkura, J.J. Adu-Gyamfi, P.K. Mohapatra, N.T. Nguyen, H. Saneoka, K. Fujita, Depression of sink activity precedes the inhibition of biomass production in tomato plant subjected to potassium deficiency stress, J. Exp. Bot 58 (2007) 2917-2928.

[21] M. Genard, S. Fishman, G. Vercambre, J.G. Huguet, C. Bussi, J. Basset, R. Habib, A biophysical analysis of stem and root diameter variation in woody plants, Plant Physiol. 126 (2001) 188-202.

[22] S.O. Link, M.E. Thiede, M.G. van Bavel, An improved strain gauge device for continuous field measurement of stem and fruit diameter, J. Exp. Bot. 49 (1998) 1583-1587.

[23] R.W. Johnson, M.A. Dixon, D.R. Lee, Water relations of the tomato during fruit growth, Plant Cell Environ. 15 (1992) 947-953.

[24] T. Simonneau, R. Habib, J.P. Goutouly, J.G. Huguet, Diurnal changes in stem diameter depend upon variations in water content: direct evidence in peach trees, J. Exp. Bot. 51 (1993) 1531-1542.

[25] J.W. Radin, J.S. Boyer, Control of leaf expansion by nitrogen nutrition in sunflower plants.

Role of hydraulic conductivity and turgor, Plant Physiol. 69 (1982) 771-775.

[26] L. Barthes, A. Bousser, J. Hoarau, E. Deleens, Reassessment of the relationship between nitrogen supply and xylem exudation in de-topped maize seedlings, Plant Physiol. Bioch. 33 (1995) 173-183.

[27] M. Carvajal, D.T. Cooke, D.T. Clarkson, Response of wheat plants to nutrient deprivation may involve the regulation of water channel function, Planta. 199 (1996) 372-381.

[28] D.T. Clarkson, M. Carvajal, T. Henzler, R.N. Waterhouse, A.J. Smyth, D.T. Cooke, E. Steudle, Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress, J. Exp. Bot. 51 (2000) 61-70.

[29] J.G. Huguet, Appreciation de l'etat hydrique d'une plante a patir des variations micrometriques de la dimension des fruits ou des tigas au cours de la journee, Agronomie 5 (1985) 733-741.

[30] K.G. Ofosu-Budu, S. Ogata, K. Fujita, Temperature effects on root nodule activity and nitrogen release in some sub-tropical and temperate legumes, Soil Sci. Plant Nutr. 38 (1992) 717-726

[31] R. Suwa, N.T. Nguyen, H. Saneoka, R.E. Moghaieb, K. Fujita, Effect of salinity stress on photosynthesis and vegetative sink in tobacco plants, Soil Sci. Plant Nutr. 52 (2006) 243-250.

[32] M.S. McIntosh, Analysis of combined experiments, Agron. J. 75 (1983) 153-155.

[33] C.C. De Groot, R. Van den Boogaard, L.F.M. Marcelis, J. Harbinson, H. Lambers, Contrasting effects of N and P deprivation on the regulation of photosynthesis in tomato plants in relation to feedback limitation, J. Exp. Bot. 54 (2003) 1957-1967.

[34] T.E. Proseus, G.L. Zhu, J.S. Boyer, Turgor, temperature and growth of plant cells: using Chara corallina as a model system, J. Exp. Bot. 51 (2000) 1481-1494.

[35] E. Steingrover, P. Ratering, J. Siesling, Daily changes in uptake, reduction and storage of nitrate in spinach grown at low light intensity, Physiol. Plantarum. 66 (1986) 550-556.

[36] E. Urbanczyk-Wochniak, A.R. Fernie, Metabolic profiling reveals altered nitrogen nutrient regimes have diverse effects on the metabolism of hydroponically-grown tomato (Solanum lycopersicum) plants, J. Exp. Bot. 56 (2005) 309-321.

[37] R. Serraj, T.R. Sinclair, Osmolyte accumulation: can it really help crop increase yield under drought conditions? Plant Cell Environ. 25 (2002) 333-341.

[38] P.S. Basu, M. Ali , S.K. Chaturvedi, Osmotic adjustment increases water uptake, remobilization of assimilates and maintains photosynthesis in chickpea under drought, Indian J. Exp. Biol. 45 (2007) 261-267.

[39] A. Krapp, M. Stitt, An evaluation of direct and indirect mechanisms for the 'sink-regulation' of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme

activities and steady-state transcript levels after cold-girdling source leaves, Planta. 195 (1995) 313-323.

[40] M.J. Paul, S.P. Driscoll, Sugar repression of photosynthesis: the role of carbohydrates in signaling nitrogen deficiency through source:sink imbalance, Plant Cell Environ. 20 (1997) 110-116.

[41] J.W. Radin, Responses of transpiration and hydraulic conductance to root temperature in nitrogen- and phosphorus-deficient cotton seedlings, Plant Physiol. 92 (1990) 855-857.

[42] K.L. Nielsen, A. Eshel, J.P. Lynch, The effect of phosphorus availability on the carbon economy of contrasting common bean (Phaseolus vulgaris L.) genotypes, J. Exp. Bot. 52 (2001) 329-339.

[43] C.E. Hartt, Effect of nitrogen deficiency upon translocation of ^{14}C in sugarcane, Plant Physiol. 46 (1970) 419-422.

Table 1. *The effect of N deficiency on ^{13}C atom % excess in various parts of tomato plants at different times after treatment*

Plant parts	9 DAT				19 DAT			
	Control		N deficiency		Contrl		N deficiency	
Fed leaf	0.557 ±	0.014	1.083 ±	0.141	0.583 ±	0.034	0.938 ±	0.118
Other leaves	0.006 ±	0.001	0.002 ±	0.001	0.038 ±	0.002	0.007 ±	0.000
Fruits	0.115 ±	0.021	0.113 ±	0.032	0.048 ±	0.005	0.045 ±	0.003
Stem, upper	0.006 ±	0.002	0.006 ±	0.001	0.034 ±	0.008	0.018 ±	0.004
Stem, lower	0.016 ±	0.001	0.028 ±	0.003	0.022 ±	0.005	0.022 ±	0.003
Roots	0.006 ±	0.001	0.006 ±	0.002	0.004 ±	0.001	0.013 ±	0.001
Average±SEM								

Figure legends

Fig. 1. The effect of N-deficiency on dry weight of tomato plant organs. 0, Initial of treatment; Cont.: control; -N: nitrogen deficiency. Vertical bars denote SEM for whole plant weight and each organ weight. Fruit (black and white checks), root (white), stem (diagonal stripe), leaves (spotted).

Fig. 2.The effect of N-deficiency on diurnal changes of stem diameter of tomato plant. Changes in stem diameter were monitored by shrinkage type micro-displacement detector. Data represent the means of three plants. Control (fine line), -N treatment (thick line) and PAR (most bottom line).

Fig. 3. The effect of N deficiency on diurnal changes of stem diameter of tomato plants. On each occasion, the diameter changes in both control and N-deficient plants were recorded with reference to 'Zero change' at midnight (00.00 h). Control (fine line), -N treatment (thick line) and PAR (most bottom line).

Fig. 4. The effect of N deficiency on changes in fruit diameter in tomato. Changes in fruit diameter was monitored as described in Fig. 2. Control (fine line), -N (thick line) and PAR (most

bottom line).

Fig. 5. The effect of N deficiency on diurnal changes of fruit diameter of tomato plants. On each occasion, the diameter changes in both control and N-deficient plants were recorded with reference to 'Zero change' at midnight (00.00 h). Control (fine line), -N treatment (thick line).

Fig. 6. The effect of N deficiency on (A) apparent photosynthetic rate, (B) stomatal conductance and (C) transpiration rate of the leaf immediately below the second truss of tomato. Control (white circle), -N treatment (black circle). Vertical bars denote SEM for each value.

Fig. 7. The effect of N deficiency on ^{13}C export rate from the leaf immediately below the first truss of tomato at 9 d and 19 d after treatment (DAT). Cont., control; -N, N deficiency.

Fig. 8. The effect of N deficiency on ^{13}C partitioning among various part of tomato plant at 9 d and 19 d after treatment (DAT). Cont., control; -N, N deficiency. 1st fruits (black and white checks), root (white), stem (diagonal stripe), leaves (spotted), other (black).

Fig. 9. The effect of N deficiency on ^{15}N partitioning among various part of tomato plant at 19 d

after treatment (DAT). Cont., control; -N, N deficiency. 1st fruits (black and white checks), root (white), stem (diagonal stripe), leaves (spotted), other (black).

Fig. 10. The effect of N deficiency on (A) N concentration and (B) sugar concentration of the leaf immediately below the second truss of tomato. Control (white circle), -N treatment (black circle). Vertical bars denote SEM for each value.

Fig. 11. Relationship between (A) leaf N concentration and apparent photosynthetic rate, (B) leaf sugar concentration and apparent photosynthetic rate. R^2 is the linear regression coefficient squared. *P* is level of significance.

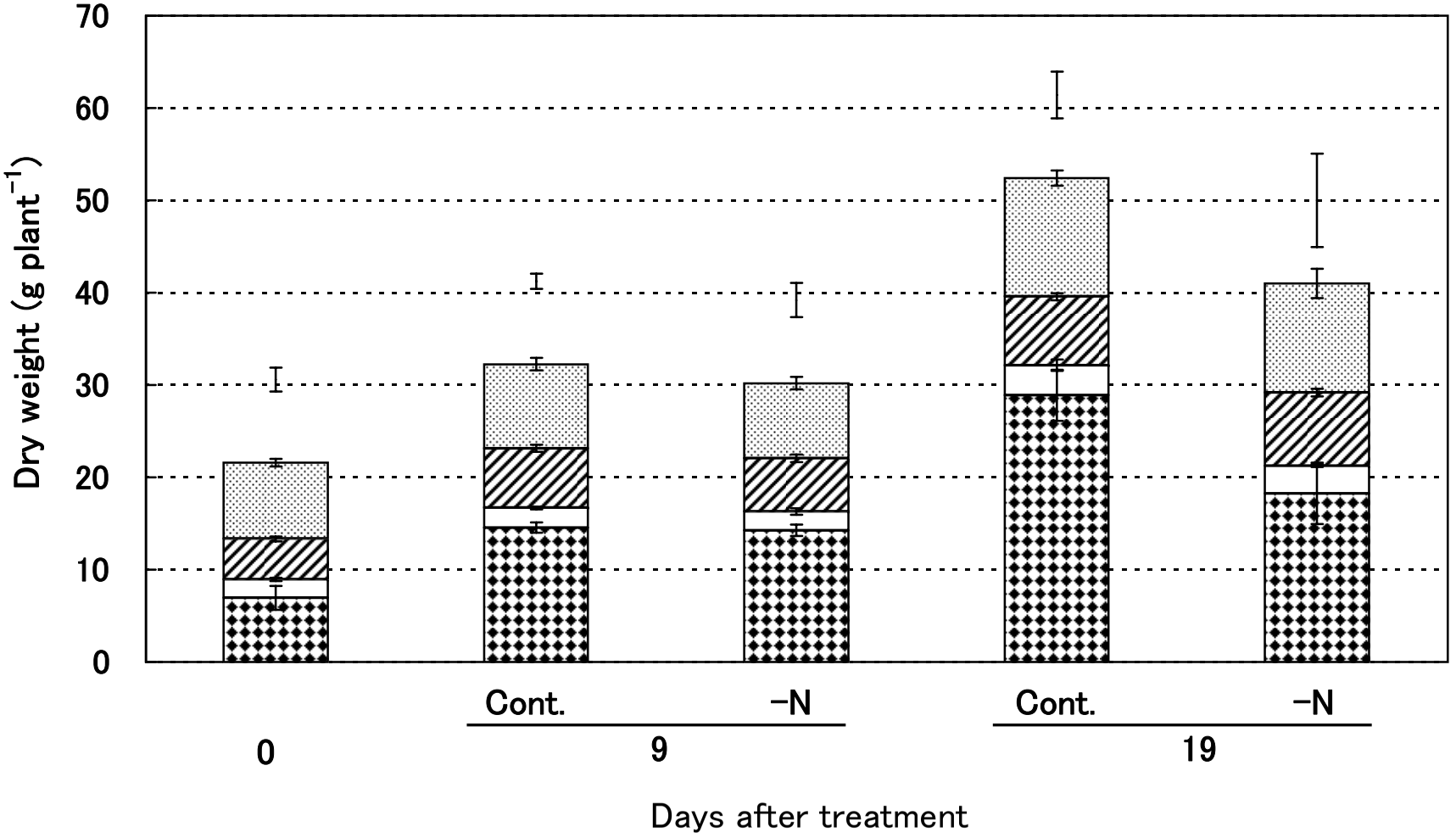

Fig. 1. The effect of N-deficiency on dry weight of tomato plant organs. 0, Initial of treatment; Cont.: control; −N: nitrogen deficiency. Vertical bars denote SEM for whole plant weight and each organ weight. Fruit (black and white checks), root (white), stem (diagonal stripe), leaves (spotted).

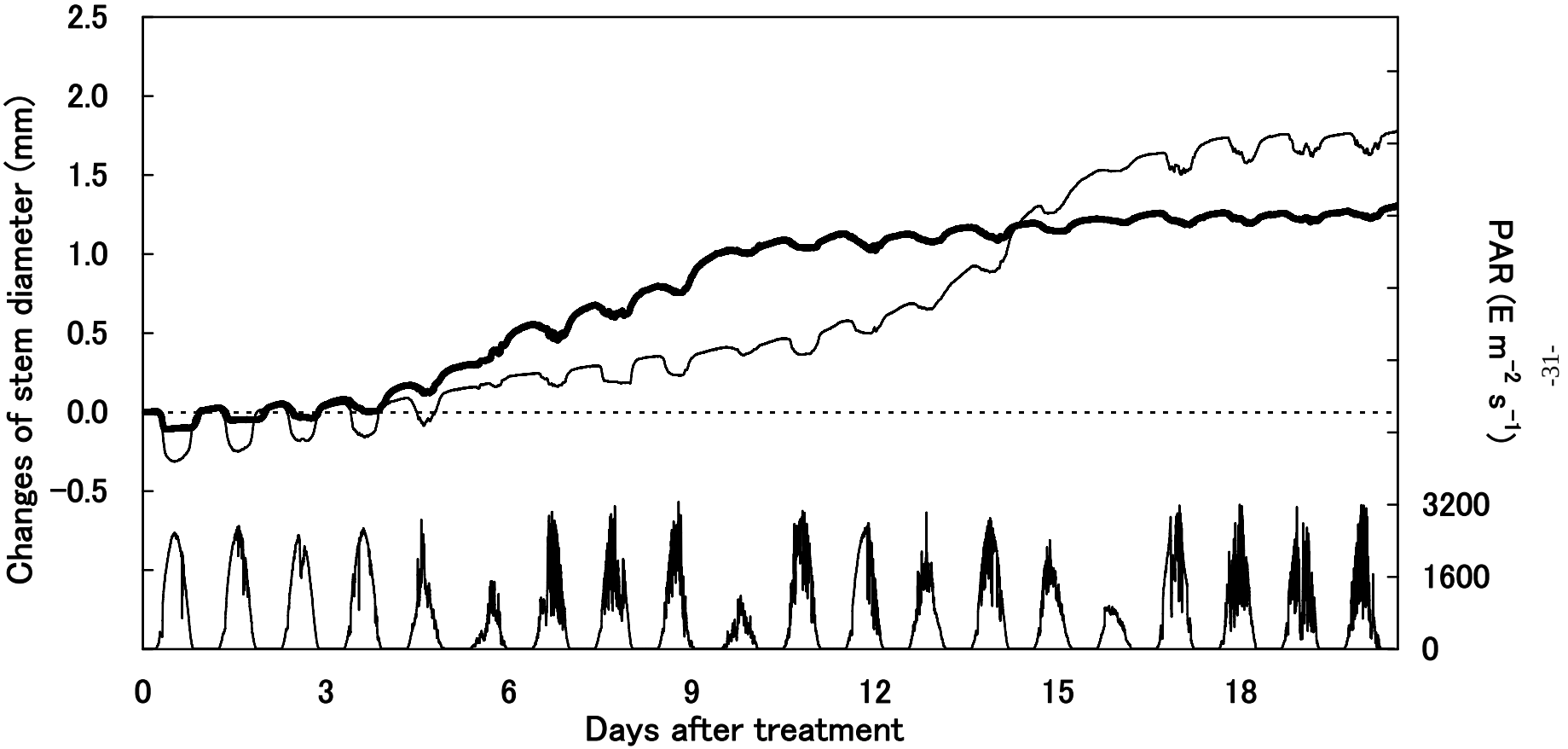

Fig. 2. The effect of N−deficiency on diurnal changes of stem diameter of tomato plant. Changes in stem diameter were monitored by shrinkage type micro−displacement detector. Data represent the means of three plants. Control (fine line), −N treatment (thick line) and PAR (most bottom line).

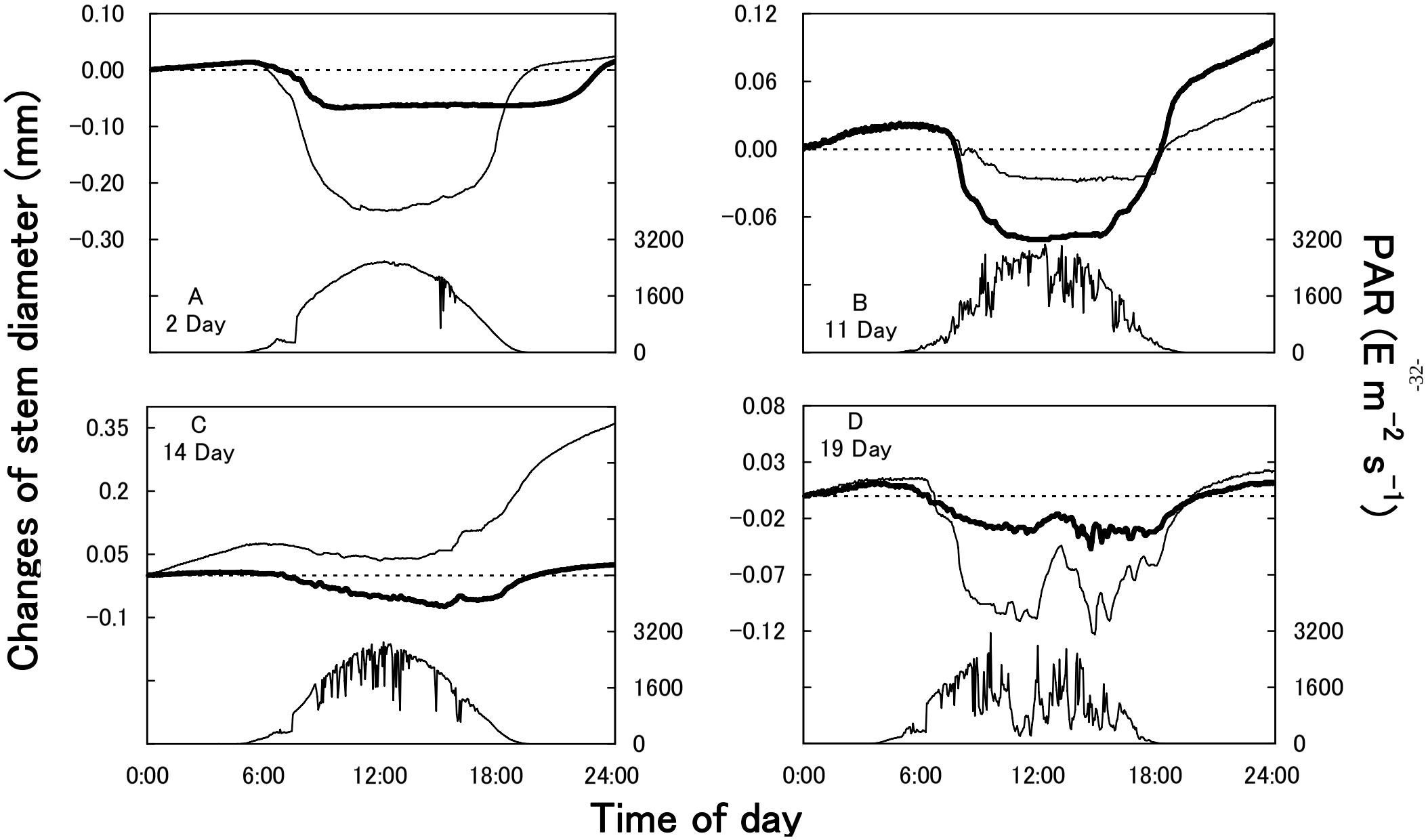

Fig. 3. The effect of N deficiency on diurnal changes of stem diameter of tomato plants. On each occasion, the diameter changes in both control and N-deficient plants were recorded with reference to 'Zero change' at midnight (00.00 h). Control (fine line), −N treatment (thick line) and PAR (most bottom line).

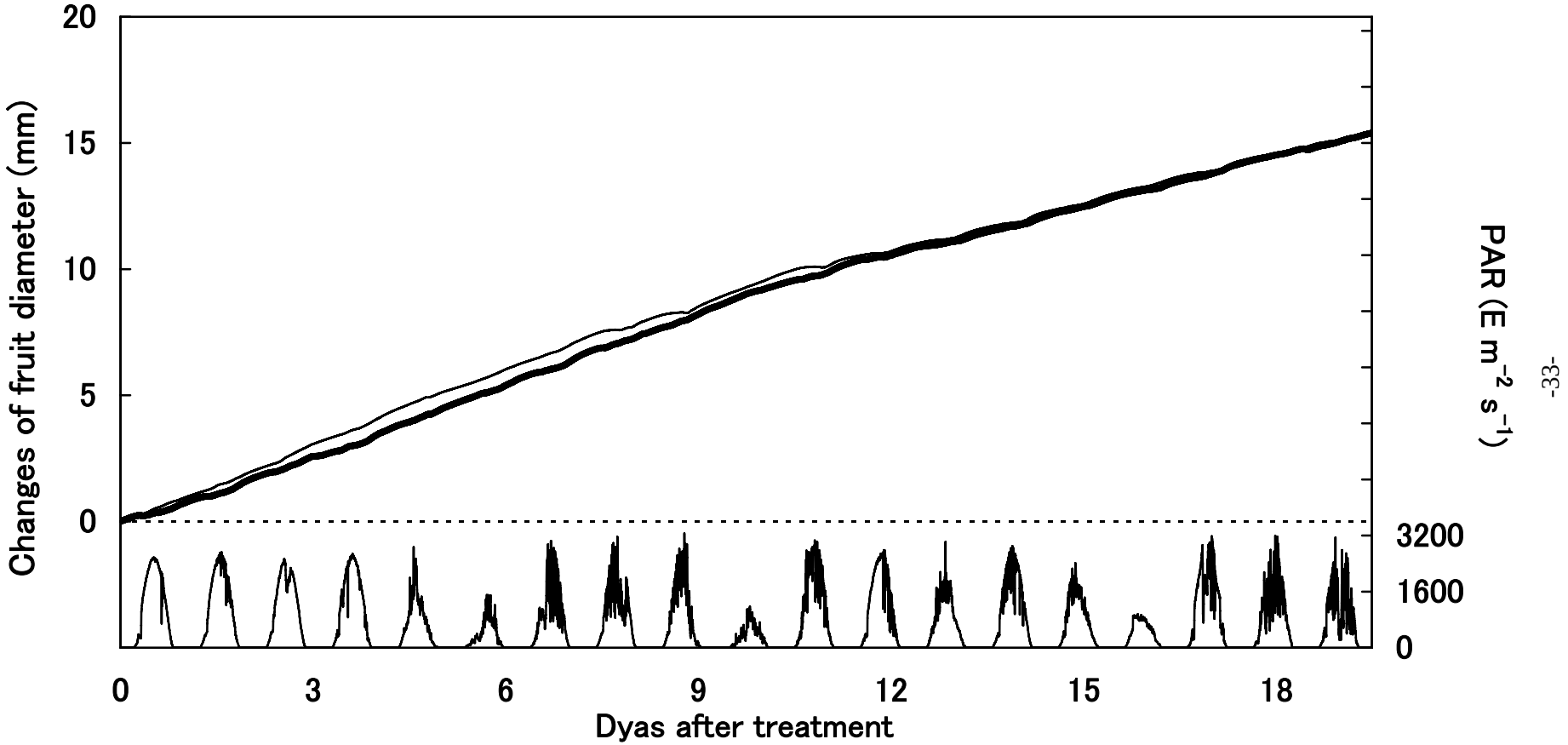

Fig. 4. The effect of N deficiency on changes in fruit diameter in tomato. Changes in fruit diameter was monitored as described in Fig. 2. Control (fine line), −N (thick line) and PAR (most bottom line).

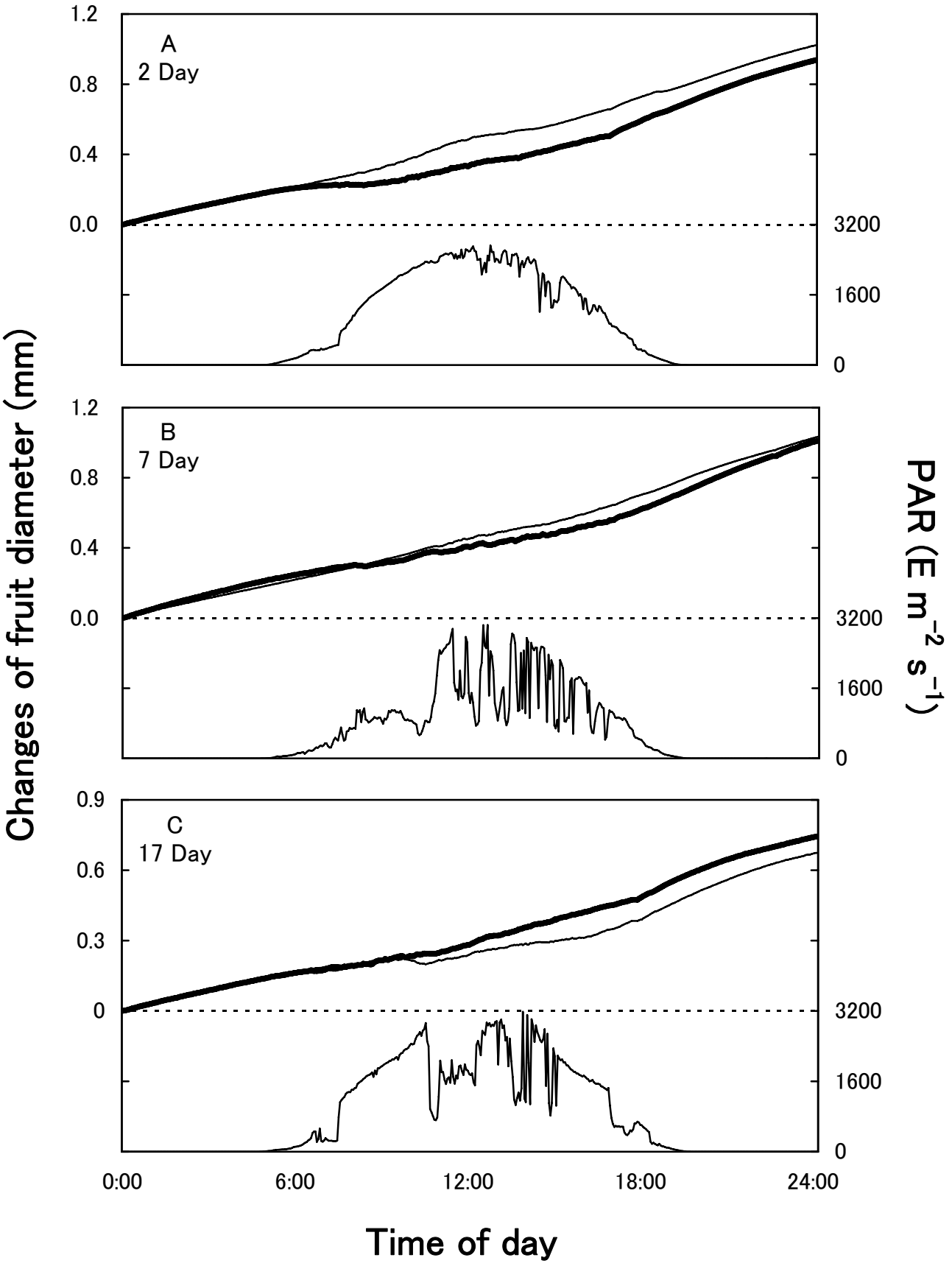

Fig. 5. The effect of N deficiency on diurnal changes of fruit diameter of tomato plants. On each occasion, the diameter changes in both control and N-deficient plants were recorded with reference to 'Zero change' at midnight (00.00 h). Control (fine line), -N treatment (thick line).

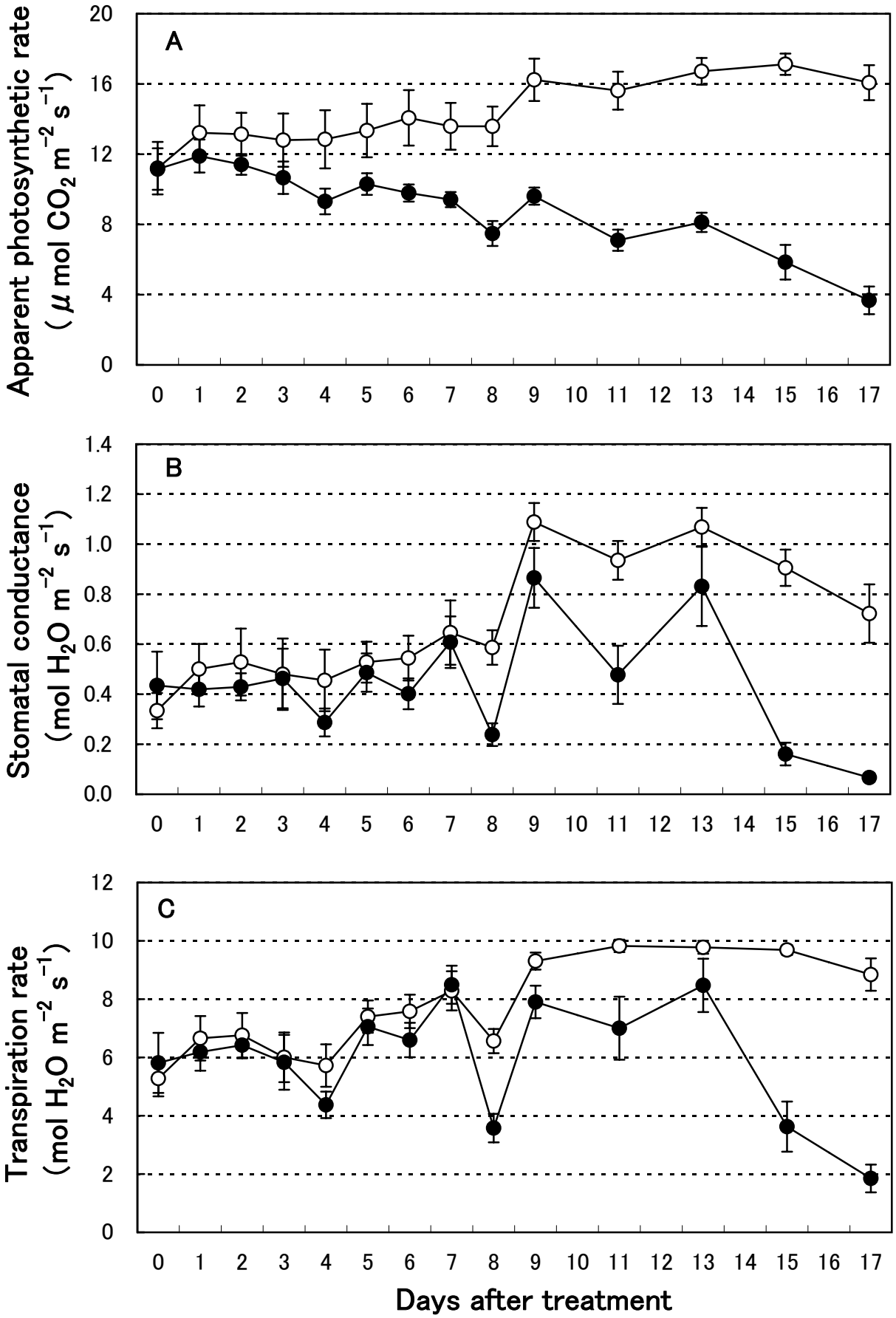

Fig. 6. The effect of N deficiency on (A) apparent photosynthetic rate, (B) stomatal conductance and (C) transpiration rate of the leaf immediately below the second truss of tomato. Control (white circle), −N treatment (black circle). Vertical bars denote SEM for each value.

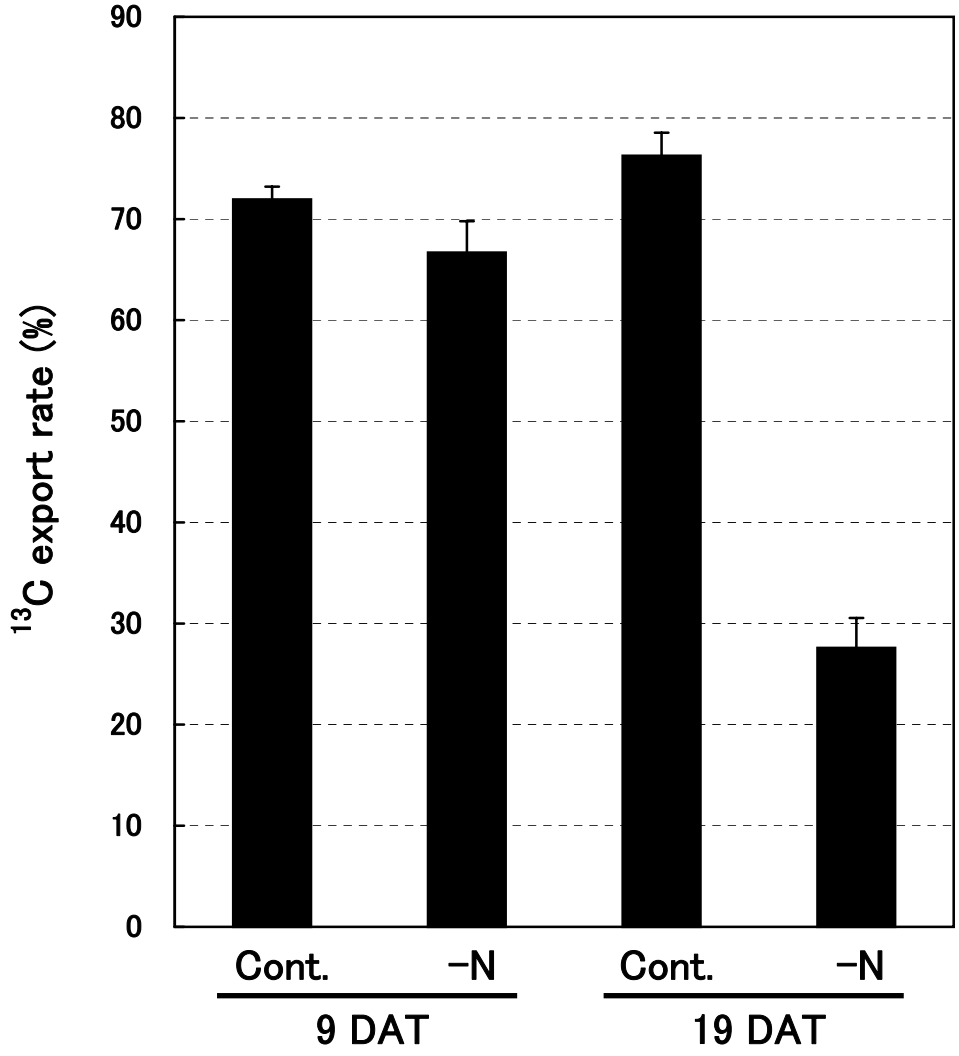

Fig. 7. The effect of N deficiency on ^{13}C export rate from the leaf immediately below the first truss of tomato at 9 d and 19 d after treatment (DAT). Cont., control; −N, N deficiency.

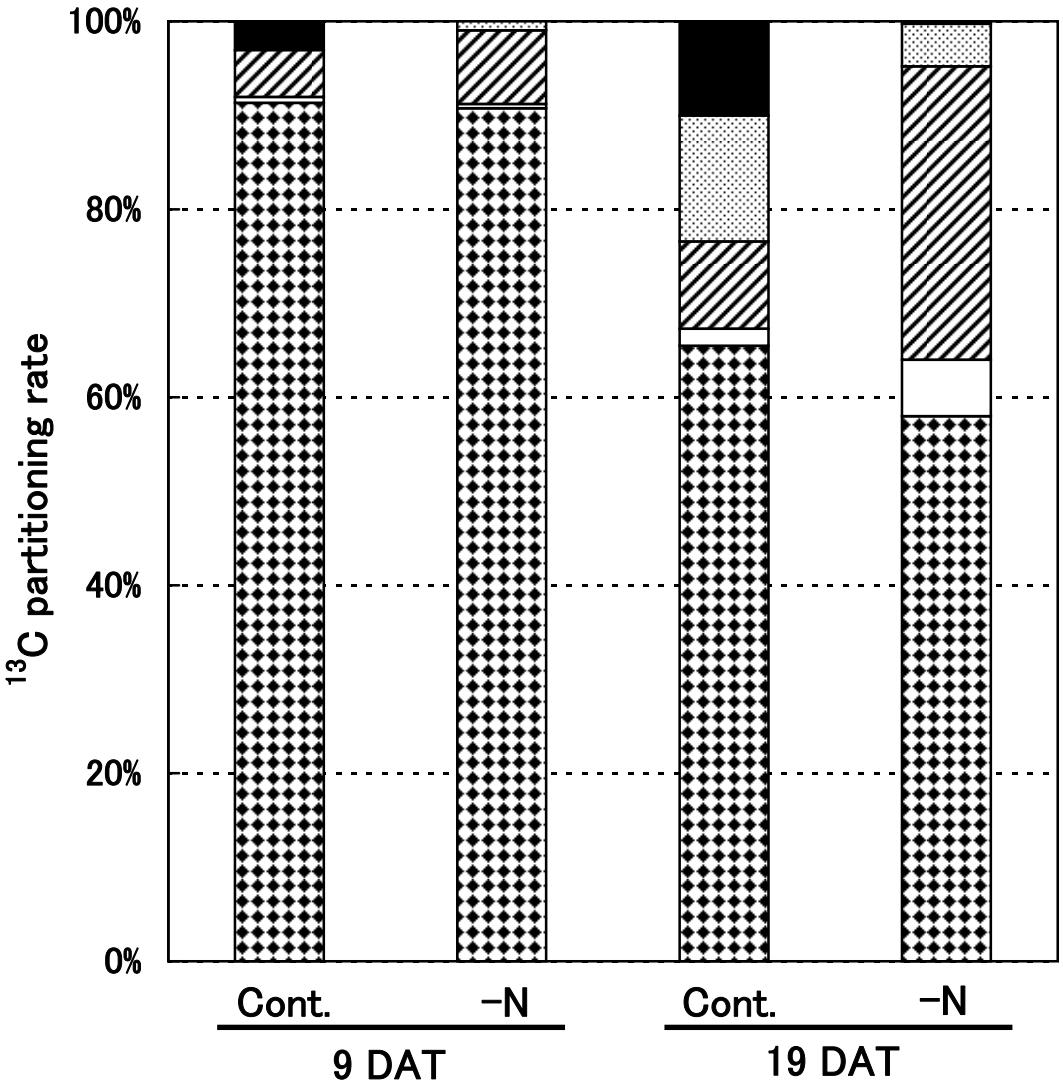

Fig. 8. The effect of N deficiency on ^{13}C partitioning among various part of tomato plant at 9 d and 19 d after treatment (DAT). Cont., control; −N, N deficiency. 1st fruits (black and white checks), root (white), stem (diagonal stripe), leaves (spotted), other (black).

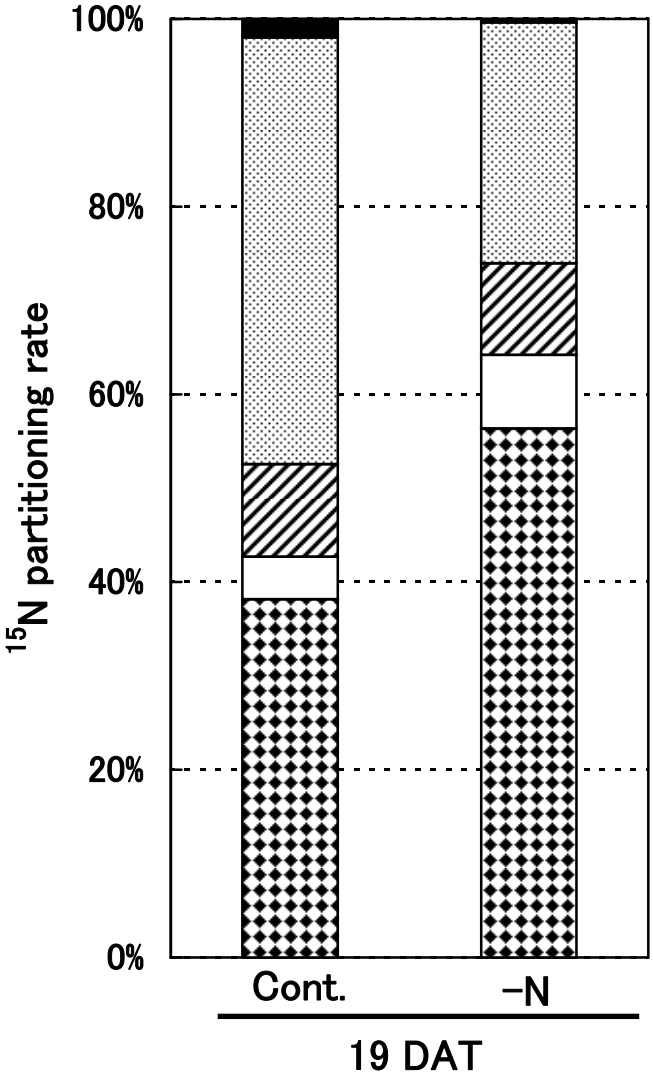

Fig. 9. The effect of N deficiency on ^{15}N partitioning among various part of tomato plant at 19 d after treatment (DAT). Cont., control; −N, N deficiency. 1st fruits (black and white checks), root (white), stem (diagonal stripe), leaves (spotted), other (black).

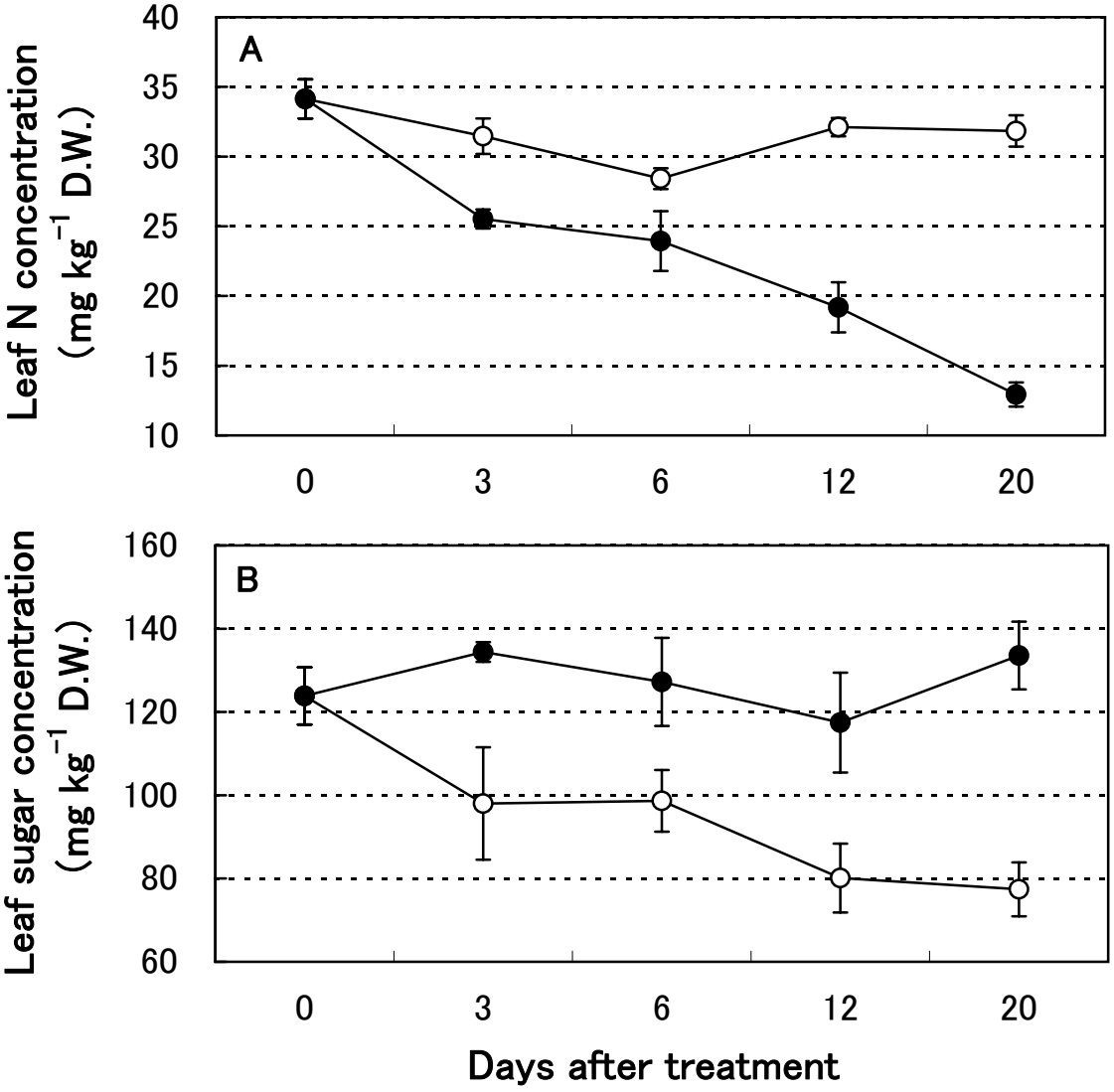

Fig. 10. The effect of N deficiency on (A) N concentration and (B) sugar concentration of the leaf immediately below the second truss of tomato. Control (white circle), −N treatment (black circle). Vertical bars denote SEM for each value.

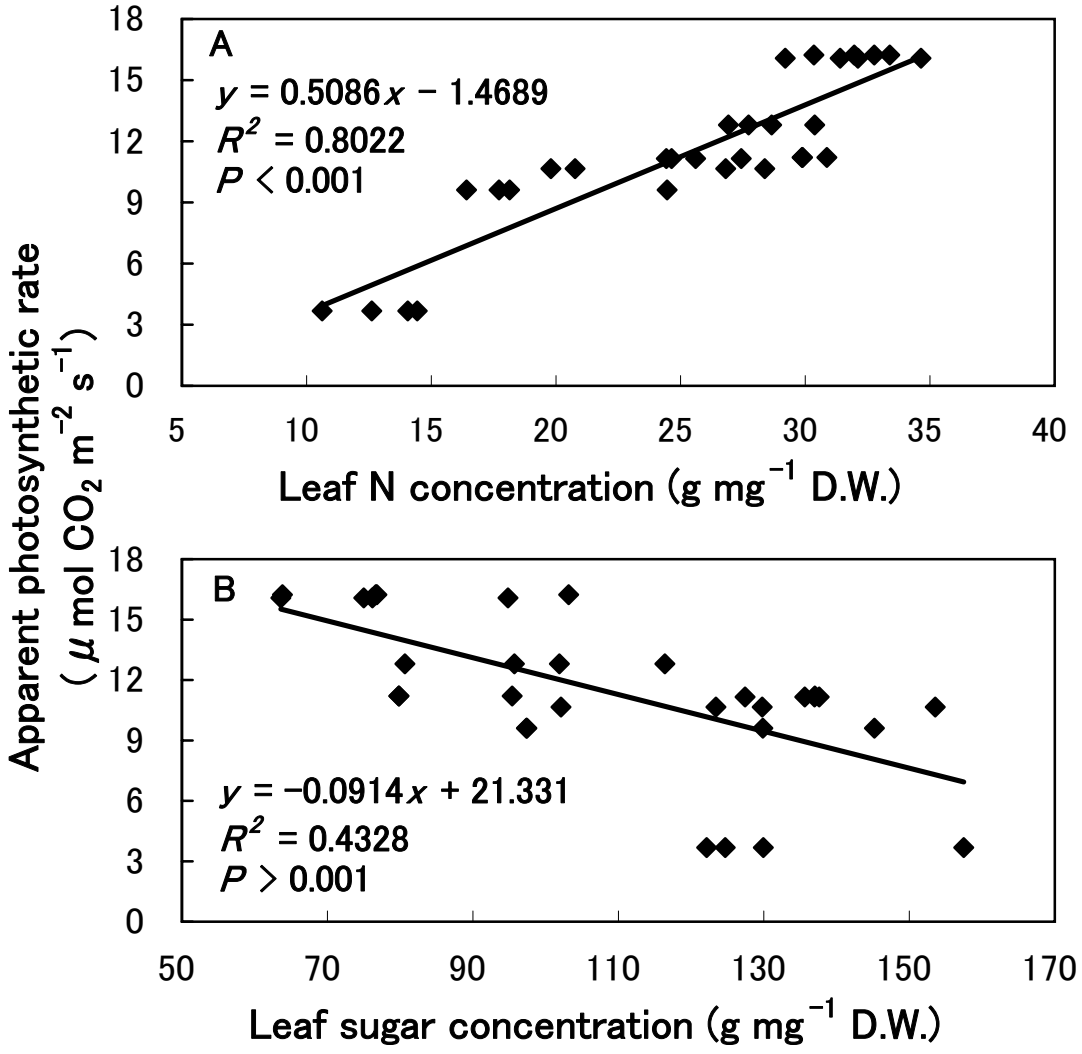

Fig. 11. Relationship between (A) leaf N concentration and apparent photosynthetic rate, (B) leaf sugar concentration and apparent photosynthetic rate. R^2 is the linear regression coefficient squared. P is level of significance.