Carbon dioxide assimilation and photosynthetic electron transport of tea leaves under nitrogen deficiency

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Abstract

Background: Tea plant is famed in humid and sub-humid of tropical regions, sub-tropical regions, and is a leaf-harvested crop. Nitrogen is the most important nutrient for increasing quality of tea leaves. Therefore, large amounts of nitrogen fertilizer are increasingly applied by tea farmers. Appropriate application of nitrogen fertilizer aroused people's concern. This research of physiological response to N deficiency stress will be helpful for appropriate application of nitrogen fertilizer for tea farmers and elucidate a mechanistic basis for the reductions in carbon dioxide (CO2) assimilation.

Results: To elucidate a mechanistic basis for the reductions in carbon dioxide (CO2) assimilation under nitrogen (N) deficiency tea leaves, changes in chlorophyll (Chl), carbohydrates, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and chlorophyll fluorescence transient were examined together with six N treatment (0, 50, 100, 300, 1200 or 6000 μM N). Root, stem and leaves dry weight (DW) increased as N supply increased from 0 to 300 μM, then remained unchanged. The reductions in CO2 assimilation of N-deficient leaves paralleled with high intercellular CO2 concentration. Rubisco activity, protein and Chl content increased linearly or curvilinearly over the range of leaf N content examined except unchanged as leaf N from 2.15 to 2.79 g m⁻². Chlorophyll fluorescence transient from N-deficient leaves displayed a depression at the P-step, accompanied by a new step at about 150 μs (L-step). Fv/Fm, REo/ETo, ETo/ABS, Snr, ETo/CS or P₁abs, P₁tot, abr were decreased in N-deficient leaves but increased DIo/CS or DIo/RC and DIo/ABS. Regressive analysis showed that CO2 assimilation decreased linearly or curvilinearly with decreasing initial rubisco, P₁abs and Leaf Chl, respectively. Therefore, we concluded the decreased photosynthetic electron transport capacity, leaf Chl content and initial rubisco activity are probably the main factors contributing to decreased CO2 assimilation under N deficiency.

Conclusions: The decreased photosynthetic electron transport capacity, leaf Chl content and initial rubisco activity are probably the main factors contributing to decreased CO2 assimilation under N deficiency.

Keywords: Tea plant, Nitrogen deficiency, CO2 assimilation, Chlorophyll fluorescence
fertilizer to tea plantations have been as high as 450–
1200 kg N ha\(^{-1}\) \(\text{year}^{-1}\), which significantly surpasses
the recommended rate of 250–375 kg N ha\(^{-1}\) \(\text{year}^{-1}\) for
high tea yields (Tokuda and Hayatsu 2004; Hirono and
Nonaka 2012; Fu et al. 2012; Zhu et al. 2014). Not surpris-
ingly, such high nitrogen inputs can easily induce excess
residual nitrogen and acidification of soil; both influence
the nitrogen cycle of tea fields in which a great deal of
nitrogenous gases are produced (Jumadi et al. 2008; Zhu
et al. 2014). Despite such use, soil N deficiency remains a
major constraint on crop productivity in many developing
countries. Therefore, increasing tea plant tolerance to
low-N conditions would improve tea production, espe-
cially in regions with low soil N levels (Wei et al. 2016).

The yield of leaves and leaf photosynthetic rate are
directly linked to plant dry matter production. The
extent of photosynthesis during tea plant growing can be
affected by many factors, including tea cultivars, altitude,
climatic condition, CO\(_2\) level, soil condition and tem-
perature. Another major factor affecting photosynthesis
is the available N level of the soil (Wei et al. 2016; Jaaffar
and Gardner 1988). Given the diverse roles that nitrogen
plays in plant physiology and development, N deficiency
has a crippling effect on plants. N deficiency significantly
reduces a plant’s capacity for photosynthesis (Boussadia
et al. 2010) by reducing the rates of leaf photosynthesis
and new leaf area expansion. Furthermore, N deficiency
leads to the degradation of photosynthetic pigments and
proteins, and reduced enzyme synthesis in plants (Poless-
kaya et al. 2004). Therefore, N deficiency leads to changes
in the expression levels of proteins, as well as the activ-
ity levels of enzymes, which invariably leads to changes
in plant metabolism (Wei et al. 2016). For example, N levels
affect the post-translational modification of phospho-
enolpyruvate carboxylase (PEPCase) (Prinsi et al. 2009).
Earlier studies in several other crops have also indicated that
N deficiency reduces ribulose bisphosphate carboxy-
lase/oxygenase (Rubisco) activity (Chen and cheng 2003,
2004), as well as reducing the actual amount of Rubisco
produced by the plant. In addition, N deficiency impacts
overall plant metabolism through wide reprogramming
of primary and secondary metabolic pathways (Scheible
et al. 2004).

The chlorophyll fluorescence transient has been found to
be a sensitive indicator of photosynthetic electron trans-
port processes (Tóth et al. 2007). The transient is con-
sidered to be determined by changes in the redox state of primary quinine acceptor (QA) (Lázár 2006; Lin
et al. 2009), but at the same time, the transient reflects
the reduction of the photosynthetic electron transport
chain (Schanzer et al. 2005; Lin et al. 2009). The chlo-
rophyll fluorescence transient was applied in numerous
studies in crop plants, e.g. to assess the environmental
effects in wheat, such as drought (Zivčák et al. 2008), high
temperature (Brestić et al. 2012), light stress (Kalaji et
al. 2012; Živčák et al. 2014). The chlorophyll fluorescence
transient were applied several times also in studies deal-
ing with nitrogen deficiency in plants and the effect of
poor nitrogen supply on photosystem II (PSII) is recently
well described (Lu et al. 2001; Redillas et al. 2011; Li et
al. 2012). Although in many of published works the rapid
chlorophyll fluorescence is denoted as a useful tool for
assessing the physiological effects of nitrogen deficiency
on plants, there is still a lack of data on the usefulness of
the method in assessment of plant photosynthetic perfor-
mane in crop trials with different nitrogen supply. Thus,
it is not well known how N deficiency affects photosyn-
thetic electron transport in tea plant.

Gaining a more complete mechanistic picture of how
plants adapt and respond to low N conditions is impor-
tant since N plays important roles in growth and physi-
ology. This is especially critical for crops like tea, which
serves as one of the most popular beverages worldwide
(Khokhar and Magnusdottir 2002; Topuz et al. 2014).
In addition, a better understanding of the proteins, CO\(_2\)
assimilation and photosynthetic electron transport that
influence responses to low N can improve the utilisation
efficiency of N fertilisers and assist in developing better
methods to evaluate plant responses to possible deficien-
cies. In this study, we aimed to determine how N defi-
ciency affects CO\(_2\) assimilation, Rubisco, non-structural
carbohydrates and photosynthetic electron transport in
tea leaves to understand the mechanism by which N defi-
ciency leads to a decrease in CO\(_2\) assimilation.

Methods
Plant materials and N treatments
The experiment was completed in 2015 in study plot of
Tea Research Institute, Fujian Academy of Agricultural
Sciences, by using 9-month-old uniform tea (Camel-
lia sinensis (L.) O. Kuntze) cv. Benshan) trees potted in
6 L argil pots that were filled with river sand, 2 seed-
lings per pot, and cultivated in the natural temperature
and light conditions. Nutrient solution was prepared by
referring to (Lin et al. 2009), and full-strength nutrient
solution contained 3 mmol L\(^{-1}\) NH\(_4\)NO\(_3\), 0.5 mmol L\(^{-1}\)
Ca(H\(_2\)PO\(_4\))\(_2\), 1.0 mmol L\(^{-1}\) K\(_2\)SO\(_4\), 0.5 mmol L\(^{-1}\) CaCl\(_2\),
0.6 mmol L\(^{-1}\) MgSO\(_4\), 46 μmol L\(^{-1}\) H\(_3\)BO\(_3\), 9 μmol L\(^{-1}\)
MnSO\(_4\), 9 μmol L\(^{-1}\) ZnSO\(_4\), 2 μmol L\(^{-1}\) CuSO\(_4\),
2.6 μmol L\(^{-1}\) Na\(_2\)MoO\(_4\) and 30 μmol L\(^{-1}\) Fe-EDTA. Seven
weeks after transplanting, the treatment was applied
for 18 weeks: until the end of the experiment, each pot
was supplied three times weekly with 500 mL of nutrient
solution at a N concentration of 0, 50, 100, 300, 1200
or 6000 μM from NH\(_4\)NO\(_3\) at pH of 5.0. At the end of
the experiment, the fully-expanded (about 7 weeks old)
leaves from different replicates and treatments were used for all the measurements. Leaf discs (0.63 cm² in size) were collected at noon under full sun and immediately frozen in liquid N₂. Samples were stored at −80 °C until they were used for the determination of Chl, Rubisco, carbohydrates, and protein. Special care was taken to ensure that all samples were transferred directly from liquid N₂ to freezer of −80 °C, at no time were any samples exposed to room temperature.

**Measurements of root, stem and leaf DW**

At the end of the experiment, 10 plants per treatment from different pots were harvested. The plants were divided into their separate parts (roots, stems and leaves). The plant material was then dried at 80 °C for 48 h and the DW measured. Specific leaf weight was measured according to Syvertsen et al. (1980).

**Determination of leaf total soluble protein, Chl, and total N**

Chl, Chl a and Chl b were assayed according to Lichtenthaler (1987). Briefly, 2 frozen leaf discs were extracted with 8 mL of 80% (v/v) acetone for 24 h in the dark. The extracts were determined using Libra S22 ultraviolet–visible spectrophotometer (Biochrom Ltd., Cambridge, UK). Leaf total soluble protein was extracted with 8 mL of 80% (v/v) acetone for 24 h in the dark. The extracts were determined using Libra S22 ultraviolet–visible spectrophotometer (Biochrom Ltd., Cambridge, UK). Leaf total soluble protein was extracted with 8 mL of 80% (v/v) acetone for 24 h in the dark. The extracts were determined using Libra S22 ultraviolet–visible spectrophotometer (Biochrom Ltd., Cambridge, UK). Leaf total soluble protein was extracted with 8 mL of 80% (v/v) acetone for 24 h in the dark. The extracts were determined using Libra S22 ultraviolet–visible spectrophotometer (Biochrom Ltd., Cambridge, UK). Leaf total soluble protein was extracted with 8 mL of 80% (v/v) acetone for 24 h in the dark. The extracts were determined using Libra S22 ultraviolet–visible spectrophotometer (Biochrom Ltd., Cambridge, UK).

**Measurements of leaf chlorophyll fluorescence transients**

Chlorophyll fluorescence transient was measured by a Handy Plant Efficiency Analyzer (Handy PEA, Hansatech Instruments Limited, Norfolk, UK) according to Strasser et al. (1995). All the measurements were done with 3 h dark-adapted plants at room temperature. Chlorophyll fluorescence transient was induced by 3400 μmol m⁻² s⁻¹ red light, which was provided by three luminous diodes (peak value of 650 nm), and the light was focused on the leaves, evenly illuminated on the exposed (diameter of 4 mm) surface. At the beginning 300 μs, data were read per 10 μs. With the slowing fluorescence dynamic signals, the time interval of data reading is prolonged. All determination work was conducted at room temperature 3 h after the plants were adapted to the dark condition, and repeat for every 5–7 (each leaf of the seeding repeats once).

For the following derivative parameters: (1) \( V_J = (F_{2ms} - F_o) / (F_m - F_o) \) denotes the relative variable fluorescence at point J (2 ms); \( V_I = (F_{30ms} - F_o) / (F_m - F_o) \) the relative variable fluorescence at point I (30 ms); \( S_m = (E_{0o}/R_C) - \text{Area}/(F_m - F_o) \) the general electronic carrier of the reaction center; and \( M_o = 4(F_{300ms} - F_o) / (F_m - F_o) \) the initial slope of chlorophyll fluorescence induction curve; (2) The energy used to capture the electron transfer of the unit reaction center (RC): dissipated energy in the RC: \( D_{I_o}/R_C = \text{ABS}/RC - TR_o/RC \); (3) the quantum yield and energy distribution ratio parameters: the maximum photochemical efficiency \( \varphi = TR_o/ABS = 1 - F_o/F_m = F_o/F_m \); quantum yield for electron transfer \( \varphi = ET_o/ABS = F_o/F_m \times (1 - V_I) \).
probability of electron transfer by the captured exciton to the electron transfer chain in exceeding other QA electron acceptors $\psi_{Eo} = \frac{ET_o}{TR_o} = (1 - V_J)$; quantum yield ratio used for heat dissipation $\phi_{Do} = \frac{DI_o}{ABS} = 1 - \phi_{Po}$; quantum yield of electron transfer in unit area ($t = 0$) $ET_o/CS_o = \psi_{Eo} \times (ABS/CS_o)$; heat dissipation in unit area ($t = 0$) $DI_o/CS_o = ABS/CS_o - TR_o/CS_o$; (5) Performance index (PI): that is based on light absorption $PI_{abs} = RC/ABS \times \left[ \phi_{Po}/(1 - \phi_{Po}) \right] \times \left[ \psi_{Eo}/(1 - \psi_{Eo}) \right]$; (6) Total PI, measuring the performance up to the PSI end electron acceptors $PI_{tot, abs} = (RC/ABS) \times (\psi_{Po}/(1 - \psi_{Po})) \times (\psi_{Eo}/(1 - \psi_{Eo})) \times (\delta_{Ro}/(1 - \delta_{Ro}))$.  

**Experimental design and statistical analysis**

There were 20 pots trees per treatment in a completely randomized design. Experiments were performed with 5–10 replicates (one tree from different pots per replicate). Differences among treatments were separated by the least significant difference (LSD) test at P < 0.05 level.

**Results**

**Leaf N content and plant growth characteristics**

As N supply decreased, leaf N content decreased curvilinearly (Fig. 1A). Root, stem and leaf dry weight increased as N supply increased from 0 to 300 μM, then remained unchanged (Fig. 1B–D), and resulted in a greater root DW/shoot DW ratio under N supply with 0 and 50 μM (Fig. 1E).

**Leaf Chl, soluble protein, gas exchange and Rubisco**

The total soluble protein contents (Fig. 2A) did not change significantly as leaf N decreased from 2.79 to 2.15 g m$^{-2}$ then decreased significantly with further decreasing leaf N contents. Leaf Chl a (Fig. 2B), Chl b (Fig. 2C) and Chl (Fig. 2D) contents did not change significantly as leaf N decreased from 2.79 to 2.15 g m$^{-2}$ then decreased with further decreasing leaf N content. The ratio of Chl a/b (Fig. 2E) remained unchanged over the range of leaf N content examined.

Leaf CO$_2$ assimilation (Fig. 3A) and stomatal conductance (Fig. 3B) increased as leaf N content increased from 1.30 to 2.15 g m$^{-2}$, then remained relatively stable with further increasing leaf N content, whereas intercellular CO$_2$ concentration decreased as leaf N content increased.
from 1.30 to 1.65 g m$^{-2}$, then increased from as leaf N content increased from 1.99 to 2.79 g m$^{-2}$ (Fig. 3C).

Both initial and total Rubisco activity kept relatively constant as leaf N content decreased from 2.79 to 1.99 g m$^{-2}$, and then decreased significantly with further decreasing leaf N content from 1.99 to 1.30 g m$^{-2}$ (Fig. 4A, C), whereas both initial and total activity expressed on a protein basis did not change significantly, except decreased significantly as leaf N content decreased from 1.33 to 1.30 g m$^{-2}$ (Fig. 4B, D). Rubisco activation state remained unchanged as leaf N content decreased from 2.79 to 1.65 g m$^{-2}$, and then dropped as leaf N content decreased from 1.33 to 1.30 g m$^{-2}$ (Fig. 4E).

**Leaf nonstructural carbohydrates**

On an area basis, contents of glucose and fructose content did not change significantly as leaf N content decreased from 2.79 to 2.15 g m$^{-2}$ and then dropped significantly as leaf N content decreased from 1.99 to 1.30 g m$^{-2}$ (Fig. 5A, B). The sucrose content increased successive over the range of leaf N content from 1.30 to 2.79 g m$^{-2}$ (Fig. 5C). Leaf starch content remained little changed as leaf N content increased from 1.30 to 1.33 g m$^{-2}$, then increased significantly with further increasing leaf N content (Fig. 5D).

**Leaf chlorophyll fluorescence transients and related parameters**

The chlorophyll fluorescence transients of leaves from 0, 50 and 100 μM N-treated trees showed a large depression at the P-step (Fig. 6A). Figure 6C and D shows the kinetics of relative variable fluorescence at any time $V_t = (F_t - F_o)/(F_m - F_o)$ and the differences of normalized N-treated transients minus 6000 μM N-treated transient ($\Delta V_t$). The differences revealed two obvious bands: increase in the 2–4 ms range J-step and in the 30–100 ms range I-step. The positive J and I-steps were very pronounced in the leaves from 0, 50 and 100 μM N-treated trees. Figure 6E and F depicts the relative variable fluorescence between $F_o$ and $F_{300 \mu s}$ ($W_{300}$) and the differences of normalized N-treated transients minus 6000 μM N-treated transient ($\Delta W_{300}$). The differences showed a clear L-step in the leaves from 0, 50
and 100 μM N-treated trees. Figure 6B depicts from 0 to 100 μM N-treated trees had decreased maximum amplitude of IP phase and rise time, and the end-levels were lowered by N deficiency. According to Fig. 7, each parameter the values were normalized on that of the sample treated with 6000 μM N-treated trees. The result showed that leaves N content from 1.30 to 1.65 g m\(^{-2}\) had decreased \(F_v/F_m\), \(RE_o/ET_o\), \(ET_o/\text{ABS}\), \(S_m\) (Fig. 7A), \(ET_o/\text{CS}_o\), \(\text{PI}_{\text{abs}}\), \(\text{PI}_{\text{tot, abs}}\) (Fig. 7B), but increased \(\text{DI}_o/\text{CS}_o\), \(\text{DI}_o/\text{RC}\) and \(\text{DI}_o/\text{ABS}\) (Fig. 7C).

Leaf \(\text{Pi}_{\text{abs}}\) initial rubisco activity and Chl content in relation to \(\text{CO}_2\) assimilation

Leaf \(\text{CO}_2\) assimilation increased linearly or curvilinearly with increasing \(\text{Pi}_{\text{abs}}\) (Fig. 8A), initial rubisco activity (Fig. 8B) and Chl content (Fig. 8C), respectively.

Discussion

Nitrogen is one of the most important nutrients for crop growth and development because it affects dry matter production by influencing the leaf area development and maintenance as well as photosynthetic efficiency (Zhu et al. 2014). Nearly all physiological and biochemical activities reached their maximum in the leaves of about 2.15 g m\(^{-2}\) from 300 μM N-treated trees (Figs. 2, 3, 4, 5, 6, 7). Based on these results, trees treated with 0, 50 or 100 μM N are considered N deficient.

The present work (Fig. 1), like that of previous workers (Chen and Cheng 2004; Chen et al. 2015; Zhu et al. 2014) indicates that N deficiency suppressed N content, plant growth and DM accumulation. N deficiency resulted in an increase in the ratio of root/shoot dry weight (Fig. 1E) which agrees with the view that plant tops are affected by N deficiency to a greater extent than root systems (Chen et al. 2015).

Leaf Chl a, b and total Chl (Fig. 2A–C) concentration were closely correlated with leaf N level decreased linearly as leaf N concentration decreased, except for leaf N content from 2.79 to 2.15 g m\(^{-2}\), indicating that leaf N no longer limited Chl. These results agree with earlier reports in Grape (Chen and Cheng 2004), sorghum (Zhao et al. 2005), apple (Chen and Cheng 2004) and in corn (Zhao et al. 2003).

\(\text{N}\) limitation significantly reduced both \(\text{CO}_2\) assimilation and stomatal conductance of tea leaves (Fig. 3A, B), but higher intercellular \(\text{CO}_2\) concentration in low N leaves (Fig. 3C). These indicates that the low \(\text{CO}_2\) assimilation under N limitation was caused by non-stomatal factors (Bondada and Syvertsen 2005). Decreases in \(\text{CO}_2\) assimilation accompanied by an increase in intercellular \(\text{CO}_2\) concentration due to N deprivation has also been reported in wheat (\textit{Triticum aestivum} L.) (Evans 1983), citrus (Bondada and Syvertsen 2005) and Grape (Chen and Cheng 2004). However, the decrease of assimilation \(\text{CO}_2\) rate under N deficiency was accompanied by
a decrease in the starch accumulation (Fig. 5D), as previously reported for P deficiency of tea (Lin et al. 2009). This indicates that the production, rather than the utilization of photosynthates, is limiting. The results showed that N-deficiency significantly reduced the contents of glucose (Fig. 5A), fructose (Fig. 5B) and sucrose (Fig. 5C). However, N deficiency was probably not the primary factor limiting CO₂ assimilation, because there was a greater decrease in CO₂ assimilation than in sugars content. Evidence shows that soluble sugars, specifically hexoses, may repress photosynthetic gene expression, particularly of the nuclear-encoded small sub-unit of Rubisco, thus decreasing Rubisco content and CO₂ assimilation (Lin et al. 2009, 2010).

Earlier studies in several other crops indicated that N deficiency reduced either Rubisco activity (Heitholt et al. 1991; Chen and cheng 2004) or the amount of the enzyme (Osaki et al. 1993; Chen and cheng 2003).

In our study, initial and total Rubisco activity expressed on an area basis were closely correlated with leaf N level decreased linearly as leaf N concentration decreased, except for leaf N content from 2.79 to 1.99 g m⁻², indicating that leaf N no longer limited Rubisco activity. Similar result has been obtained.
for apple (Chen and Cheng 2004) and Grape (Chen and Cheng 2003). The decrease in CO₂ assimilation in N-deficient leaves can not be attributed to a decrease in protein contents, because the decrease in leaf total soluble protein (Fig. 2A) contents was much less than CO₂ assimilation. Similar results have been reported for maize (Wei et al. 2016), sorghum (Zhao et al. 2005), Olive (Boussadia et al. 2010).

It was well documented that the nitrogen nutrition influences the plant photosynthetic capacity (Terashima and Evans 1988; Živčák et al. 2014). Thus, the membrane processes must be balanced to maintain high efficiency in the conversion of energy and to avoid the over-reduction of photosynthetic electron chain in conditions with different nitrogen supply (Tóth et al. 2007). The decrease of Fç/Fm in N-deficient leaves was caused by a decrease in Fm (Fig. 6A, B), as previously found for corn (Jin et al. 2015), maize (Wei et al. 2016) and wheat (Živčák et al. 2014). The decrease in Fç/Fm under stress is considered to reflect the photo inhibitory damage to PSII complexes (Baker and Eva-Rosenqvist 2004). The J-step, I-step and IP phase of chlorophyll fluorescence transients are correlated with the redox state of QA, the redox state of plastoquinone, and the redox state of end acceptors at PSI electron acceptor side, respectively (Lazár 2006; Schansker et al. 2005). The finding that N-deficient leaves had increased Vç and VJ (Fig. 6C, D), but decreased maximum amplitude of IP phase (Fig. 6B) suggests that acceptor side of PSII became more reduced under N deficiency, but the acceptor side of PSI become more oxidized. A positive L-step appeared at ca. 150 μs in the chlorophyll fluorescence transients in N-deficient leaves (Fig. 6E). This means that the oxygen evolving complex (OEC) is damaged (Hakala et al. 2005). A positive L-step has also been found in N-deficient cowpea leaves (Strasser et al. 1995).

We found that N deficiency decreased Fç/Fm, REo/ETo, ETo/ABS, S_m, ETo/CSo, PI吸收, PI总吸收 (Fig. 7A, B) and increased DIo/CSo, DIo/RC and DIo/abs (Fig. 7C). This means that N-deficient leaves damaged all of the photochemical and non- photochemical redox reactions and had a decreased capacity for electron transport, thus limiting ATP synthesis and RuBP regeneration. Regressive analysis showed that CO₂ assimilation decreased linearly

![Figure 5](image_url)
or curvilinearly with decreasing initial rubisco (Fig. 8A), 
PIabs (Fig. 8B) and Leaf Chl (Fig. 8C), respectively. Therefore, we concluded the decreased photosynthetic 
electron transport capacity, leaf chl content and initial 
rubisco activity are probably the main factors contributing to decreased CO₂ assimilation under N deficiency.
Conclusions
Assessing the impact of N deficiency on CO₂ assimilation and photosynthetic electron transport of tea plant is important to improve the utilisation efficiency of N fertilisers and assist in developing better methods to evaluate plant responses to possible deficiencies. This study demonstrated how N deficiency affects CO₂ assimilation, Rubisco, non-structural carbohydrates and photosynthetic electron transport in tea leaves to understand the mechanism by which N deficiency leads to a decrease in CO₂ assimilation. The results indicated that the decreased photosynthetic electron transport capacity, leaf chl content and initial rubisco activity are probably the main factors contributing to decreased CO₂ assimilation under N deficiency.

Fig. 7 Nine fluorescence parameters (A Fv/Fm, REo/ETo, ETo/ABS; B ETo/CS, PI_{abs}, PI_{tot,abs}, C DIo/CS, DIo/ABS, DIo/RC) derived from the average chlorophyll fluorescence transients of Fig. 6A in relation to N content (1.30, 1.32, 1.65, 1.99, 2.15 and 2.79 g m⁻²) in tea leaves. Fv/Fm: Maximum quantum yield of primary photochemistry; REo/ETo: Efficiency with which an electron can move from the reduced intersystem; ETo/ABS: Quantum yield for electron transport; ETo/CS: Electron transport flux per CS; PI_{abs}: Performance index (PI) on absorption basis; PI_{tot,abs}: Total PI, measuring the performance up to the PSI end electron acceptors; DIo/CS: Dissipated energy flux per CS; DIo/ABS: Quantum yield for energy dissipation; DIo/RC: Dissipated energy flux per RC. All the values were expressed relative to the sample treated with 6000 μM N set as 1.

Fig. 8 Leaf initial rubisco activity (A), PI_{abs} (B) and Chl content (C) in relation to CO₂ assimilation in tea leaves. All the values were expressed relative to the sample treated with 6000 μM N set as 1. Regression equations: A y = 1.07 + 1.4lnx (r² = 0.9317); B y = 1.54x - 0.65 (r² = 0.9329); C y = 1.6x - 0.63 (r² = 0.9932)
Abbreviations
Rubisco: ribulose-1,5-bisphosphate carboxylase/oxygenase; PSI: photosystem II, P<sub>max</sub>: performance index (PI) on absorption basis; N deficiency: nitrogen deficiency; Chl: chlorophyll; OEC: oxygen evolving complex; DW: dry weight; BSA: bovine serum albumin; RuBP: ribulose-1,5-bisphosphate.

Authors' contributions
ZHL performed most of the experiments and wrote the manuscript. QSZ helped in designing the study. CSC designed and directed the study and revised the manuscript. QCR performed the experiments and analyzed the data. ZHC and XMY analyzed data. All authors have read and approved the final manuscript.

Acknowledgements
This work was supported by the National Natural Science Foundation of China (31570690) and Modern Agro-Industry Technology Research System (nycytx-23).

Competing interests
The authors declare that they have no competing interests.

Received: 26 August 2016 Accepted: 5 November 2016
Published online: 17 November 2016

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