Ecosystems (2021) 24: 1468–1484
https://doi.org/10.1007/s10021-020-00595-4

ECOSYSTEMS

Impacts of Canopy and Understory Nitrogen Additions on Stomatal Conductance and Carbon Assimilation of Dominant Tree Species in a Temperate Broadleaved Deciduous Forest

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ABSTRACT

Excess N deposition has aroused concerns about its negative impacts on forest ecosystems. A two-year study was conducted to assess the responses of stomatal conductance (G_c) and carbon assimilation (A_{net}) of dominant tree species (*Liquidambar formosana*, *Quercus acutissima* and *Quercus variabilis*) to increased N deposition at a canopy and understory N additions experimental platform in a temperate deciduous broadleaved forest. Five treatments included N addition of 25 and 50 kg ha^{-1} y^{-1} onto either the canopy (C25 and C50) or the understory (U25 and U50), and a control treatment (CK, without N addition). Our results showed that neither canopy nor understory N addition had an impact on carboxylation capacity (V_{cmax}), the light saturated rate of electron transport (J_{max}) and leaf-level net assimilation (A_{nL}) of the studied tree species. Higher concentrations of N addition (U50 and C50 treatments) exerted negative impacts on G_c and A_{net} of *L. formosana* and *Quercus acutissima* under lower precipitation conditions, while lower concentrations of N addition (U25 and C25 treatments) had minimal impacts on overall ecophysiological function. The U50 treatment increased tree water use efficiency (*WUE*) of *L. formosana* in the second experimental year. Canopy and understory N addition generated differential effects on forest vegetation. The traditional approach with understory addition could not fully reflect the effects of increased N deposition on the canopy-associated assimilation processes.

Received 5 July 2020; accepted 6 December 2020;

Supplementary Information: The online version contains supplementary material available at (https://doi.org/10.1007/s10021-020-00595-4).

Author contributions: PZ, KVRS, SF and WS conceived and designed the research; YH, LZ, KVRS, XZ, GN, YZ, HY and WZ conducted the experiment; YH analyzed the data and wrote the manuscript; KVRS and PZ revised the manuscript.

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Key words: Nitrogen deposition; Net photosynthetic rate; Carboxylation capacity; Sap flux density; Stomatal conductance; Water use efficiency.

HIGHLIGHTS

- Negative impacts on Gc and Anet merely occurred at higher N addition rate.
- Negative effects of N addition mainly occurred when the precipitation was lower.
- Canopy leaf Vcmax and Jmax were unaffected by external N addition.
- Understory N addition failed to fully reflect the effect of increased N deposition.

INTRODUCTION

Atmospheric deposition of reactive nitrogen (N) has increased dramatically over the past century due to extensive fossil fuel utilization, heavy fertilizer application, expansion of animal husbandry, and other anthropogenic forcing. (Gruber and Galloway 2008; Janssens and others 2010; Liu and others 2013). Previous research has shown that N deposition has increased from 32 Tg y^{-1} in 1860 to 103 Tg y^{-1} in 1993 globally and will continue to increase up to 195 Tg y^{-1} by 2050 (Galloway and others 2004; Galloway and others 2008). N deposition in China has also been among the greatest globally, and the averaged N deposition rate in China has increased from 13.2 kg ha^{-1} in the 1980s to 20.6 kg ha^{-1} in 2014 (Liu and others 2013). Excess nitrogen deposition has aroused concerns about its negative impacts on forest ecosystems (Nakaji and others 2001; Sutton and others 2008; Bowman and others 2008; Fleischer and others 2013). Forest ecosystems play an important role in the global carbon cycle and are estimated to be a carbon sink with a rate of 1.1 ± 0.8 Pg C y^{-1} (Pan and others 2011). The perturbation of increased N deposition, which affects the processes that determine the forest carbon balance, would potentially influence the carbon sequestration in global forests and the terrestrial carbon cycle (Högberg 2007; Thomas and others 2010). Thus, analyzing the responses of carbon uptake processes to increased N deposition will help elucidate how N depostition influences the ability of forest ecosystem to store carbon in the long-term woody pools.

Due to the geographic and species differences, forest ecosystems have diverse responses to increased N deposition (Thomas and others 2010; Chen and others 2015). In the N limited forests, some studies showed increased N deposition enhanced the net primary production (*NPP*) and drove carbon sequestration, for example, boreal *Pinus sylvestris* forest and northern hardwood forest (Pregitzer and others 2008; Reay and others 2008; Lim and others 2015); other research focusing on the responses of mature N-poor boreal *Pinus sylvestris* to increasing N supply showed no effects on the long-term shoot-scale carbon uptake (Tarvainen and others 2016). In the N saturated forest ecosystems, external N addition generally had a minimal or negative impact on *NPP* (Nadelhoffer and others 1999; Hyvönen and others 2007; Thomas and others 2010; Chen and others 2015). Whether the forests located at the climate transition zone from the warm temperate to subtropical region are N limited or saturated forest, and how these forests would respond to increased N deposition remains uncertain.

Many studies addressing the influence of N deposition on forest carbon assimilation rate and primary productivity have been conducted; some of these studies investigated the impacts of N addition on leaf-level chlorophyll content, Rubisco content and net photosynthetic rate (Bauer and others 2001; Grassi and others 2002; Liu and others 2018); some studies explored the effects of N addition through observing the variation of forest biomass, stem basal area or branch growth (Xia and Wan 2008; Bedison and McNeil 2009; Eastaugh and others 2011); while some studies analyzed the response of regional forest carbon storage to N addition using models (De Vries and others 2006; Churkina and others 2010; De Vries and Posch 2011). The impact of increased N deposition has been investigated at a specific time point through the above-mentioned approaches at the leaf, individual tree and ecosystem level; however, the process on how carbon assimilation would respond to increased N deposition across a period of time has seldom been reported.

Sap flow measurements have been tested as a robust approach to estimate canopy stomatal conductance in large numbers of studies (Pataki and others 1998a; Ewers and others 2005; Tang and others 2006), and can be applied to calculate carbon assimilation (A_{net}) together with physiological and environmental parameters (Köstner and others 2008). Some studies consider the whole canopy as a layer to estimate assimilation rate without taking the environmental heterogeneity inside the canopy into account (Hu and others 2010; Wang and others 2014), which would cause estimation errors. The Canopy Conductance Constrained Carbon Assimilation (4C-A) model, which is a multilayer model based on sap flux-based canopy stomatal conductance, has been developed and provides a feasible approach to estimate A_{net} continuously (Schäfer and others 2003; Hu and others 2019a).

In reality, N retention by the forest canopy is also a key issue in determining the effects of atmospheric N deposition on forest ecosystems (Guerrieri and others 2010; Guerrieri and others 2011; Fenn and others 2013). However, the traditional understory N addition approach, in which N solution or fertilizer was sprayed directly onto the understory plants or forest floors (Mo and others 2008; Ward and others 2012; Lu and others 2014), did not include the influences of N deposition on the canopy-associated processes (Zhang and others 2015). For purpose of better simulating the realistic N deposition in forest canopies, some studies used aircraft to spray N solution onto the canopy (Adams and others 2007; Gaige and others 2007; Dail and others 2009). Nevertheless, these experiments had the disadvantage that the N solution may not be sprayed uniformly onto the canopy (Zhang and others 2015).

A manipulative experimental platform with both canopy and understory N additions has been established in a deciduous broadleaved forest located in Central China to investigate the effects of increased atmospheric N deposition on a forest ecosystem. Our experiment was carried out based on this platform, and the main objectives of this study were: (1) to examine the possible effects of canopy and/or understory N addition on stomatal conductance (G_c) of dominant tree species; (2) to analyze how canopy and understory N additions influence the canopy carbon assimilation (A_{net}) and tree water use efficiency (*WUE*); (3) to compare the effects of traditional understory N addition and canopy N addition on G_c and A_{net} of dominant tree species.

METHODS

Study Site

The experimental site is located in the Jigongshan National Nature Reserve, Henan Province, Central China (31° 46′–31° 52′ N, 114° 01′–114° 06′ E). The region is located at the climate transition zone from warm temperate to subtropical, with an annual precipitation of 1119 mm and annual average temperature of 15.2 °C (Zhang and others 2015). Our experiment was conducted in a broadleaved deciduous forest with the dominant tree species of *Liquidambar formosana* Hance, *Quercus variabilis* Blume and *Quercus acutissima* Carruth. The importance values of these species, which were calculated as the average value of relative dominance, density and frequency (Kent 2011), were estimated at this site. The three selected dominant species had an importance value of 0.20, 0.15 and 0.14, respectively, which are far higher than those of the coexisting tree species *Acer buergerianum* (0.06) and *Celtis sinensis* (0.05) and of the other species in the community. The main shrub layer species include *Lindera glauca*, *Vernicia fordii* and *Acer buergerianum*, and the herb plants including *Lygodium japonicum* and *Ophiopogon japonicas* are sparsely distributed. The soil is a sandy loam with a pH of 4.6. The soil total nitrogen content and organic matter at 20-cm depth are 0.08% and 2.1%, respectively (Zhu and others 2017).

Experimental Design

The nitrogen addition treatments, which were initiated in April 2013 and are still being conducted until the present, are as follows: (i) canopy addition of 25 (C25) and 50 (C50) kg N ha^{-1} y^{-1}; (ii) understory addition of 25 (U25) and 50 (U50) kg N ha^{-1} y^{-1}; and (iii) control treatment (CK, without N addition). Four blocks were established at the forest site, and the 5 treatments were assigned to 5 plots in each block in a completely randomized block design. The spraying radius of the canopy N addition is 17 m, leaving the central area of 400 m^2 for sampling. The plots were separated by at least 20 m buffer zone to minimize lateral contamination of N solution (Zhang and others 2015). In addition, the plots were not set at the same slope aspect to minimize the effects of upper plots impacting lower plots to the greatest extent.

The canopy spraying system was built in the center of each canopy N addition plot to simulate N deposition onto the canopy. Details about the spraying system are presented in Zhang and others (2015). Nitrogen solution for understory N addition plots was sprayed 1.5 m above the ground by an automatic irrigation system, which was made up of 5 sprinklers evenly distributed in each plot. NH_4NO_3 solution of targeted concentration was added to the plot as low intensity precipitation ($\sim$ 3 mm per event) monthly from April to October. The first addition was carried out about a week before all the buds had burst (around April 15th), and the final addition was performed in the middle of October during leaf senescence. There was no nitrogen solution added to the CK plots. The total added N solution amount was 21 mm y^{-1}, accounting for less than 2% of annual precipitation. Thus, the confounding effect caused by water addition is negligible in this study.

Environmental Monitoring

A temperature (T, °C) and relative humidity (RH, %) sensor (HygroClip2, Rotronic, Switzerland) and a photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) sensor (XST-SQ110, Davis Instruments, USA) were mounted on a supporting frame and connected to a data logger (DL2e, Delta-T Devices, UK) at an open site approximately 50 m away from the experimental platform. The sensors read every 30 s, and the 10-min averaged data were recorded. Precipitation (P, mm) was provided by the Jigongshan meteorological station located about 2 km away from the plots. The vapor pressure deficit (VPD, kPa) was calculated according to Campbell and Norman (1998):

$$\mathrm{VPD} = ae^{\left(\frac{bT}{T+c}\right)}(1 - \mathrm{RH}) \qquad (1)$$

where a, b and c are fixed parameters (0.611 kPa, 17.502 (unitless) and 240.97 °C, respectively), T is temperature in degrees Celsius, and RH is relative humidity as a ratio.

Physiological and C Isotope Measurements

The leaf samples of the three dominant tree species in the canopy for all the plots were obtained using a pole pruner in the summer of 2018. Daytime mitochondrial respiration rate (R_d, μmol m^{-2} s^{-1}), carboxylation capacity (V_{cmax25}, μmol m^{-2} s^{-1}) and the light saturated rate of electron transport (J_{max25}, μmol m^{-2} s^{-1}) scaled to 25 °C were obtained from the response curve of leaf net assimilation (A_{nL}) to internal CO_2 concentration ($A_{nL} - C_i$ curve) which was measured with a Li-Cor 6400 portable photosynthesis system (Li-Cor Instruments Inc., Lincoln, NE, USA). The photosynthetic photon flux density was set to be 1200 μmol m^{-2} s^{-1} when measuring the $A_{nL} - C_i$ curve. The method to estimate R_d, V_{cmax} and J_{max} is described in Sharkey and others (2007). In total, the leaves of 18 *L. formosana* (n under CK, U25, U50, C25 and C50 treatment was 3, 5, 3, 4 and 3), 20 *Q. acutissima* (n under CK, U50, C25 and C50 was 4, 5, 6 and 5) and 21 *Q. variabilis* (n under CK, U25, U50, C25 and C50 was 3, 5, 3, 5 and 5) trees in all the experimental plots were obtained and measured.

The leaves with for gas exchange measurements were scanned with a commercial scanner to determine the leaf area and then dried at 60 °C to a constant dry weight. The specific leaf area (SLA, m^2 kg^{-1}) was calculated by dividing leaf area by leaf dry weight. Then the dried leaves were ground into powder using a ball bearing mill and encapsulated in tin capsules for N content determination and ^{13}C isotopic analysis (IsoPrime100, Elemental, Germany). In addition, the leaf N content and SLA of the three dominant tree species in the canopy for all the plots in 2015 were also measured.

Sap Flow Measurement and Scaling Up to Canopy Stomatal Conductance

In total, 41 *L. formosana*, 25 *Q. acutissima* and 52 *Q. variabilis* trees in all the experimental plots were selected for sap flow monitoring during the growing season (from Apr. 1st to Nov. 14th) of 2014 and 2015. Information on the sample trees of *L. formosana*, *Q. acutissima* and *Q. variabilis* under each treatment is shown in Table S1 in Supplementary Material. The one-Way ANOVA showed no remarkable differences ($P > 0.05$) in diameter at breast height (DBH) among the five treatments for the three species. The self-made Granier-type sensors were inserted into the sapwood of the sample trees and connected to the data logger (DL2e, Delta-T Devices, UK) to monitor stem sap flow. Details about the installation of the sensors and the method of converting temperature difference data to sap flux density (J_s, g m^{-2} s^{-1}) were described in Hu and others (2019b).

The original empirical Granier equation has been tested to be applicable for most but not all species (Bush and others 2010). Even so, the original Granier equation has been applied to estimate J_s of other sweetgum and oak trees (Granier and others 1994; Phillips and others 1996; Pataki and others 1998b; Bovard and others 2005; Oishi and others 2008), indicating the practicability of using the Granier method in this study. Furthermore, we used the original Granier equation for the three species and assumed that there existed ignorable impacts on the comparison of the N addition treatment effects because the source of estimation error was the same for the 5 treatments. However, the limited applicability of the original empirical Granier equation would restrict the J_s and g_c comparison with other tree species measured in previous studies using the calibrated Granier equation.

All the sap flow sensors on the same trees were replaced by new sensors in April 2015 before we began the second-year monitoring. As an insect attack damaged some sap flow sensors during the middle of July in 2014, the data during this period were not analyzed. Additionally, there was only one *Q. acutissima* selected for sap flow measurement under the U25 treatment as there existed no *Q. acutissima* for several plots, we could not conduct comparative analysis and did not analyze the effect of the U25 treatment on *Q. acutissima*.

After converting temperature difference data to sap flux density, the whole-tree transpiration (E_t, g s^{-1}) was calculated as follows

$$E_t = J_s A_s \tag{2}$$

where A_s is sapwood area and J_s is the converted sap flux density, and A_s was determined based on the established relationship between *DBH* and A_s (Hu and others 2019b; Oishi and others 2008). The radial variation of J_s should be taken into consideration (Phillips and others 1996; Schäfer and others 2000) when using Eq. (2), and the radial variation of J_S was calculated according to equation (3) in Pataki and others (2011). In addition, the sapwood area may also be influenced by N addition. The maximal annual radial growth was about 4 mm for the three studied tree species (Zhang and others 2018; Yu and others 2019). If N addition influenced annual radial growth by 50% (that is, 2 mm) for a tree with the *DBH* of 30 cm, the sapwood area would be only altered by 1.8%. Therefore, the effects of N addition on sapwood area were minimal, and A_s was estimated using the same equation for each tree species under different N addition treatments.

To analyze the stomatal responses to N addition, sap flux-based canopy stomatal conductance (G_c) which reflects the stomatal behavior and is closely related to carbon uptake was estimated in our study. G_c for each individual tree was calculated using a simplified Penman–Monteith equation (Köstner and others 1992):

$$G_c = E_L \rho G_V \frac{T + 273}{\text{VPD}} \tag{3}$$

where ρ is the density of water (998 kg m^{-3}), G_V is the universal gas constant adjusted for water vapor (0.462 m^3 kPa K^{-1} kg^{-1}), T (°C) is air temperature, and E_L (m s^{-1}) is the transpiration per unit of leaf area with consideration to the time lags between sap flux measured on the stem at breast height and the canopy environmental variables (Phillips and others 1997), and calculated by dividing E_t by tree leaf area. Total leaf area of each individual tree was calculated as the product of the SLA and leaf weight (*W*, kg) which was estimated according to biomass equations for each dominant tree species. The detailed method for determining leaf area is described in Hu and others (2019b).

The leaf area index (LAI) was also measured using a LI-2000 plant canopy analyzer (LI-2000, LI-COR, USA) during May 2015 among the five treatments, and was not significantly different among the treatments. Likewise, there was no remarkable difference in the litterfall weight measured in December 2015 among the five treatments (Figure S1 in the Supplementary Material; $P > 0.05$), indicating that N addition treatments exerted no impact on leaf biomass and area at that time. Thus, the leaf area was estimated using the same biomass equation for each tree species under different N addition treatments.

Estimation of A_{net} and WUE Using the 4C-A Model

Photon flux density varies at different layers in the canopy, thus the canopy was divided into several layers (each layer has a depth of 1 m), and leaves were divided into two light categories, that is, sunlit and shaded leaves. The proportion of foliage in each light category along with its incident PPFD at each layer was calculated according to Campbell and Norman (1998) in half hour increments. Carbon assimilation rate (A_{net}) for both sunlit and shaded leaves at each layer can be acquired by solving the following two equations (Vico and others 2013):

$$A_{net} = \frac{k_1(c_i - \Gamma^*)}{k_2 + c_i} - R_d \tag{4}$$

$$A_{net} = g_c(c_a - c_i) \tag{5}$$

where C_i is intercellular CO_2 concentration, C_a is the ambient atmospheric CO_2 concentration, Γ^* is the CO_2 compensation point in absence of dark respiration, R_d is the daytime mitochondrial respiration rate, k_1 and k_2 are parameters related to the photosynthetic parameters (the detailed calculation is explained in Vico and others (2013)), and g_c is the stomatal conductance to CO_2. The g_c was obtained by constraining the estimated optimal conductance using the stomatal optimization approach to the sap-flow-based conductance, and the detailed calculation process was presented in Hu and others (2019a). The leaf physiological parameter V_{cmax} in 2015 was obtained from the N content (g m^{-2}) measured in 2015 and the established relationship between N content and V_{cmax} from the measurements in 2018 (Figure S2; Kattge and others 2009), and the $J_{\max}$ (or R_d) in 2015 was then obtained from the following relationship between V_{cmax} and $J_{\max}$ (or R_d) from the measurements in 2018: $J_{\max} = 0.92 \times V_{\mathrm{cmax}} + 18.26$ ($P < 0.001$, $R^2 = 0.36$), $R_d = 0.039 \times V_{\mathrm{cmax}} - 0.55$ ($P < 0.001$, $R^2 = 0.35$). To take the seasonal variation of V_{cmax} and $J_{\max}$ into consideration, the day length-based scalar was used to modify the measured V_{cmax} and $J_{\max}$ to obtain their values for a given day (Bauerle and others 2012):

$$V_{\mathrm{cmax\_modified}} = V_{\mathrm{cmax\_measured}} \times \left(\frac{dayl}{dayl_{\max}}\right)^2 \tag{6}$$

$$J_{\mathrm{max\_modified}} = J_{\mathrm{max\_measured}} \times \left(\frac{dayl}{dayl_{\max}}\right)^2 \tag{7}$$

where *dayl* and $dayl_{\max}$ are the day length for a given day and the annual maximum day length, respectively. In addition, both the measured and modified V_{cmax} and $J_{\max}$ were the values scaled to 25 °C; therefore, the temperature adjustment was made according to Katul and others (2010) and Hikosaka and others (2007).

The tree canopy-level water use efficiency (WUE) was calculated as follows:

$$\mathrm{WUE} = \frac{A_{\mathrm{net,total}}}{E_{\mathrm{t,total}}} \tag{8}$$

where $A_{\mathrm{net,total}}$ and $E_{\mathrm{t,total}}$ represent the total species-specific tree canopy carbon assimilation estimated by the 4C-A model and transpiration calculated from sap flow measurements during the growing season (from Apr. 1st to Nov. 14th), respectively.

Statistical Analysis and Gap Filling

One-way ANOVA followed by a Duncan's test was used to test the differences in V_{cmax}, $J_{\max}$, N content, G_c, A_{net} and *WUE* among the five treatments. The trees under each specific treatment in all the four blocks were compared to each other, that is, the n for the statistical analysis was the number of trees instead of the block number. Some sap flow data are missing because of power or equipment failure, and the percentage of missing data for each individual tree was 23.6 ± 0.06%. The relationship between the J_s and environmental factors (*PPFD* and *VPD*) was established seasonally for each individual tree by a nonlinear regression analysis and used to fill the J_s gaps ($P < 0.05$). The equation form was as follows: $J_s = a(1 - e^{-m \times \mathrm{VPD}})(1 - e^{-n \times \mathrm{PPFD}})$. The average determination coefficient of these equations was 0.82 ± 0.01 (mean value ± standard error) ranging from 0.41 to 0.97, with 91.3% of the determination coefficients above 0.70.

RESULTS

Environmental Factors

This experiment was conducted during the growing season (from Apr. 1st to Nov. 14th) in 2014 and 2015. The daily average photosynthetic photon flux density (*PPFD*) ranged from 6 to 516 μmol $\mathrm{m}^{-2}\,\mathrm{s}^{-1}$ for the entire measurement period, with daily mean vapor pressure deficit (*VPD*) in the range of 0–2.1 kPa and daily average temperature (*T*) from 4 to 33 °C (Figure 1). The total precipitation (*P*) during the growing season in 2014 and 2015 was 966 and 894 mm, respectively. The average PPFD and *P* in autumn were lower than those in spring and summer (Figure 1; Hu and others 2019b).

Effects of N Addition on G_c

Results showed that there was no remarkable difference ($P > 0.05$) in *DBH* among the five treatments for the three studied species (Table S1), and daily average G_c was not correlated with *DBH* for trees in the CK plots ($P > 0.05$). Thus, the difference in G_c among the treatments should result from the N addition rather than from tree size. During the first year (2014), both canopy and understory N addition had no influence on the daily average G_c of *L. formosana* and *Q. variabilis* ($P > 0.05$; Figure 2). However, C50 treatment reduced G_c of *Q. acutissima* in April and June ($P < 0.05$). However, the case during the second year (2015) was different. The understory N addition exerted a greater impact on G_c than canopy N addition. The U50 treatment reduced G_c of *L. formosana* in autumn ($P < 0.05$).

Effects of N Addition on Physiological Properties

The N addition had no influence on leaf N content measured in 2018 for all the three studied tree species ($P > 0.05$; Figure 3). Carboxylation capacity (V_{cmax}) and the light saturated rate of electron transport ($J_{\max}$) of the three species were obtained in 2018 from the response curve of leaf net assimilation to internal CO_2 concentration. Neither canopy N addition nor understory addition exerted an impact on V_{cmax} or $J_{\max}$ of the three dominant tree species ($P > 0.05$; Figure 4). Similar to the responses of V_{cmax} and $J_{\max}$, the leaf-level A_{nL} un-

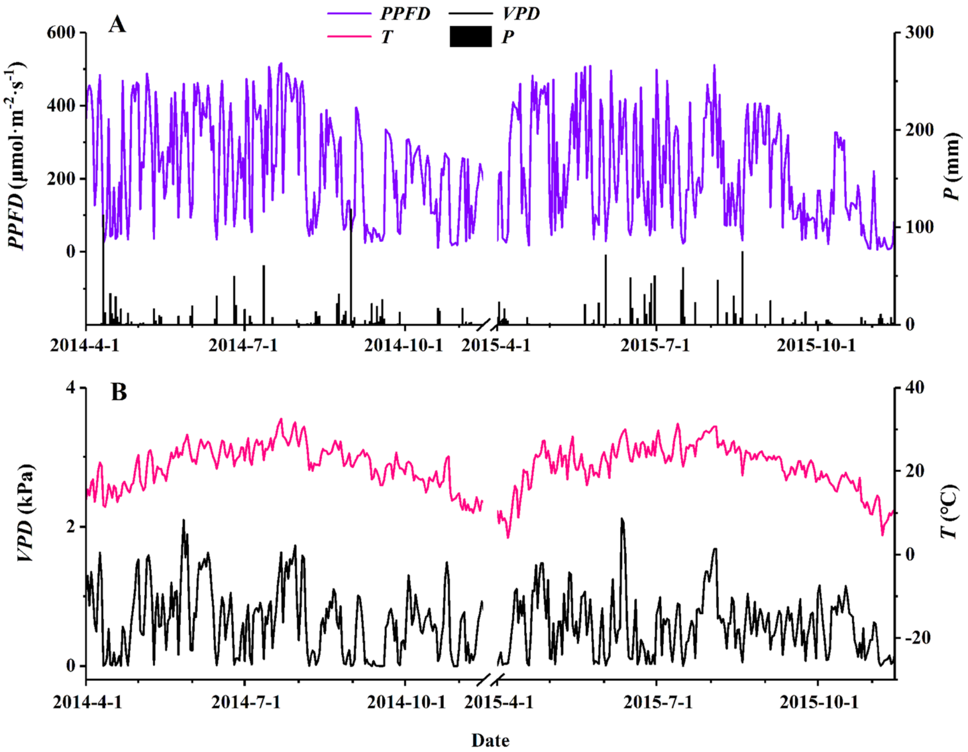

Figure 1. Daily mean photosynthetic photon flux density (*PPFD*), vapor pressure deficit (*VPD*), temperature (*T*) and daily precipitation (*P*) during the growing season (from Apr. 1st to Nov. 14th) in 2014 and 2015 at the experimental site (Hu and others 2019b).

der *PPFD* of 1200 μmol m^{-2} s^{-1} and CO_2 concentration of 400 ppm was also unaffected by N addition ($P > 0.05$; Figure 4).

Effects of N Addition on Tree-Level A_{net}

The canopy instantaneous A_{net} within a day ranged from 0 to 7.6 μmol m^{-2} s^{-1}, and the average of A_{net} during the whole day was estimated to analyze the effects of N addition on carbon assimilation (Figure 5). During the first year, both canopy and understory N additions had no impacts on the daily average A_{net} modeled with 4C-A for the three species ($P > 0.05$; Figure 5). The understory N addition exerted greater impacts in the second year, whereby the U50 treatment reduced A_{net} of *L. formosana* and *Q. acutissima* in autumn ($P < 0.05$, Figure 5).

Effects of N Addition on WUE

Both canopy and understory N additions did not exert impacts on tree water use efficiency (*WUE*) of *Q. acutissima* and *Q. variabilis* during the two experimental years ($P > 0.05$; Figure 6). N addition treatments had no influence on the *WUE* of *L. formosana* in the first year, whereas *L. formosana* with U50 treatment presented a higher *WUE* than those with CK and other treatments in the second year ($P < 0.05$; Figure 6).

DISCUSSION

Influences of N Addition on G_c and A_{net}

Higher concentrations of N addition decreased stomatal conductance and carbon assimilation of the dominant species during some periods across the two full growing seasons of this experiment, while lower concentrations of N addition exerted no impact on plant physiological function investigated here. It is generally acknowledged that there is a dose–response relationship between external N input amount and tree physiological behavior such as net primary production (Magill and others 1998; Bobbink and Hettelingh 2011; de Vries and others 2014; Chen and others 2015). Increased N deposition enhanced the NPP and drove carbon sequestration in N limited forests (Magnani and others 2007; Pregitzer and others 2008), whereas external N addition had a minimal or negative influence on NPP in N saturated systems (Nadelhoffer and others 1999; Hyvönen and others 2007; Chen and others 2015). The negative impacts of high N addition on

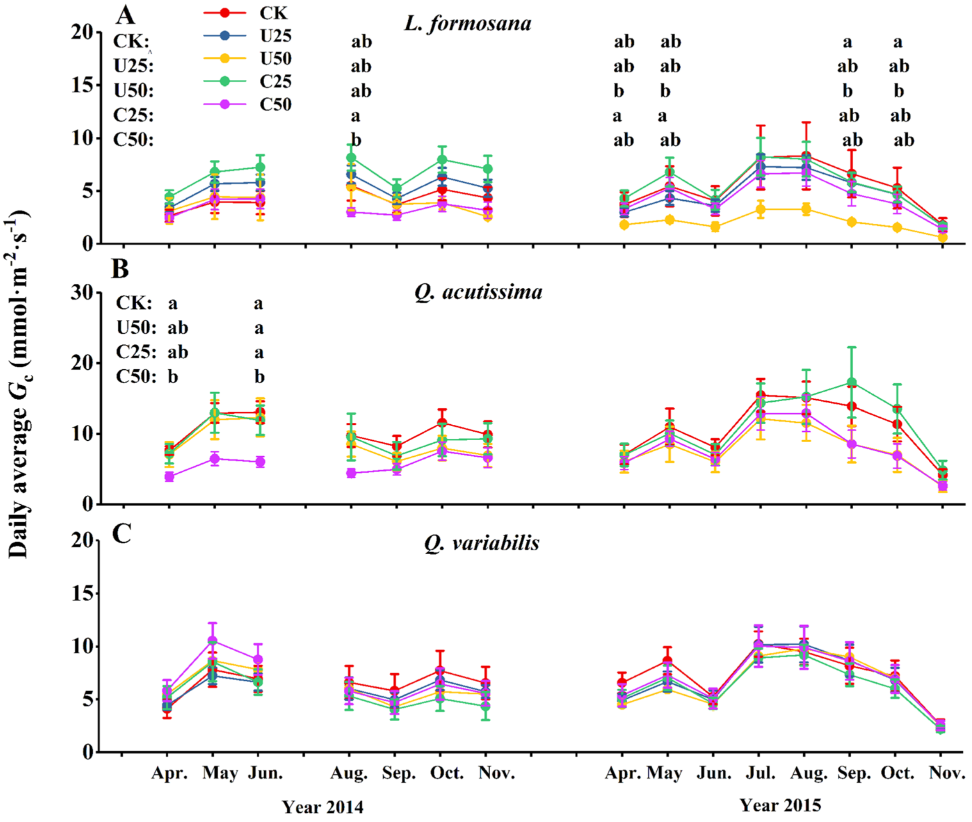

Figure 2. Effects of canopy and understory nitrogen additions on daily average sap flux-based stomatal conductance (G_c) of *L. formosana* (**A**), *Q. acutissima* (**B**) and *Q. variabilis* (**C**). CK, U25, U50, C25 and C50 represent control treatment, understory addition of 25 kg N ha^{-1} y^{-1}, understory addition of 50 kg N ha^{-1} y^{-1}, canopy addition of 25 kg N ha^{-1} y^{-1} and canopy addition of 50 kg N ha^{-1} y^{-1}, respectively. Different lowercase letters denote significant differences (α = 0.05) in G_c among five treatments during a specific month using one-way ANOVA followed by a Duncan's test.

G_c and A_{net} during some periods and minimal impacts of low N addition indicated that the studied forest was under conditions of nitrogen saturation. In addition, some studies (Aber and others 1989; Magill and others 1998) have demonstrated that excess N addition would increase the emissions of nitrous oxide after the forest had become N saturated. Zhang and others (2015) reported that U50 treatment increased N_2O emission in 2013–2014 at the same study site, which also indicated that the studied forest was under conditions of N saturation.

The V_{cmax} and J_{max} of all the three dominant species were not affected by N addition in our research, which effectively preserved photosynthetic capabilities (Fernández-de-Uña and others 2016). Likewise, N addition had no influences on V_{cmax} and J_{max} of hybrid larch saplings (Mao and others 2012) and *Acer saccharum* (Talhelm and others 2011). However, some studies demonstrated that higher N supply led to higher V_{cmax} of *Eucalyptus grandis* seedlings (Grassi and others 2002) and old growth shoots of spruce (Tomaszewski and Sievering 2007). Many studies have shown V_{cmax} and J_{max} had a positive correlation with N content for broadleaved trees (Bauer, Berntson and Bazzaz 2001; Ripullone and others 2003; Calfapietra and others 2005; Kattge and others 2009; Walker and others 2014). Leaf N content was not affected by N addition treatments in our research (Figure 3), and consequently photosynthetic parameters (V_{cmax} and J_{max}) were also unaffected by N addition.

The A_{net} of the three dominant species was decreased by N addition during some periods in our study, indicating that the ability of the broadleaved deciduous forests to sequester carbon would be weakened. According to the estimation method of photosynthesis rate, A_{net} was determined by two aspects including: (1) photosynthetic parameters (for example V_{cmax} and J_{max}) and (2) the stomatal conductance (G_c) (Vico and others 2013). Our results showed that photosynthetic capacity indicated by V_{cmax} and J_{max} was not affected by N addition, and, thus, N addition treatments inhibited A_{net} primarily by decreasing G_c, which is highly corre-

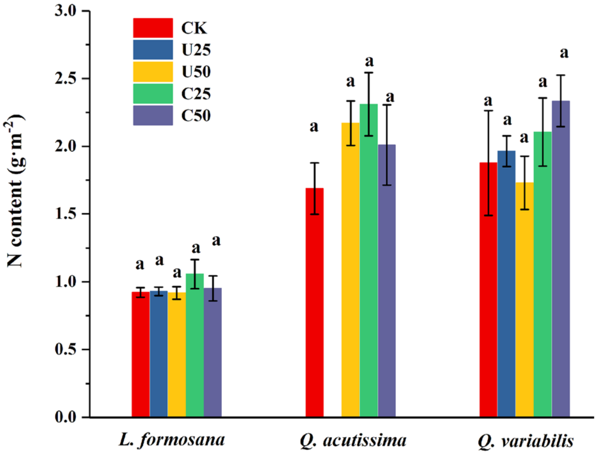

Figure 3. Effects of canopy and understory nitrogen additions on leaf nitrogen content (N; g m^{-2}) in 2018. CK, U25, U50, C25 and C50 represent control treatment, understory addition of 25 kg N ha^{-1} y^{-1}, understory addition of 50 kg N ha^{-1} y^{-1}, canopy addition of 25 kg N ha^{-1} y^{-1} and canopy addition of 50 kg N ha^{-1} y^{-1}, respectively. Different lowercase letters denote significant differences ($\alpha = 0.05$) in N content among the five treatments.

lated with the transpiration process, instead of affecting photosynthetic capacity.

Previous research conducted at the same N addition experimental site has confirmed that N addition significantly reduced pH and increased exchangeable Al^{3+} (Shi and others 2016). Thus, the negative impacts of N addition on carbon assimilation induced by the suppression on transpiration and G_c may result from the soil acidification caused by excess N addition (Bobbink and Hettelingh 2011; Phoenix and others 2012; Shi and others 2016; Carter and others 2017). Furthermore, soil acidification caused by N addition would sequentially lead to increased dissolution of aluminum, which could damage fine root development and consequently reduce water uptake (Schulze 1989; Godbold and others 2003; Bobbink and Hettelingh 2011). Moreover, the decreased soil water potential resulting from the pulsed N addition under low soil moisture situations (Lohm and others 1977; Qin and others 2014) might exert negative influences on transpiration and carbon assimilation as well, as it is generally recognized that transpiration is positively related to soil water potential (Jarvis and Jarvis 1963; Novák and others 2005). The soil water content at this research site was low during some periods because the monthly precipitation was as low as 28–34 mm for several months (Figure 1).

Apart from the altered soil chemical properties, the effects of canopy N addition on canopy properties were also responsible for the negative impacts on G_c. Canopy uptake of N compounds by the foliage from wet deposition has been confirmed in many studies (Harrison and others 2000; Ignatova and Dambrine 2000; Sievering and others 2000; Sievering and others 2007; Sparks 2009). Foliar uptake of external N solution might lead to the less negative leaf water potential (φ_L) initially as the concentration of sprayed NH_4NO_3 solution was low, and then leaf water potential usually would adjust to approximately 0 MPa at night (Koch and others 1994; Fisher and others 2006). However, NO_3^- or NH_4^+ might exist in the apoplast or cellular vacuoles for a period before eventual assimilation (Britto and Kronzucker 2002; Sparks 2009). When transpiring the same amount of water in the following few days, the leaves with accumulated NO_3^- or NH_4^+ would exhibit a more negative φ_L which had a higher potential to induce partial stomatal closure and reduce stomatal conductance (Cochard and others 2002; Brodribb and Holbrook 2003; Brodribb and others 2003). Hence, the retained NO_3^- or NH_4^+ resulting from canopy uptake of external N solution would result in an earlier stomatal closure and reduce carbon assimilation eventually.

Species-specific responses of A_{net} to N addition have been observed in this research. Canopy N addition only impacted the A_{net} of *Q. acutissima*, while that of *Q. variabilis* and *L. formosana* was unaffected. The differences in tree height of the studied three tree species may be responsible for the different response patterns. In our research, *Q. acutissima* (21.2 m) was higher than *Q. variabilis* (17.9 m) and *L. formosana* (17.4 m; $P < 0.05$). The N deposition was first intercepted by *Q. acutissima* due to its higher tree height, and consequently the A_{net} of *Q. acutissima* was more influenced by canopy N deposition. However, understory N addition negatively affected both *Q. acutissima* and *L. formosana*, while the effects of N addition on *Q. variabilis* were minimal. The A_{net} responses to excess N addition may be determined by root resistance to acidification and Al^{3+} (Godbold and others 2003; Lu and others 2010; Bobbink and Hettelingh 2011), and which factor played the key role needs to be further investigated.

WUE was analyzed based on the measured E_t and A_{net} in our research, instead of intrinsic WUE from the stable C isotope composition measurements which could not help elucidate whether the WUE response was caused by assimilation, water consumption or both. The N addition treatments

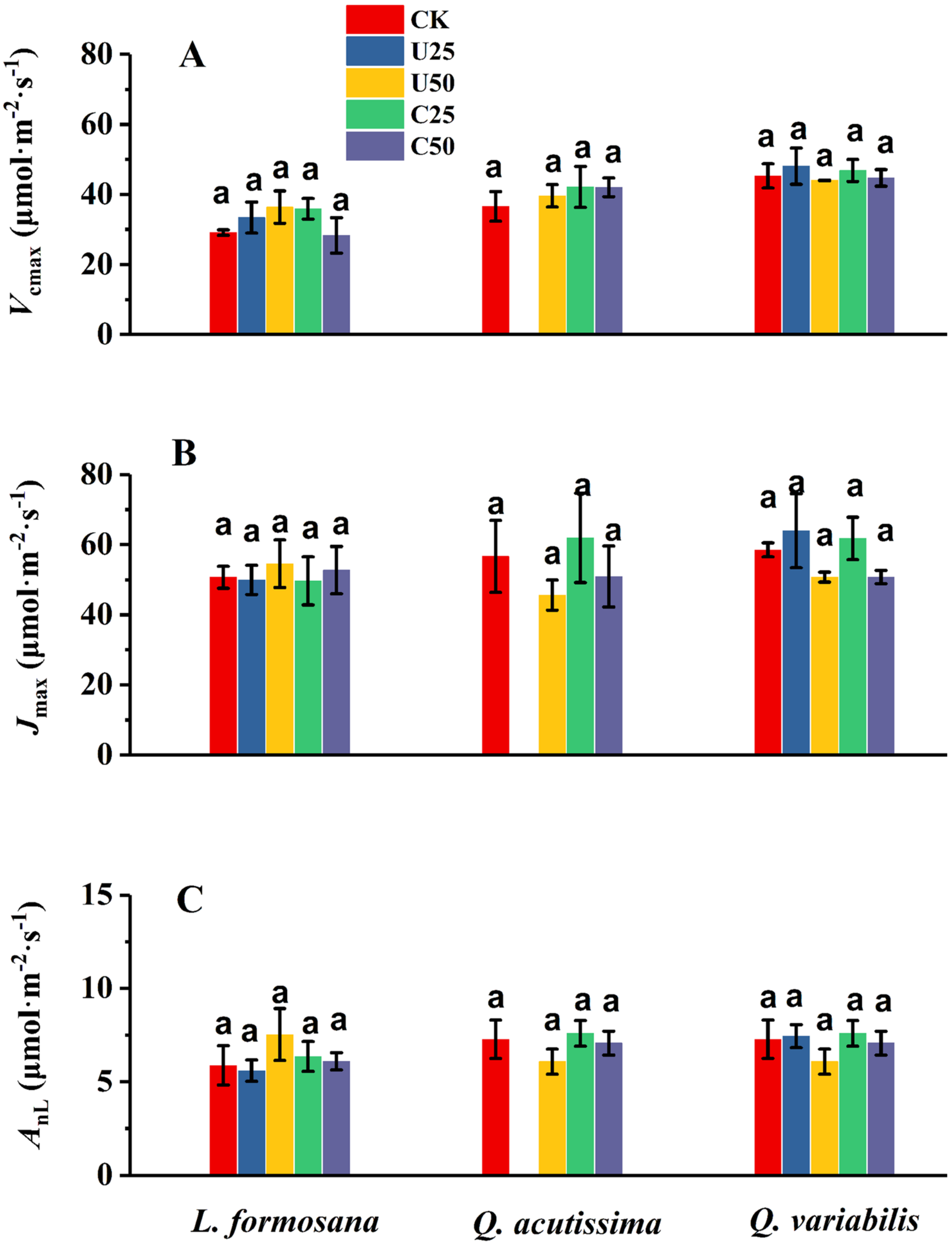

Figure 4. Effects of canopy and understory nitrogen addition on carboxylation capacity (V_{cmax}), light saturated rate of electron transport (J_{max}) and leaf-level carbon assimilation (A_{nL}) of *L. formosana* (**A**), *Q. acutissima* (**B**) and *Q. variabilis* (**C**) in 2018. CK, U25, U50, C25 and C50 represent control treatment, understory addition of 25 kg N ha^{-1} y^{-1}, understory addition of 50 kg N ha^{-1} y^{-1}, canopy addition of 25 kg N ha^{-1} y^{-1} and canopy addition of 50 kg N ha^{-1} y^{-1}, respectively. Different lowercase letters denote significant differences ($\alpha = 0.05$) in V_{cmax}, J_{max} or A_{nL} among five treatments. The leaf-level A_{nL} was measured under *PPFD* of 1200 μmol m^{-2} s^{-1} and CO_2 concentration of 400 ppm.

had no impacts on the WUE of *Q. variabilis*, while WUE of *L. formosana* was increased in the U50 treatment in the second year. Although there was no significant difference in WUE of *Q. acutissima* between CK and C50 treatments using one-way ANOVA followed by a Duncan's test, WUE with C50 treatment was significantly higher than that with CK treatment in the first year using the independent sample *t* test ($P < 0.05$). Several previous studies also showed that plants with higher N supply presented a higher WUE (Ripullone and others 2004; Dordas and Sioulas 2008; Vadeboncoeur and others 2016), which is in accordance with our results. The V_{cmax} and J_{max}

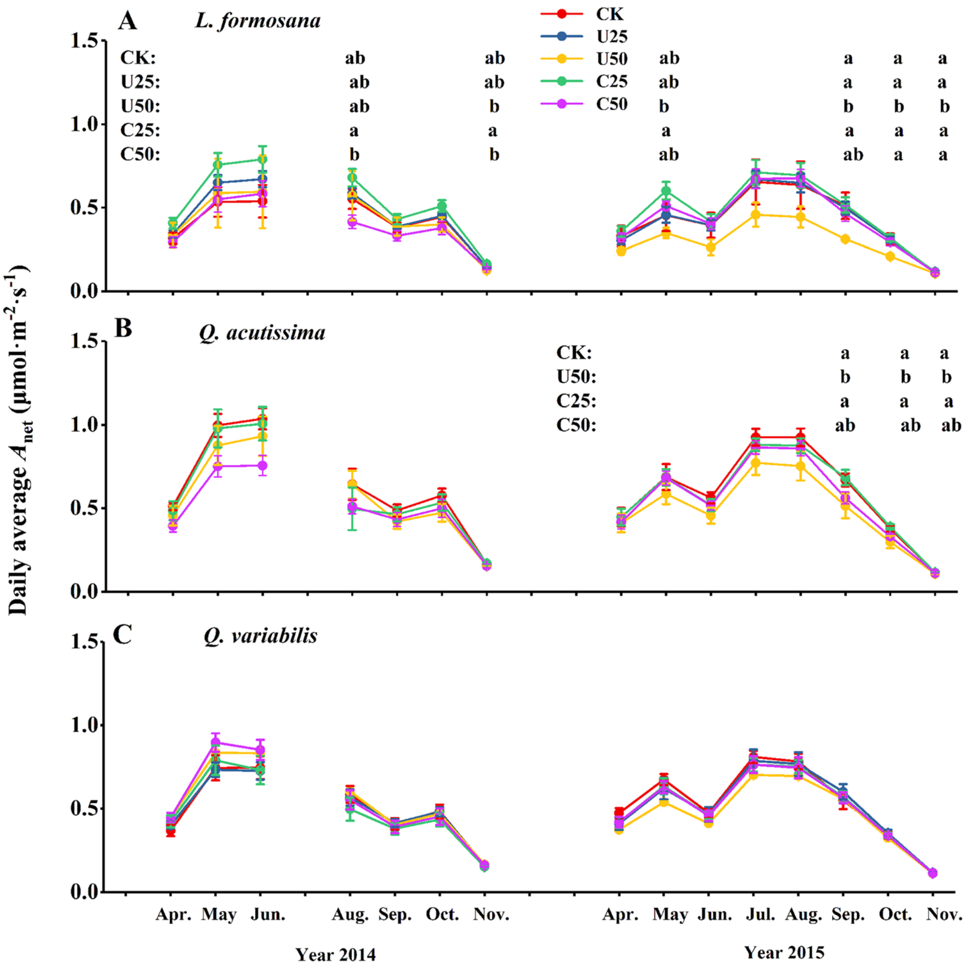

Figure 5. Effects of canopy and understory nitrogen additions on daily average carbon assimilation (A_{net}) of *L. formosana* (**A**), *Q. acutissima* (**B**) and *Q. variabilis* (**C**). CK, U25, U50, C25 and C50 represent control treatment, understory addition of 25 kg N ha^{-1} y^{-1}, understory addition of 50 kg N ha^{-1} y^{-1}, canopy addition of 25 kg N ha^{-1} y^{-1} and canopy addition of 50 kg N ha^{-1} y^{-1}, respectively. Different lowercase letters denote significant differences ($\alpha = 0.05$) in A_{net} among the five treatments during a specific month.

were not affected by N addition treatments in our research, indicating the photosynthetic capacity was unaffected. However, U50 treatment decreased G_c of *L. formosana* in the second year based on the sap flow measurements, and C50 treatment reduced G_c of *Q. variabilis* during the first year (Figure 2). It is generally accepted that lower G_c would give rise to an increased WUE for a given photosynthetic capacity (Martin and others 2010; Flexas and others 2016; Franks and Britton-Harper 2016). Consequently, the reduced G_c caused by U50 or C50 treatment resulted in an increase in WUE, while the photosynthetic capacity was unaffected.

The negative effects of N addition on A_{net} mainly occurred in May, Sep., Oct. and Nov. during which the monthly rainfall was lower than 100 mm (Figure 5), and N addition seldom exerted negative influences under the condition of adequate rainfall. The potential reasons for the minimal effects were: (i) the rainfall could wash away the external N retained on the canopy, reducing the negative effects on leaves; (ii) greater precipitation contributes to higher soil moisture levels, which in turn allows for greater stomatal conductance and may thereby weaken the effects of external N addition on photosynthesis (Sievering and others 2007); (iii) the higher soil moisture induced by larger rainfall could decrease the H^+ concentration and thereby alleviate soil acidification.

Canopy and understory N additions affected this forest ecosystem through different patterns: understory N addition treatment had intense impacts on soil chemical properties (Lu and others 2014; Shi and others 2016; Carter and others 2017), while canopy addition exerted influences on leaf physiological properties directly as well as soil

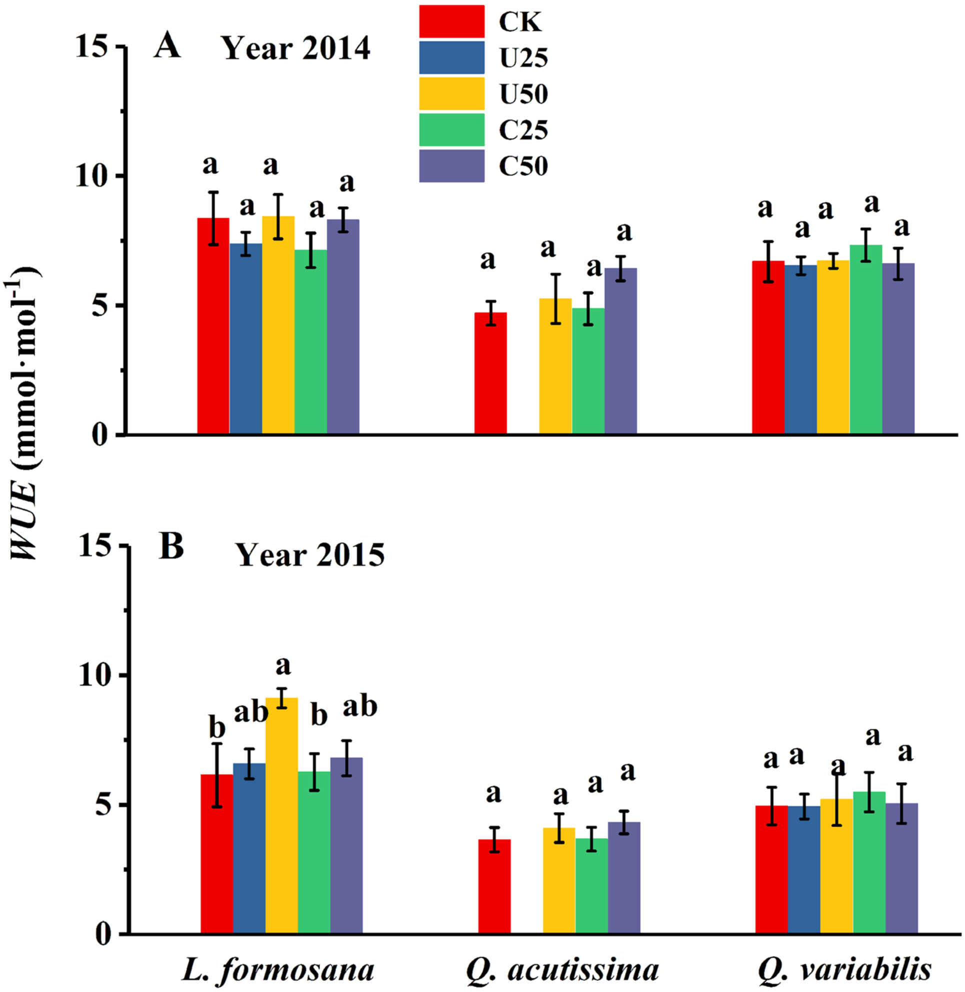

Figure 6. Responses of tree water use efficiency (*WUE*) to different nitrogen addition treatments for three studied tree species in 2014 and 2015. CK, U25, U50, C25 and C50 represent control treatment, understory addition of 25 kg N ha^{-1} y^{-1}, understory addition of 50 kg N ha^{-1} y^{-1}, canopy addition of 25 kg N ha^{-1} y^{-1} and canopy addition of 50 kg N ha^{-1} y^{-1}, respectively. Different lowercase letters denote significant differences ($\alpha = 0.05$) in the *WUE* among five treatments examined with one-way ANOVA followed by a Duncan's test.

chemical properties, but with a lesser amount affecting the soil due to canopy interception (Harrison and others 2000; Zhang and others 2015). Due to the different influencing patterns, the canopy and understory N addition consequently generated different impacts on G_c and A_{net}. Canopy N addition treatment exerted negative effects on G_c in the first experimental year for *Q. acutissima*, whereas understory addition treatment had no influences. The case in the second experimental year was different, that is, the understory N addition reduced G_c and A_{net} of *L. formosana* and *Q. acutissima*. The negative effects of understory N addition occurred during the second year may be explained by the cumulative effects of prolonged N addition, for example, more severe acidification (Phoenix and others 2012; Mao and others 2017; Zeng and others 2017), which can be indicated by the lower daily average A_{net} in 2015 compared to that in 2014 under N addition treatments (Figure S3). Thus, the traditional understory addition approach could not fully reflect the effects of increased N deposition on the canopy-associated photosynthetic process, which was indicated by the different responses of G_c and A_{net} to canopy and understory N addition.

Uncertainty Analysis

The leaf physiological parameters (V_{cmax}, J_{max} and R_d) in 2014–2015 were obtained from the measured N content in 2014–2015 along with the established relationship between N content and leaf physiological parameters from data collected during

2018 in our research. To analyze the estimation errors, the influences of the V_{cmax} and G_c variation on A_{net} were investigated. Our results showed that 10% underestimation of V_{cmax} would lead to a 1.8 ± 0.2% underestimation of A_{net}, while 10% V_{cmax} overestimation may cause a 1.4 ± 0.2% A_{net} overestimation. However, the influences of G_c variation on A_{net} were larger than those of V_{cmax} variation, that is, 10% underestimation and overestimation of G_c may lead to 5.1 ± 0.7% and 4.6 ± 0.7% A_{net} variation, respectively. Overall, G_c plays a more important role in governing the A_{net} variation, and the influences of V_{cmax} on A_{net} were minimal.

Leaf area, which plays an important role governing G_c and A_{net}, may also be influenced by N addition (Ewers and others 2001; Hubbard and others 2004; Samuelson and others 2008; Bartkowiak and others 2015; Maggard and others 2016). In this research, LAI was not affected by N addition, which was in accordance with the research on the effects of fertilization on *Pinus taeda* (Ward and others 2015). Although there were no significant differences in total leaf area among different N addition treatments, there may exist species-specific leaf area response to N addition, for example, N addition increased the leaf area of one tree species, while it decreased that of another tree species. If the leaf area was altered by N addition, the responses of A_{net} to N addition would be different to what was observed in this research. Only the harvest method can obtain the leaf area accurately, and suitable non-destructive method of species specific leaf area estimation was not found due to the overlap of leaves from surrounding trees. Thus, a developed non-destructive method of tree leaf area estimation in the future research would help better elucidate the responses of leaf area and A_{net} to N addition for individual tree.

Responses of carbon assimilation to increased N deposition depend upon the experimental year due to the cumulative effects of prolonged N addition. Our results showed that the daily average A_{net} in 2015 was lower than that in 2014 under N addition treatments (Figure S3), indicating the more intense negative effects on tree-level A_{net} resulting from the prolonged N addition. Additionally, there were no significant differences in N content between 2014–2015 and 2018 for the three studied tree species ($P > 0.05$), suggesting the potential photosynthetic activity to N addition were both unaffected with either 1–2 years or 5 years of N addition. As the stomatal conductance was merely measured during 2014–2015, only the short-term effects of N addition on tree-level A_{net} were analyzed in this research, and whether the long-term effects of N addition on tree-level A_{net} would be different needs further investigation.

There were only two *L. formosana* individuals measured with sap flow density under the U50 treatment in this study, which might make a weak statistical analysis. More sap flow measurements and carbon assimilation estimation of *L. formosana* under U50 treatment would provide stronger evidence to support the conclusion that U50 treatment reduced A_{net} and G_c of *L. formosana* in 2015. However, the observed significant differences in A_{net} and G_c of *L. formosana* between CK and U50 treatment ($P < 0.05$) in 2015 could still prove that U50 treatment reduced the A_{net} and G_c of the studied two *L. formosana* individuals in this study.

CONCLUSION

Higher concentrations of N addition exerted negative impacts on G_c and A_{net} of *L. formosana* and *Q. acutissima* during periods of lower precipitation in the broadleaved deciduous forest located at the climate transition zone from the warm temperate to subtropical zone, and increased WUE of *L. formosana* during the growing season. Lower concentrations of N addition had no impacts on dominant tree species. Canopy and understory N additions affected carbon assimilation through different ways, and consequently generated distinct impacts on vegetation. The traditional understory addition approach could not fully reflect the effects of increased N deposition on the canopy-associated assimilation processes.

ACKNOWLEDGEMENTS

This study was financially supported by the National Natural Science Foundation of China (Grant Nos. 41630752, 31800329, 31770493 and 31770646). Sincere thanks go to the CAS President's International Fellowship Initiative (PIFI: 2018VBB0006) program which provided Dr. Karina VR Schäfer funds to visit the laboratory of Dr. Ping Zhao at South China Botanical Garden.

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