

Title: Impact of nitrogen fertilization on carbon and water fluxes in a chronosequence of three Douglas-fir stands in the Pacific Northwest

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Abstract

This study examined the response of carbon (C) sequestration and evapotranspiration (ET) to nitrogen (N) fertilization during the four post-fertilization years (2007–2010) in a Pacific Northwest chronosequence of three Douglas-fir stands 61, 22 and 10 yr old in 2010 (DF49, HDF88 and HDF00, respectively). Artificial neural network (ANN) for time series analysis was employed to identify and estimate the complex nonlinear relationships between C and water exchanges and environmental variables. To test the performance of the ANN model, it was trained against multi-year monthly climate variables and eddy covariance (EC)-measured C and water fluxes for 1998–2004 and the trained model was then verified using data obtained in 2005 and 2006. The optimized model which showed high reliability (linear regression analysis: for C and water fluxes, $R^2 > 0.93$, slope = 0.96–0.99, and $p < 0.0001$) was then used to calculate the C and water fluxes for 2007–2010 assuming the three stands were not fertilized. The calculated C and water fluxes (for non-fertilized conditions) were compared with the measured fluxes to quantify the effects of fertilization during the post-fertilization during the post-fertilization four years. Results showed that N fertilization increased gross primary productivity for all three sites in all four years with the largest absolute increases in the 10 yr-old stand (HDF00) followed by the 22 yr-old stand (HDF88). Ecosystem respiration increased in all four years at HDF00, but decreased over the last three years at 22 yr-old HDF88, and over four years in the 61 yr-old stand (DF49). As a result, fertilization increased the net ecosystem productivity of all three stands with the average increase being the largest at 22 yr-old HDF88 followed by 61 yr-old DF49. In addition, fertilization caused a small increase in annual ET in all four years in 61 yr-old stand (DF49); a small increase in the first year but a decrease in the next three years at 22 yr-old HDF88; and no consistent effect at 10 yr-old HDF00. Consequently, fertilization exerted only a small impact on water use efficiency in the oldest stand (DF49) but a significant increase in the two

younger stands (HDF00 and HDF88). Furthermore, N fertilization increased light use efficiency in all three stands, especially in the 10 yr-old stand.

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1. Introduction

The factors affecting the physiological processes controlling the amount of terrestrial carbon (C) stored in terrestrial ecosystems mainly include atmospheric CO₂ concentration, climatic variability, land use change and nitrogen (N) deposition (Houghton, 2002; Piao et al., 5 2009; Tian et al., 2011). In terrestrial ecosystems, the important processes of determining whether an ecosystem is a C sink or a source are C fixation and release through photosynthesis and respiration, respectively. For N-limited terrestrial ecosystems, additional N supply can affect these processes, and in turn affect the strength of C sinks or sources (Reich et al., 2006; Högberg, 2007; Yang et al., 2011; Hilker et al., 2012; Cameron et al., 10 2013).

Research has shown that the response of C exchange in terrestrial ecosystems to N fertilization is positive or negative depending on ecosystem N status (Aber et al., 1998; Magill et al., 2004; Krause et al., 2012). Many researchers have studied the effects of N enrichment on C sequestration in the forest ecosystem, but it remains uncertain as to how 15 much N deposition contributes to the magnitude of terrestrial C sequestration (Houghton, 2002; Reay et al., 2008; Fleischer et al., 2013).

Many studies have found that N enrichment increases C sequestration (Berg and Matzner, 1997; Franklin et al., 2003; Olsson et al., 2005; Leggett and Kelting, 2006; Xia and Wan, 2008). Reay et al. (2008) suggested that about 10% of the annual anthropogenic C 20 emissions could be sequestered due to the increased atmospheric N deposition over the following decades. Magnani et al. (2007) found C sequestration was highly correlated with N deposition in forest ecosystems and the response of net C sequestration to N in wet deposition was approximately 725. However, Sutton et al. (2008) found that the response of net C sequestration to N was about 50–75, by model re-analysis across 22 European forest sites and 25 taking account of the impacts of climatological differences among stands. de Vries et al.

(2008) also found that the response of net C sequestration to N would be 30–70 by a multi-factor analysis of European forest measurements at nearly 400 intensively monitored forest plots in Europe. The results of both Sutton et al. (2008) and de Vries et al. (2008) are much smaller than that estimates by Magnani et al. (2007). Therefore, there is still
5 some controversy over the magnitude and sustainability of C sequestration resulting from N enrichment and its potential mechanisms (Dezi et al., 2010; Janssens et al., 2010; Högberg, 2012).

However, other studies have reported that N enrichment may cause a relatively small increase in C sequestration (Krause et al., 2012; Morell et al., 2011), or have no effect
10 (Körner, 2000), or a negative effect by altering plant and microbial communities, including threatened and endangered species (Fenn et al., 2003). Nadelhoffer et al. (1999) pointed out that the influences of N enrichment on ecosystem C storage should be minor in forest ecosystems in the northern temperate zone. Currie et al. (2004) found that small increases in C storage occurring primarily in living and dead wood result from elevated N deposition over
15 the next few decades. Using meta-analysis from global N addition experiments, Liu and Greaver (2010) concluded that the increases of short-term C sequestration below ground caused by N enrichment is due to increased C storage in surface organic layer, but it appeared to be hard to predict the reponse of C storage to N enrichment through a long-term experiment. Harpole et al. (2007) reported that the ability of grassland ecosystems to
20 sequester C late in growing season was decreased by N enrichment because of increased growing season water use and earlier leaf senescence.

Furthermore, water and C cycles in terrestrial ecosystems are closely coupled. Nutrients may not only affect productivity and foliar biomass but also associated with evapotranspiration (ET) in forest and other ecosystems (Felzer et al., 2011). Water use
25 efficiency (WUE) is the ratio of C gain (usually gross primary productivity, GPP) to water

loss, i.e., ET (Hu et al., 2008; Ito and Inatomi, 2011). Studies have reported that by increasing improving WUE, N addition could enhance plant productivity (Brix and Mitchell, 1986; Dordas and Sioulas, 2008; Livingston et al., 1999). On the contrary, other researchers have found that N enrichment has no impact on WUE (Mitchell and Hinckley, 1993; Jassal et al., 5 2009; Korol et al., 1999) or a negative effect on WUE (Claussen, 2002; Castellanos et al., 2013). Consequently, it is difficult to account for the differences in N-induced effects on WUE (Ripullone et al., 2004). Furthermore, since absorbed photosynthetically active radiation (APAR) is an important control of GPP, light use efficiency (LUE) (the ratio of GPP to APAR) has been receiving increasing attention primarily because by knowing LUE it 10 is possible to estimate regional or global GPP using remote sensing techniques (Running et al., 2004; Zhao et al., 2005; Yuan et al., 2010). Nevertheless, the biophysical controls of LUE in terrestrial ecosystems, especially soil N, remain poorly understood, especially in Douglas-fir stands on the west coast of Canada.

15 Long term measurements of C fluxes using the eddy-covariance (EC) technique, and fertilization with urea in early 2007 in a single dose in all three Pacific Northwest Douglas-fir stands, offer an opportunity to examine the effects of N fertilization on C and water fluxes.

Using multiple-year EC measurements in the same three stands above as we used in this study, Jassal et al. (2010a) found that in the two older stands in two years after fertilization both GPP and ecosystem respiration (R) increased compared to pre-fertilization values with 20 the increase in GPP being greater than in R . However, in the youngest stand R increased more than GPP. As a result, net ecosystem productivity (NEP) (i.e., $GPP - R$) increased in the two older stands but decreased slightly in the youngest stand. Using the same measurements, Chen et al. (2011) also found that annual NEP of the oldest stand in the first year after fertilization increased significantly; however, it was due to an increase in GPP and a decrease 25 in R . To estimate the C fluxes without fertilization, Chen et al. (2011) used the process-based

model, Boreal Ecosystem Productivity Simulator (BEPS), while Jassal et al. (2010a) used an empirical model ([multiple linear regression, MLR](#)). The artificial neural network (ANN) technique has been successfully used in identifying the complex nonlinear relationships between C and water fluxes and environmental factors in terrestrial ecosystems (Van Wijk and Bouten, 1999; Papale and Valentini, 2003; Jahanbani and El-Shafie, 2010; Moffat et al., 2010). [The main goal of this study is to estimate the effects of N fertilization on carbon and water fluxes in three different-aged stands during four post-fertilization years, and compare the performance of ANN and MLR models.](#)

2. Materials and methods

2.1. Site descriptions

Three stands were located on the east coast of Vancouver Island, BC, between Campbell River and Denman Island. In 2010, the ages of the stands were 61, 22 and 10 yr old, which spanned the typical ages of stands in this area. The three stands are dominated by Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) with relatively similar stand densities, soil, topography, elevation, and biogeoclimatic classification (Humphreys et al., 2006). On the whole, stand age and corresponding stand structural characteristics were considered to be largely responsible for the differences among these stands in C uptake and ET. The oldest stand (49°52'7.8"N, 125°20'6.3"W, flux tower location), DF49, was planted with Douglas-fir seedlings in 1949 and occupied an area of 130 ha. The stand comprised, 80% Douglas-fir and 17% western red cedar (*Thuja plicata*), and 3% western hemlock (*Tsuga heterophylla*). The 22 yr-old (pole-sapling) stand (49°32'10.49"N, 124°54'7.18"W), HDF88, 110 ha in size, was situated about 30 km southwest of DF49. The stand comprises 75% Douglas-fir, 21% western red cedar and 4% grand fir (*Abies grandis*). The youngest stand (49°52'1.08"N, 125°16'43.80"W), HDF00, was located about 2 km southeast of DF49. This 32-ha stand was harvested in the winter of 1999/2000 and in the following spring planted with one-year-old

seedlings (93 % Douglas-fir, 7 % western red cedar). Much of the leaf area at this site was due to the growth of pioneer and understory species from the previous stand. Additional details on stand history, vegetation and soil can be found in Chen et al. (2009a), Krishnan et al. (2009), Humphreys et al. (2006) and Morgenstern et al. (2004)..

5 2.2. Stand fertilization

Urea fertilizer was spread aerially at 200 kg N ha⁻¹ on about 110 ha at DF49 and 20 ha at HDF88, which included most of the respective tower flux footprints, on 13 January and 17 February 2007, respectively. Because of the young age of the planted trees and competing understory at HDF00, 80 g urea per tree (~60 kg N ha⁻¹) was manually applied along the tree drip line of trees located in the 5 ha tower flux footprint on 13–14 February 2007. The fertilized areas at the respective towers accounted for more than 80% of the EC fluxes measured during unstable atmospheric conditions occurring during the daytime and nighttime (Chen et al., 2009a).

2.3. Climate and EC measurements

The EC technique has been used to measure continuously half-hourly CO₂ and water vapour fluxes since 2000, 2001 and 1997 at DF49, HDF88 and HDF00, respectively. Weather variables that were also measured included precipitation, solar and net radiation (R_n), photosynthetically active radiation (PAR), wind speed, air temperature (T_a) and humidity, soil temperature (T_s) and soil moisture content (θ). Details on EC instrument characteristics, measurements and calculation procedures for these stands are described in Chen et al. (2009a), Humphreys et al. (2006) and Morgenstern et al. (2004). Half-hourly EC-measured CO₂ fluxes above the stand were corrected by adding the estimated rate of change in CO₂ storage in the air column below the EC sensor height to give the net ecosystem exchange (NEE). NEP was obtained using NEP = -NEE (Humphreys et al., 2006).

The methodologies of gap-filling for EC-measured NEE and its separation into GPP and R were adopted from the Fluxnet-Canada Research Network procedure (Barr et al., 2007; Chen et al., 2009a). Missed R at night and day time were obtained using an exponential function (Q_{10} model) rather than using the relationship between daytime NEP and PAR as used in Jassal et al. (2009). The estimates of GPP were equal to $NEP + R$ (warm season periods) or zero (cold season periods). An empirical light response curve was used to fill gaps in GPP. The details on NEE gap-filling and its separation into GPP and R as well as the uncertainties in the annual sums of NEE, GPP and R can be found in Chen et al. (2009b).

2.4. Artificial neural networks

An ANN is characterized by flexible mathematical structures, which can be used to investigate complicated non-linear relationships between inputs and outputs (Melesse and Hanley, 2005). For training for time series prediction, the artificial neural networks (ANNs) are conducted through propagating the input data and then back-propagating the error by means of self-adjustment of the weights with least squares residuals, in order that the simulated outputs best approximate the target outputs (observed data). The performance of an ANN as a purely empirical non-linear regression model is commonly affected by the quality of the training dataset, network architecture and network training (Moffat et al., 2007). More mathematical background on the ANN can be found in White (1989), Jain et al. (1996), Zhang et al. (1998) and Basheer and Hajmeer (2000).

In the fields of ecological and environmental science, especially in ecosystem C flux estimation, the ANN technique has been successfully applied as a data-driven modeling method with the purpose of exploring the relationships between C exchanges and environmental variables. Melesse and Hanley (2005) applied the ANN technique to simulate CO_2 flux by network training using energy fluxes and temperature for multi-ecosystems (grassland, forest and wheat) and found that CO_2 flux corresponded well with observed

values. To assess the European forest C fluxes at a continental scale, Papale and Valentini (2003) applied an optimized ANN with flux tower data to investigate the spatial and temporal patterns and found that the ANN technique was superior to traditional modeling methods.

Furthermore, the ANN technique has been widely applied in the gap-filling of EC C flux data for EUROFLUX and AmeriFlux sites (Ooba et al., 2006; Moffat et al., 2007; Richardson et al., 2008). However, very little research on simulating ET using the ANN approach has been conducted using EC water vapor flux measurements.

In this study, the back-propagation (BP) algorithm for network training was used because it is regarded as the most widely applied algorithm in the ANN literature (Rumelhart et al., 1986; Maier and Dandy, 2000). A three-layer BP neural network (BPNN) was employed with one hidden layer because Funahashi (1989) and Cybenko (1989) have demonstrated that, even with one hidden layer, a BPNN can approximate any continuous multivariate function with a reasonable precision. In the calibration phase, the optimization approach applied was based on Levenberg–Marquardt (Moré, 1978), which is known to outperform the simple gradient descent and other conjugate gradient methods. A hyperbolic tangent sigmoid function (tansig) and a linear function (purelin) in MATLAB for the hidden and output layers were used as the respective transfer functions. Aiming at obtaining the hidden node number and avoiding over-fitting in the training period, we used a trial and error method to select the optimal solution through altering the number of hidden nodes.

2.5. Comparison of ANN with MLR methods

In this study, six variables including T_s at the 5-cm depth, T_a , total downwelling PAR (Q), water vapor saturation deficit (D), R_n , θ in the 0-30 cm layer, all of which can significantly affect photosynthesis, respiration and ET, were selected to train an ANN using pre-fertilization flux measurements. Environmental variables that worked best for GPP, R and ET were chosen as inputs after trying several networks. Three different variable groups (T_a ,

θ , Q , D), (T_a, θ, T_s) , and (T_a, θ, R_n, D) were then used to train the ANN and predict monthly GPP, R and ET, respectively, for the three sites assuming the stands had not been fertilized. To ensure high precision in the period of model prediction, we used multi-year monthly climate variables and EC-measured C fluxes and ET before 2005 to train the ANN and then verified the trained model with measurements in 2005 and 2006. When we were convinced that the optimized ANN model successfully simulated the multiyear seasonal variations of C fluxes and ET, the input values for the post-fertilization period were brought into the trained ANN to predict the GPP, R and ET for 2007 to 2010. The resulting differences between the measurements and predictions were used to discern the impact of fertilization.

At the beginning of training, inputs and outputs were normalized between 0 and 1 which is a common preprocessing method for variables with different values. Moreover, we also used MLR to simulate C fluxes and ET with the same variables to assess the advantages of the ANN approach over traditional regression modeling techniques. Because of the differences of stand age among these three stands, it is important to note that the models both ANN and MLR were run separately for each stand. The performances of the ANN and MLR approaches were mainly evaluated by comparing predicted with measured data in the calibration and validation periods. In this study, computer programming and data analysis were performed using MATLAB (version 7.13, R2011b).

3. Results

3.1. Comparisons of observed C fluxes and ET before and after fertilization in the chronosequence

Fig. 2 shows how monthly values of EC-measured C fluxes and ET varied in the three stands before and after fertilization. For DF49, the monthly GPP values during the post-fertilization 4 years were similar to the means during non-fertilized 9 yr (Fig. 2a). on the

other hand, the monthly values of R during the post-fertilization 4 yr were close to the pre-fertilization 9 yr means for Jan–May but lower for the later months except for June and July in 2009 (Fig. 2b). Therefore, the monthly NEP values during the post-fertilization 4 yr were greater than the pre-fertilization 9 yr average values for March–December, especially in 5 June, August and September (Fig. 2c). Similarly, monthly NEP values during the post-fertilization 4 yr at HDF88 were generally greater than the pre-fertilization multi-year average values from 2002 to 2006 (Fig. 2g). In the case of HDF00, compared with the 2001–2006 means, the higher monthly values of NEP during 2007–2010 occurred in June, July and August (Fig. 2k). The monthly GPP values during 2007–2010 at HDF88 and HDF00 10 were higher than the mean values for the pre-fertilization periods (Fig. 2e and i), partly because these two stands are at the period of rapid growth for their entire life cycle. Furthermore, at DF49, the maximum NEP occurred in April and May (Fig. 2c), while maximum NEP at HDF88 and HDF00 occurred in June and July (Fig. 2g and k).

Most of monthly ET values during 2007–2010 at DF49 and HDF88 were close to the 15 multi-year monthly means in the pre-fertilization period (Fig. 2d and h). However, at HDF00, monthly ET values for May–July during 2009 to 2010 were significantly higher than the means for the pre-fertilization period (Fig. 2l). Moreover, for DF49, the monthly values of ET during January to April in 2010 were higher than the previous 9 yr average values because of higher precipitation during these months in this year (Fig. 2d). Monthly ET values in all years 20 in all three stands exhibited similar seasonal and interannual variability as GPP suggesting a strong coupling between GPP and ET irrespective of N fertilization (Fig. 2a, d, e, h, i and l).

3.2. ANN model validation and its comparison to MLR model

Since all three stands were exposed to similar weather conditions (warm dry summers and wet cool winters), we described only the variations in environmental variables and C fluxes 25 and ET in the 61 yr-old stand (DF49) at seasonal and inter-annual scales during the pre- and

post-fertilization periods. Fig. 1 shows that the seasonal patterns of environmental variables T_a , T_s , θ , D , Q and R_n during 2007–2010 were similar to those measured before fertilization from 1998 to 2006 in the 61 yr-old stand. Compared with the 9-year means during the pre-fertilization period, the annual average values of T_a during 2007–2010 were lower by about 0.7, 1.1, 0.4 and 0.8 °C, respectively, and the annual average values of T_s were higher by about 0.3, 0.1, 0.1 and 0.6 °C, respectively (Fig. 1a and b). Annual values of total Q in 2007, 2008 and 2010 were lower by about 9%, 3% and 6%, respectively, suggesting possible growth limitation in those years, but higher by about 8% in 2009 (Fig. 1f). Annual average values of θ in the 0–30 cm soil layer in the growing season (May–September) in the post-fertilization period were 0.22, 0.20, 0.19 and 0.20 $\text{m}^3 \text{m}^{-3}$, respectively, which were higher than the pre-fertilization 9 yr mean (0.16 $\text{m}^3 \text{m}^{-3}$), especially in 2007 which had a noticeably wet growing season (Fig. 1c).

Figs. 4a and 5a-c illustrate the differences among EC-measured, ANN and MLR simulated monthly GPP, R , and NEP for 1998–2004 in 61 yr-old stand, respectively. ANN simulations showed strong agreement with the observed values and explained about 99%, 99% and 92% of the variance of monthly GPP, R , and NEP, respectively (Fig. 5a-c and Table 1). The linear regression analysis between ANN-modeled and measured values in Fig. 5a-c show that $P < 0.0001$ for all C component fluxes and RMSE = 6, 12, and 11 $\text{g C m}^{-2} \text{mon}^{-1}$ for GPP, R and NEP, respectively. These results indicate that through the trained network parameters, the ANN model achieved reasonable simulation of monthly photosynthesis and respiration mechanisms. Figs. 4b and 5e-g illustrate the differences among EC-measured, ANN- and MLR- modeled monthly GPP, R , and NEP for the 2005–2006 validation period at DF49. The linear regression analysis between ANN-modeled and measured values in Fig. 5e-g shows that $R^2=0.99$, 0.99 and 0.93; RMSE=5, 11 and 11 $\text{g C m}^{-2} \text{mon}^{-1}$ for GPP, R , and

NEP, respectively, which are similar to the assessment of model performance during the model calibration stage from 1998 to 2004 (Fig. 5a-c and Table 1).

MLR simulations for the model calibration period, however, explained about 96%, 92% and 77% of the variance of monthly GPP, *R*, and NEP, respectively (Figs. 4a and 5a-c), which are all significantly lower than ANN simulations (Fig. 5a-c and Table 1). The linear regression analysis also showed that $P < 0.0001$ for all C component fluxes and RMSE = 21, 29, and 19 g C m⁻² mon⁻¹ for GPP, *R* and NEP, respectively, which are all significantly higher than those for the comparison of ANN simulated and EC-measured values (Fig. 5a-c and Table 1). In addition, for the period of verification (2005 and 2006), the linear regression analysis, i.e. comparison of MLR-calculated with observed values in Figs. 5e–g, shows that RMSE=32, 34 and 24 g C m⁻² mon⁻¹ for GPP, *R*, and NEP, respectively, which are all significantly higher than those for the comparison of MLR simulated and EC-measured values during the calibration period, indicating that it is difficult for the MLR model to reveal the actual interactions of C exchanges and climatic variables.

Furthermore, for the two younger stands, the linear regression analysis comparing ANN-modeled and EC-measured values were superior to those comparing MLR-modeled and EC-measured values, especially for the youngest stand (HDF00) (Table 1). In addition, the MLR model often underestimated the values of the C component fluxes in winter in all three stands, especially in the case of *R*.

Since ANN model was confirmed to be clearly superior to MLR model, in the rest of this section we discuss only the use the ANN approach together with EC measurements to ascertain the responses of C fluxes and ET and to N fertilization. Figs. 4 and 5d, h and i and Table 1 show the differences between EC-measured, ANN and MLR simulated monthly ET in the pre- and post-fertilization periods in the 61 yr-old stand. During the calibration and verification periods, the linear regression results comparing ANN-modeled and EC-measured

ET were superior to those comparing MLR-modeled and EC-measured values. Similar to the above results for C component fluxes, we also focused on describing the simulation results of the ANN approach in predicting fertilization effects on ET.

3.3. Effects of N addition on C exchange fluxes, WUE and LUE in the chronosequence

5 Fig. 3 shows the annual variations of EC-measured C exchange fluxes, ET, WUE and LUE. The results suggest that EC-measured NEP during the post-fertilization four years significantly increased in all three stands (Fig. 3c). Annual NEP in the 61 yr-old stand remained stable over post-fertilization period (2007–2010), and increased by about 200 g C m⁻² y⁻¹, despite this period being cooler and wetter than pre-fertilization years (Fig. 1a and c).
10 However, at HDF88, annual NEP consistently increased with increasing stand age (Fig. 3c).

An examination of the inter-annual variability of GPP and *R* in the 61 yr-old stand since 1998 showed that there was a much larger decrease in *R* during 2007–2010 (Fig. 3a-b). However, for the two younger stands, annual *R* increased in the first post-fertilization year and decreased in the next three years (Fig. 3b). Furthermore, significant increases in GPP and *R* occurred in 2004 and 2005 in all three stands (Fig 3a and b), resulting from the
15 unusual climatic conditions alternating between warm and wet seasons in both years (data not shown), which may be challenging for the ANN training.

Annual ET during the post-fertilization period was no apparently different from the previous 9 yr means in all three stands (Fig. 3d). WUE, calculated as the ratio of GPP to ET,
20 its annual magnitude varied greatly among the three stands (Fig. 3e). Annual WUE of the oldest stand (DF49) was highest, ranging from 4.5 to 5.7 g C m⁻² kg⁻¹, and steadily decreased during 2008–2010. LUE, calculated as the ratio of GPP to APAR, its annual values of HDF88 and HDF00 increased with age, from 0.0131 to 0.0214 mol C mol⁻¹ photons and 0.0026 to 0.0146 mol C mol⁻¹ photons, respectively, but in the 61 yr-old stand, it remained
25 relatively high with values ranging from 0.0208 to 0.0282 mol C mol⁻¹ photons (Fig. 3f). We

found that the LUE followed trends similar to GPP in all three stands between pre-and post-fertilization years regardless of the PAR level.

To further examine the influences of fertilization on C exchange fluxes, Fig. 6 shows monthly EC-measured and ANN modeled C fluxes during four post-fertilization years, and the responses of C fluxes to fertilization as a result from the differences between observed and simulated values.

For the post-fertilization period, ANN modeled monthly values of GPP were lower than measurements in the 61 yr-old stand (Figs. 5i and 6a, $R^2 = 0.91$), but were higher for R (Figs. 5j and 6d, $R^2 = 0.95$), and consequently, predicted monthly values of NEP were lower than observed values (Figs. 5k and 6g, $R^2 = 0.45$). Table 2 shows the responses of C exchanges to N enrichment during the post-fertilization four years from 2007 to 2010 in the three stands. We found that fertilization consistently increased annual GPP in the 61 yr-old stand of 8% (from 1939 to 2095 g C m⁻² y⁻¹), 11% (from 1906 to 2111 g C m⁻² y⁻¹), 5% (from 2002 to 2103 g C m⁻² y⁻¹) and 11% (from 1924 to 2138 g C m⁻² y⁻¹) during 2007 to 2010, respectively, and with respective decreases in annual R of about 3% (from 1591 to 1543 g C m⁻² y⁻¹), 3% (from 1623 to 1580 g C m⁻² y⁻¹), 11% (from 1755 to 1558 g C m⁻² y⁻¹) and 6% (from 1616 to 1520 g C m⁻² y⁻¹). As a result, N-addition increased annual NEP by about 59% (from 348 to 552 g C m⁻² y⁻¹), 88% (from 283 to 531 g C m⁻² y⁻¹), 122% (from 246 to 545 g C m⁻² y⁻¹) and 101% (from 308 to 618 g C m⁻² y⁻¹) during 2007 to 2010, respectively, and showed a rising trend in annual NEP from 2007 to 2010.

Furthermore, in the 22 yr-old stand (HDF88), fertilization resulted in greater relative increases in annual GPP than in the 61 yr-old stand with values of 32.9% (from 1393 to 1851 g C m⁻² y⁻¹), 29% (from 1302 to 1675 g C m⁻² y⁻¹), 11% (from 1419 to 1572 g C m⁻² y⁻¹) and 12% (from 1527 to 1716 g C m⁻² y⁻¹) during 2007 to 2010, respectively (Table 2). Annual R in the 22 yr-old stand increased by 11% (from 1544 to 1714 g C m⁻² y⁻¹) in 2007, but

decreased by 17% (from 1757 to 1457 g C m⁻² y⁻¹), 19% (from 1634 to 1326 g C m⁻² y⁻¹) and 20% (from 1701 to 1365 g C m⁻² y⁻¹) during 2008 to 2010, respectively. This caused annual NEP increases from -151 to 137 g C m⁻² y⁻¹, -455 to 218 g C m⁻² y⁻¹, f -215 to 246 g C m⁻² y⁻¹ and -174 to 351 g C m⁻² y⁻¹ during 2007 to 2010, respectively.

5 For the [10 yr-old stand \(HDF00\)](#), fertilization increased both GPP and *R* during the four years with the increase in GPP being larger than in *R* in all years (Table 2). Consequently, fertilization led to annual NEP increases at HDF00 of 35% (from -672 to -440 g C m⁻² y⁻¹), 34% (from -589 to -391 g C m⁻² y⁻¹), 62% (from -610 to -230 g C m⁻² y⁻¹) and 38% (from -567 to -350 g C m⁻² y⁻¹) during 2007 to 2010, respectively. The results show that N addition led to
10 the greatest increases in GPP [in the 10 yr-old stand](#) during the post-fertilization four years, followed in decreasing order by [the 22 yr-old stand](#) and [the 61 yr-old stand](#). However, it caused greatest increases in NEP at HDF88.

Table 3 indicates the overall N effects on annual ET, WUE and LUE in the post-fertilization four years during 2007 to 2010 in the three stands. There were no
15 discernible effects of fertilization on annual ET in the post-fertilization period in all three stands. N addition led to substantial increases in annual WUE for [the 22 yr-old stand](#) and [the 10 yr-old stand](#), especially at the youngest stand (HDF00) with increases ranging from 1.14 to 1.89 g C (kg water)⁻¹, due to the great impacts on GPP compared to the slight impacts on ET, and remained relatively stable for both stands during the post-fertilization four years.
20 However, N fertilization had little effect in annual WUE [in the 61 yr-old stand](#) due to its relatively small effect on annual GPP (Tables 2 and 3). Table 3 shows that N fertilization had a positive impact on annual LUE for all three stands, especially at the [10 yr-old stand](#) with values ranging from 0.0058 to 0.0064 mol C mol⁻¹ photons.

To further examine the influences of fertilization on WUE and LUE, [Fig. 7](#) shows
25 monthly EC-measured and ANN modeled WUE and LUE in the post-fertilization four years,

and the responses of WUE and LUE to fertilization as the results from the differences between observed and simulated values. There were no prominent responses of monthly WUE and LUE to fertilization at DF49, while at 22 yr-old HDF88 and 10 yr-old HDF00, fertilization had positive impacts on WUE and LUE for most months during 2007 to 2010.

5 4. Discussion

4.1. Effects of N fertilization on gross primary productivity

N fertilization generally results in GPP and NPP increases in terrestrial ecosystems through promoting growth and biomass of terrestrial plants (Dezi et al., 2010; Hogberg, 2007; Thomas et al., 2010; Niu et al., 2010; Xia and Wan, 2008). Our results that N addition caused GPP increase by 8% (=156 g C m⁻² y⁻¹) in the 61 yr-old stand in the post-fertilization first year are in excellent agreement with a 8% (=157 g C m⁻² y⁻¹) increase obtained by Chen et al. (2011) using a model-data synthesis approach and a 10% (184 g C m⁻² y⁻¹) increase obtained by Jassal et al. (2010a) using a simple empirical model (Fig. 8 and Table 2). It is also similar to the 14% increase obtained by Grant et al. (2010) using a process-based model. In addition, our results that N fertilization led to an increase in GPP in the three stands during the first two years after fertilization are consistent in sign with the findings by Jassal et al. (2010a), although the magnitude of the increases in GPP were somewhat different, especially at HDF00. The difference may partly result from the reported uncertainty due to the use of the empirical relationships used for gap-filling, resulting in about 50 g C m⁻² y⁻¹ in the EC-measured GPP with uncertainty of 75 and 25 g C m⁻² y⁻¹ in *R* and NEP, respectively (Humphreys et al., 2006).

Furthermore, from the longer time series of four years after fertilization, we found N fertilization had positive impact on GPP for all three stands, and the response in GPP to fertilization among the three stands was the highest in the youngest stand (HDF00), followed by the younger stand (HDF88) (Fig. 8 and Table 2). This agrees with the response of LUE to

fertilization among the three stands (Table 3). In addition, we also found N fertilization showed a decreasing trend in GPP in the post-fertilization four years only at 10 yr-old HDF88, maybe resulting from the decreasing trend in LUE (Table 3).

4.2. Effects of N fertilization on ecosystem respiration

5 Our results indicated that N fertilization for the 61 yr-old stand reduced R by approximately $48 \text{ g C m}^{-2} \text{ y}^{-1}$ (about 3%) in the post-fertilization first year, which is somewhat less than the $93 \text{ g C m}^{-2} \text{ y}^{-1}$ (6%) decrease calculated by Chen et al. (2011), but disagrees with the result with $35 \text{ g C m}^{-2} \text{ y}^{-1}$ increment in R induced by N application at DF49 found by Jassal et al. (2010a). In addition, during the next three years (2008-2010) after
10 fertilization in the 61 yr-old stand, ANN modeled R remained greater than measured R by 43 to $197 \text{ g C m}^{-2} \text{ y}^{-1}$, implying that N fertilization suppressed R . In this study, however, the increase in R due to N fertilization at the 10 yr-old HDF00 during the two years after fertilization agrees with the findings in Jassal et al. (2010a) for the same stand. Our ANN modeling indicated that this positive effect on R at the 10 yr-old HDF00 continued for the
15 remaining two years (Fig. 8 and Table 2). It should be noted that Jassal et al (2010a) partitioned NEE by estimating daytime R using the relationship between daytime NEP and PAR rather than using the relationship between nighttime NEE and T_s .

N addition suppressed R in the two younger stands (DF49 and HDF88), especially at HDF88 suggesting that the magnitudes of decrease in R induced by N fertilization were
20 almost 2 times that of the increase in GPP during the post-fertilization 2009-2010. It is important to investigate the mechanisms of R reduction in response to N fertilization. Chen et al. (2011) reported that the reduction in aboveground R might mainly result from stem respiration. On the other hand, Jassal et al. (2007) found that soil respiration (R_s) accounted for about 62% R at DF49, which is similar to 67% by (Chen et al., 2011) in the first year after
25 fertilization. Some of the reduction in annual R may result from annual R_s decrease caused by

N enrichment (e.g., Bowden et al., 2004; Mo et al., 2007; Olsen et al., 2005). Based on soil CO₂ flux measurements using automated- and manual-chamber systems at DF49, Jassal et al. (2010b) found that N led to significantly increase in R_s over about 4 months resulting from the increase in autotrophic respiration (R_a) accompanied by a slight decrease in heterotrophic respiration (R_h). Olsson et al. (2005) reported the results of a long-term and large-scale tree girdling experiment showing that N addition had a great potential to increase boreal forest C sequestration largely due to increased forest biomass, but would decrease both R_a and R_h . Using gas chromatography and static chamber techniques, Mo et al. (2007) found that N deposition of tropical forests has caused R_s to decrease, but it might change depending on the rates of atmospheric N deposition. On the basis of weekly R_s measurements, Bowden et al. (2004) reported that continuous 13 yr N application reduced R_s by 41% in the plots with high N content during the growing season.

4.3. Effects of N fertilization on C sequestration

In this study, N fertilization significantly increased NEP in the four years following fertilization in all the three stands, especially for the 22 yr-old stand, largely due to N-induced GPP increases and R decreases, followed by the 61 yr-old stand (Fig. 8). These results are consistent with the results obtained in the 61 yr-old stand in the post-fertilization first year by Chen et al. (2011), and are also consistent in sign with the results obtained by Jassal et al. (2010a) in the 61 yr-old stand and the 22 yr-old stand in the two post-fertilization years, although the magnitude of the increase in NEP was less than in this study. However, at HDF00, a substantial increase in NEP in the four years after N application was primarily due to the increase in GPP rather than R (Fig. 8). In summary, fertilization led to increase in NEP, in accordance with a number of previous studies (Adams et al., 2005; de Vries et al., 2009). Moreover, the prediction results of the ANN model indicated that fertilization resulted in an increasing trend (60 to 120%) in annual NEP in the post-fertilization three years but decline

in the fourth year after fertilization both at DF49 and HDF00 (Fig. 8 and Table 2). Niu et al. (2010), from results of a 4-year experiment study in a temperate steppe, reported that N enrichment stimulated NEE with the increases of 60% and 21% in the first two years post-fertilization, respectively, but it had no significant impact in the last two years. Several studies have suggested that the diminishing N stimulation of NEP and plant growth over time may be caused by many factors such as soil acidification (Niu et al., 2010) and the slight deficiency of potassium (Jassal et al., 2010a).

C:N response [$\text{kg C (sequestered) kg}^{-1} \text{ (N added)}$] defined as the increase in the net C sequestration (i.e., NEP) per unit of N application has been widely used to estimate the response of C sequestration to N addition (de Vries et al., 2008; Magnani et al., 2007; Sutton et al., 2008). The estimate of C:N ratio varies depending on geographical location, soil status, tree species, stand age and fertilizer composition and dose (Hyvönen et al., 2008). High N fertilization level (200 kg N ha^{-1}) in one single dose used in this study is a common forest management activity in N-limited forests (Chen et al., 2011), which is different from N deposition by the experiments of long-term low-dose N addition studied by Hyvönen et al. (2008). Therefore, it is also important to investigate the responses of net C sequestration to this common N fertilization activity. Moreover, it should be noted that the effects of N fertilization on net C sequestration may be attenuated due to atmospheric N deposition resulting in C:N response lower than expected, especially in N-limited forests. Our results suggested that C:N responses varied with stand age being the highest ($14 \text{ kg C (kg N)}^{-1}$) for the younger stand (HDF88), followed by the youngest stand (HDF00 ($12 \text{ kg C (kg N)}^{-1}$)) and the oldest stand (DF49 ($10 \text{ kg C (kg N)}^{-1}$)) during the post-fertilization first year. Our results are consistent with the total carbon sequestration range of $5\text{--}75 \text{ kg C (kg N)}^{-1}$ reported by de Vries et al. (2009). C:N response in the oldest stand (DF49 ($23 \text{ kg C (kg N)}^{-1}$)) is higher than in the same stand ($13 \text{ kg C (kg N)}^{-1}$) during the post-fertilization two years estimated by

Jassal et al (2010a). The 4-yr cumulative C:N response was 53, 97 and 51 kg C (kg N)⁻¹ for DF49, HDF88 and HDF00, respectively. The findings by this study and Jassal et al (2010a) are similar to the findings by de Vries et al. (2008), Högberg (2012) and Sutton et al. (2008), which are significantly smaller than the assessment by Magnani et al. (2007), with an extremely high C:N response (approximately 725 kg C (kg N)⁻¹) in wet deposition.

Furthermore, for the youngest stand (HDF00), it seems that 60 kg N ha⁻¹ application to individual trees was more efficient with respect to C sequestration than the larger application (200 kg N ha⁻¹) for the two older stands, consistent with the studies by Hyvönen et al. (2008), which resulted from more GPP stimulated from N application in younger stand.

It is widely held that increased N deposition from atmospheric may enhance globally terrestrial C sequestration, especially in N limited temperate and boreal forest ecosystems (Magnani et al., 2007; Sutton et al., 2008). Moreover, Komarov and Shanin (2012) concluded that nitrogen deposition for forest ecosystems of European Russia played different relative roles acting together with climate changes in different climatic zones. Therefore, it is important to assess the effect of nitrogen deposition on forest C sequestration in the context of various future management and climate change.

4.4. Effects of N fertilization on ET, WUE and LUE

Our study demonstrated that N application had no significant change in annual ET in the 61 yr-old stand. The N fertilization decreased ET in the 22 yr-old stand in the last three years and had no clear effect on ET in the 10 yr-old stand in the four post-fertilization years (Table 3). As a consequence, it is difficult to summarize the responses of ET to N enrichment due to its complexity. N application had significantly promotional effects on WUE for the 22 yr-old stand and the 10 yr-old stand, due to its major positive effect on GPP compared to its small effect on ET, but had no significant effect on the oldest stand (DF49), which agrees with results of the study by Jassal et al. (2010a) (Table 3). Similar findings were obtained by

Halitligil et al. (2000) at four winter wheat sites in Central Anatolia showing that N application caused significant increases in WUE. Ripullone et al. (2004) found that N addition increased both WUE and biomass production. They concluded that this was mainly due to the positive response of photosynthesis to N addition with it having no impact on either stomatal conductance or transpiration.

The effect to fertilization on WUE seems to be associated with the amount of N applied (Morell et al., 2011). Cantero-Martínez et al. (2003) reported that moderate N supply caused increases in barley yield and biomass in three successive growing seasons at three locations in Northeast Spain, but under the semiarid conditions, there were no significant increases with high N application rates. They also pointed out that N addition led to an increase in crop WUE, but excessive N supply resulted in increased water consumption without enhancing WUE when available water was adequate. Wu et al. (2008) suggested that low or appropriate N application might improve the survivability of *Sophora davidii* seedlings under water deficit condition, resulting in promoting WUE and stimulating growth and biomass production, but excessive N application should not be advisable. Consistent with above studies, our results showed that the supply of 60 kg N ha⁻¹ applied to individual trees in the youngest stand (HDF00) appears to be more efficient in terms of WUE than a higher application of 200 kg N ha⁻¹ for the two older stands, especially in the oldest stand (DF49).

We found that N fertilization resulted in the greatest LUE increases in the youngest stand (HDF00) in the post-fertilization four years, followed by the 22 yr-old stand (HDF88). We also found a decrease in the effect of N application on LUE under high PAR (data not shown), which was also found by Ibrom et al. (2008) and Schwalm et al. (2006). Besides, there remains some uncertainty in the relationships between canopy LUE and N content, partly due to the use of the different definitions of LUE and the differences in spatial and temporal scales (Kergoat et al., 2008; Ollinger et al., 2008; Green et al., 2003; Peltoniemi et

al., 2012). Using EC measurement technique in temperate and boreal ecosystems, Kergoat et al.(2008) reported that there were significant correlations between maximum daily LUE and foliar N content. To the contrary, Schwalm et al. (2006) reported that canopy LUE was no significant correlation with foliar nitrogen content during the peak growing season in forest ecosystems. Furthermore, Still et al. (2004) suggested that due to the effect of deciduous forests having higher N content levels, LUE for North America was lower than for continental Eurasia.

4.5. Modeling uncertainty and limitation

Several studies have found that there was observable interannual variability of C component fluxes and ET in these stands which to a large extent results from interannual variability in climate (Humphreys et al., 2006; Chen et al., 2009b; Jassal et al., 2009; Krishnan et al., 2009). We used both ANN and MLR approaches as modeling methods of our experiment with the purpose of removing the interannual climatic variability impacts on C fluxes and ET in the three stands during the pre-fertilization period. This is consistent with the previous results obtained by Jassal et al. (2010a) and Chen et al. (2011) using MLR and a process-based ecosystem model, respectively, for the same stands. Our results show that in modeling the effect of climate on pre-fertilization monthly C and water fluxes in the three stands, the ANN approach, compared with the MLR approach, resulted in acceptably small values of RMSE with slopes close to 1.0 (Fig. 5). However, the three approaches produced significantly different effects of fertilization on C and water fluxes (Fig. 8 and Table 2).

Krishnan et al. (2009) found that environmental variables in the same three stands as this study presented a strong effect on C fluxes across half hourly to daily and monthly timescales. Jassal et al. (2010a) found that environmental variables explained about 95% of variance of monthly GPP, *R* and ET in 61 yr-old DF49 stand. The results from Krishnan et al. (2009) and Jassal et al. (2010a) above were characterized by using the MLR model at monthly steps.

It was very impressive that ANN model with its powerful advantage in investigating the complicated non-linear processes grasped the relationship between environmental variables and C and water fluxes during the pre-fertilization period in the three stands, with R^2 close to 1, even in the youngest stand (HDF00) with $R^2 = 0.92$ (Table 1 and Fig. 5). ANN modeling was more effective to simulate the C and Water fluxes during the post-fertilization period, due to its strong generalization ability.

There was a significant effect of stand development on C fluxes in the two younger stands as we demonstrated in Fig. 3. However, Jassal et al. (2010a) found that adding age as another variable to describe C fluxes in the two growing stands (i.e., HDF88 and HDF00) did not improve the model fits. We further verified that, regardless of stand age, the ANN model for the calibration period in this study was still able to strongly capture the relationship between environmental variables and C and water fluxes with explaining approximately 99% of the variance in monthly C fluxes and ET. On the other hand, although we didn't consider stand age as an input variable in ANN model, we believe that the ANN model considers the information on stand development in responses to C and water fluxes by the variations of C fluxes and ET in a chronosequence in an implicit way. Because we used C fluxes and ET as the output values of the ANN model to train the network during the period of pre-fertilization. It is worthy to note that the C fluxes varied with the stand development in a chronosequence, especially in the two younger stands, which was different from environmental variables. As a consequence, we may deduce that the trained ANN model had captured the information of stand development.

Therefore, in this study, we focused on removing the inter-annual climatic variability impacts on C fluxes and ET in the three stands during the pre-fertilization period to estimate the effects of N fertilization on carbon and water fluxes during the post-fertilization four

years and reduce uncertainties in the two previous studies (Chen et al., 2011 and Jassal et al., 2010a).

Regarding the time step used in modeling fluxes, Keenan et al. (2012) used hourly C flux data and ancillary data to drive the ANN model. Since one of our objectives was to compare our results with the MLR approach in Jassal et al. (2010), we decided to be consistent in using the monthly time step. We should emphasize that for such long data sets the monthly time step has been found to be effective in modeling seasonal and interannual variability in C fluxes in these stands (Jassal et al. 2010), which is supported by the high values of the coefficient of determination and the low values of the RMSE. To further examined the performance of ANN in assessing the effect of N fertilization at finer time scales, multi-years C fluxes and environmental variables in the mature and stable DF49 stand were conducted at half-hour time step. Half-hourly simulations for the model calibration period before 2005, explained about 87%, 74% and 80% of the variance of monthly GPP, R , and NEP, respectively, which are all significantly lower than monthly simulations in this study, with R^2 close to 1 in all three C fluxes in the same stand. Therefore, it was worthwhile to assess the impacts of N application at monthly time scale as a alternative step, and compared to higher resolution time scales such as half-hourly and hourly, using monthly time step may be more effective in this study.

In addition, the determination of BPNN model parameters such as the hidden layer node number, momentum coefficient and learning rate and its shortcomings such as slow convergence rate and local minimum have extremely restricted its application and lead to uncertainties in the estimate of N effect. Because of the limitation of BPNN model, a hybrid BPNN with optimized algorithm such as genetic algorithm and particle swarm optimization can possibly be used to further improve the estimate of N addition effect.

These limitations above could lead to some uncertainties in the estimates of N fertilization effects in this study. Nevertheless, we believe this study contributes to quantify the effects of N fertilization to C and water fluxes and reduce the uncertainty in model simulation.

5 5. Conclusions

1. Pre-fertilization monthly C fluxes and ET were better described using the ANN approach as compared to multiple linear regression (MLR) modeling.
2. N fertilization increased GPP in all three stands during the 4 yr period with the greatest increment occurring in the youngest (10 yr-old) stand followed by the 22 yr-old stand, suggesting that the effectiveness of fertilization on GPP may be associated with the stand age. In addition, fertilization decreased R in all four years in the 61 yr-old stand, and in the last three years in the 22 yr-old stand, but increased R in the youngest stand in all four years. As a result, N fertilization increased NEP in all three stands, with the greatest in the 22 yr-old stand and followed by the oldest (61 yr-old) stand.
3. N fertilization resulted in very small changes in annual ET in all three stands. These effects led to significant increments in WUE in the two younger stands but only a small increments in the oldest stand (DF49).
4. N fertilization increased LUE in all three stands, especially in the 10 yr-old stand.

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Table 1. Comparisons of artificial neural network (ANN) and multiple linear regression (MLR) model performances between calibration and verification periods for monthly gross primary productivity (GPP, $\text{g C m}^{-2} \text{y}^{-1}$), ecosystem respiration (R , $\text{g C m}^{-2} \text{y}^{-1}$) and evapotranspiration (ET, mm y^{-1}).

Stand	DF49				HDF88				HDF00				
	MVMLR		MVANN		MVMLR		MVANN		MVMLR		MVANN		
	Period1	Period2											
GPP													
Slope	0.96	0.88	0.99	1	0.91	0.86	1.01	0.97	0.79	0.37	0.94	0.92	
Intercept	6.14	9.16	1.84	-1.74	9.3	-21.14	-0.64	1	9.81	18.53	4.71	-3.11	
RMSE	21.15	31.52	6.23	4.85	21.54	48.89	8.27	8.3	25.94	68.29	16.21	17.16	
R^2	0.96	0.95	0.99	0.99	0.91	0.9	0.99	0.99	0.79	0.59	0.92	0.98	
R													
Slope	0.92	0.89	0.99	0.98	0.92	0.73	1.02	0.96	0.82	0.71	0.98	1	
Intercept	11.76	10.36	1.52	3.49	9.73	24.18	-2.74	4.7	16.33	34.6	1.42	-0.22	
RMSE	29.26	33.76	11.63	11.27	16.39	26.11	3.87	4.57	23.88	35.4	5.87	4.81	
R^2	0.92	0.91	0.99	0.99	0.92	0.92	0.99	0.99	0.82	0.78	0.99	0.99	
ET													
Slope	0.96	0.91	0.99	0.99	0.93	1.26	0.99	1.03	0.92	0.92	0.99	0.04	
Intercept	1.47	2.69	0.23	0.09	2.32	-10.04	0.14	-1.49	1.88	1.21	0.01	1.01	
RMSE	4.07	5.22	1.68	2.15	6.22	10.35	1.16	3.19	5.33	7.44	1.68	1.74	
R^2	0.96	0.94	0.99	0.99	0.93	0.91	0.99	0.98	0.92	0.87	0.99	0.99	

MVMLR, measured versus MLR modeled; MVANN, measured versus ANN modeled. Period1 refers to calibration step; Period2 refers to verification step.

Table 2. Effects of nitrogen fertilization on annual gross primary productivity (GPP, g C m⁻² y⁻¹), ecosystem respiration (*R*, g C m⁻² y⁻¹) and net ecosystem productivity (NEP, g C m⁻² y⁻¹) from different study results in the three West Coast Douglas-fir stands.

Stand	Flux	2007			2008			2009			2010		
		<i>a</i>	<i>b</i>	<i>c</i>									
DF49	GPP ^①	2095	1939	+156	2111	1906	+205	2103	2002	+101	2138	1924	+214
	GPP ^②	1944	1760	+184	1889	1750	+139						
	GPP ^③	2095	1938	+157									
	<i>R</i> ^①	1543	1591	-48	1580	1623	-43	1558	1755	-197	1520	1616	-96
	<i>R</i> ^②	1397	1362	+35	1364	1303	+61						
	<i>R</i> ^③	1453	1636	-93									
	NEP ^①	552	348	+204	531	283	+248	545	246	+299	618	308	+310
	NEP ^②	547	398	+149	525	447	+78						
	NEP ^③	552	302	+250									
HDF88	GPP ^①	1851	1393	+458	1675	1302	+373	1572	1419	+153	1716	1527	+189
	GPP ^②	1713	1098	+615	1526	1160	+366						
	<i>R</i> ^①	1714	1544	+170	1457	1757	-300	1326	1634	-308	1365	1701	-336
	<i>R</i> ^②	1633	1200	+433	1358	1171	+187						
	NEP ^①	137	-151	+288	218	-455	+673	246	-215	+461	351	-174	+525
	NEP ^②	80	-102	+182	168	-11	+179						
HDF00	GPP ^①	1126	641	+485	1005	459	+546	1083	597	+486	1068	539	+529
	GPP ^②	813	508	+305	815	486	+329						
	<i>R</i> ^①	1566	1313	+253	1396	1048	+348	1314	1207	+107	1418	1106	+312
	<i>R</i> ^②	1338	1030	+308	1399	988	+411						
	NEP ^①	-440	-672	+232	-391	-589	+198	-230	-610	+380	-350	-567	+217
	NEP ^②	-525	-522	-3	-584	-502	-82						

a Measured with N fertilization;

b ANN modeled without N fertilization;

c N effect (= *a* - *b*);

① This study;

② Jassal et al (2010);

③ Chen et al (2011).

Table 3. Effects of nitrogen fertilization on annual evapotranspiration (ET, mm y⁻¹), water use efficiency (WUE, g C (kg water)⁻¹) and light use efficiency (LUE, mol C mol⁻¹ photons) from different study results in the three West Coast Douglas-fir stands.

Stand		2007			2008			2009			2010		
		<i>a</i>	<i>b</i>	<i>c</i>									
DF49	ET ^①	421	388	+33	394	377	+17	421	405	+16	472	382	+91
	ET ^②	426	397	+29	400	394	+6						
	WUE ^①	4.97	5	-0.03	5.35	5.06	+0.29	4.99	4.95	+0.04	4.53	5.04	-0.51
	WUE ^②	4.56	4.43	+0.13	4.72	4.44	+0.28						
	LUE ^①	0.0254	0.0235	+0.0019	0.0241	0.0218	+0.0023	0.0216	0.0206	+0.001	0.0253	0.0228	+0.0025
HDF88	ET ^①	448	428	+20	385	468	-83	397	455	-58	425	443	-18
	ET ^②	450	419	+31	386	404	+2						
	WUE ^①	4.14	3.26	+0.88	4.36	2.79	+1.57	3.96	3.12	+0.84	4.04	3.45	+0.59
	WUE ^②	3.81	2.62	+1.19	3.95	2.87	+1.08						
	LUE ^①	0.0214	0.0161	+0.0053	0.0176	0.0137	+0.0039	0.0145	0.0131	+0.0014	0.0176	0.0157	+0.0019
HDF00	ET ^①	283	307	-24	288	234	+54	360	320	+40	364	423	-59
	ET ^②	279	273	+6	291	277	+14						
	WUE ^①	3.97	2.09	+1.89	3.48	1.96	+1.52	3.01	1.87	+1.14	2.93	1.27	+1.66
	WUE ^②	2.91	1.86	+1.05	2.8	1.75	+1.05						
	LUE ^①	0.0146	0.0083	+0.0063	0.0117	0.0053	+0.0064	0.0129	0.0071	+0.0058	0.013	0.0066	+0.0064

a Measured with N fertilization;

b ANN modeled without N fertilization;

c N effect (= *a* - *b*);

① This study;

③ Jassal et al (2010).

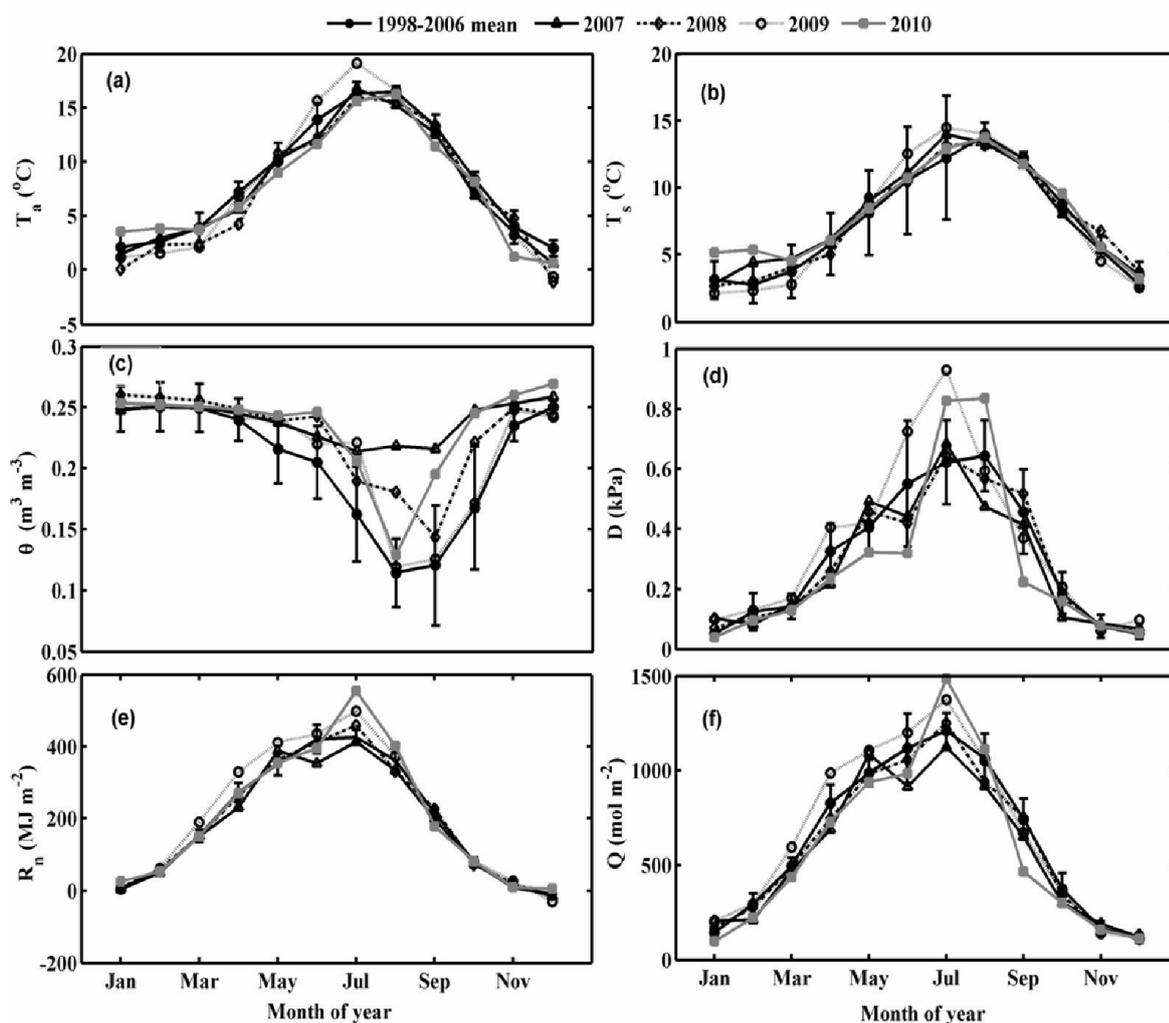


Fig. 1. Comparisons of mean monthly values of environmental variables observed at DF49 for the pre-fertilization means (1998–2006) and for the fertilized years 2007–2010. The error bars are $\pm 1SD$ for all the pre-fertilization 9-year data. (a) air temperature above the canopy (T_a), (b) 5-cm depth soil temperature (T_s), (c) 0–30 cm soil water content (θ), (d) water vapor pressure deficit(D), (e) total downwelling photosynthetically active radiation (Q) and (f) net radiation (R_n).

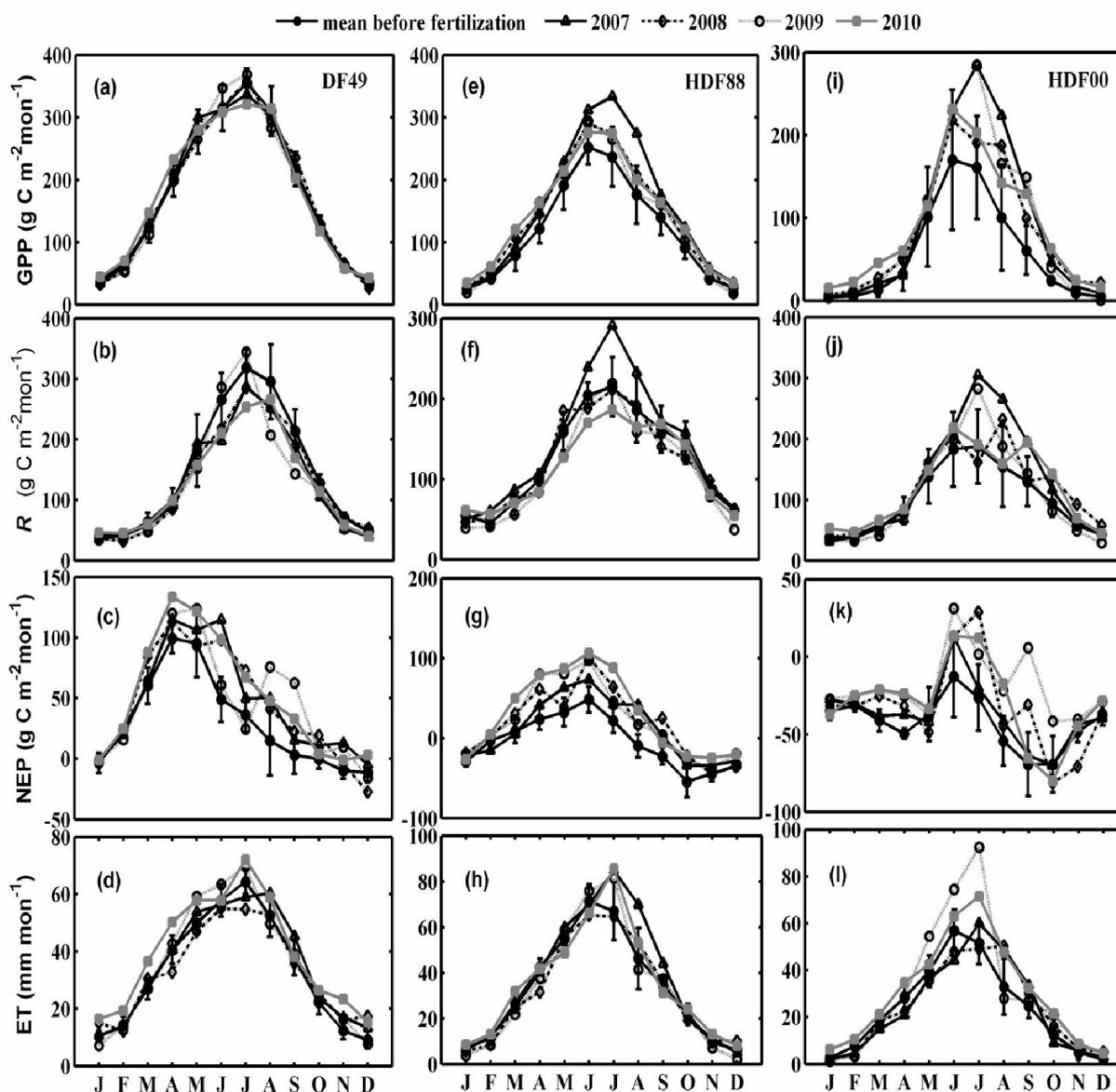


Fig. 2. Comparisons of mean monthly values of EC-measured C fluxes and ET in all three stands for the pre-fertilization means and for the post-fertilization years 2007–2010. The means of EC-measured C component fluxes and ET at DF49, HDF88 and HDF00 are during 1998 to 2006, 2002 to 2006 and 2001 to 2006, respectively. (a)–(d) for DF49, (e)–(h) for HDF88, and (i)–(l) for HDF00. The error bars are $\pm 1SD$ for all the pre-fertilization multi-year data.

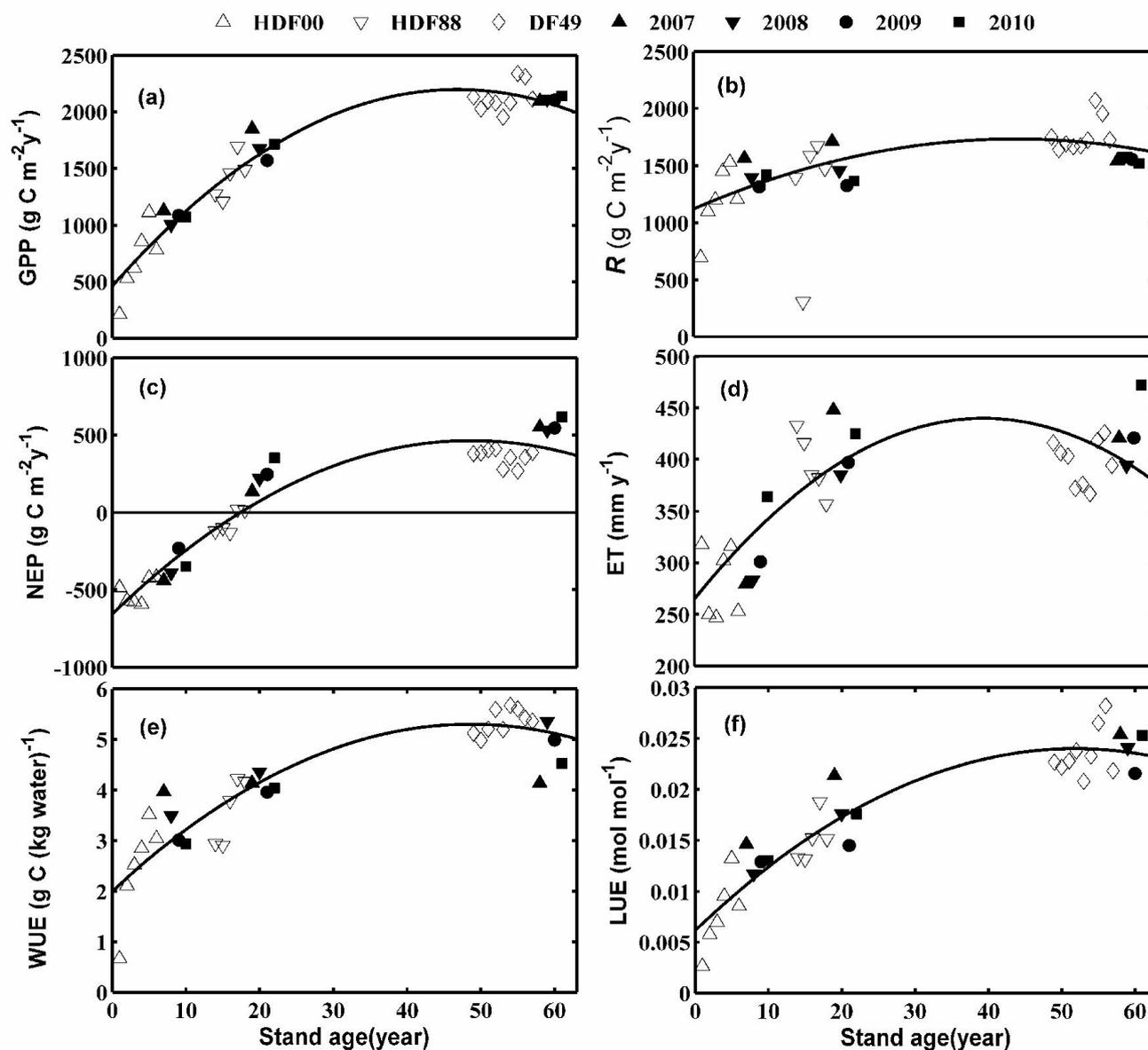


Fig. 3. Effects of N fertilization and stand age on EC-measured C component fluxes, WUE and LUE in the three West Coast Douglas-fir stands. (a) gross primary productivity (GPP), (b) ecosystem respiration (R), (c) net ecosystem productivity (NEP), (d) evapotranspiration (ET), (e) water-use efficiency (WUE) and (f) light-use efficiency (LUE).

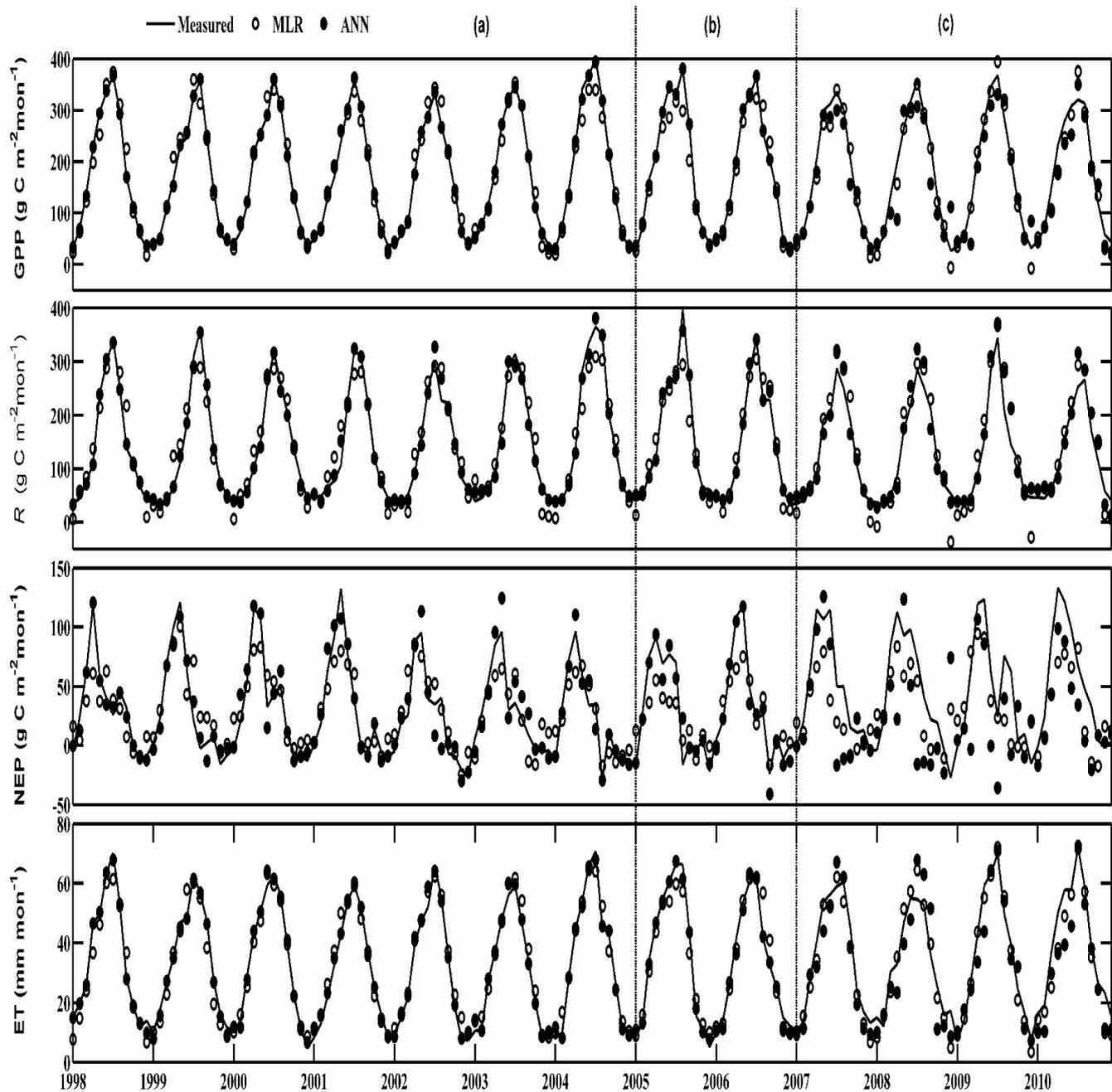


Fig. 4. ANN, MLR simulated and EC-measured monthly C component fluxes and evapotranspiration (ET) at DF49 from 1998 to 2010. (a) for the ANN and MLR calibration years 1998–2004, (b) for the model validation years 2005–2006, and (c) for the post-fertilization four years 2007–2010.

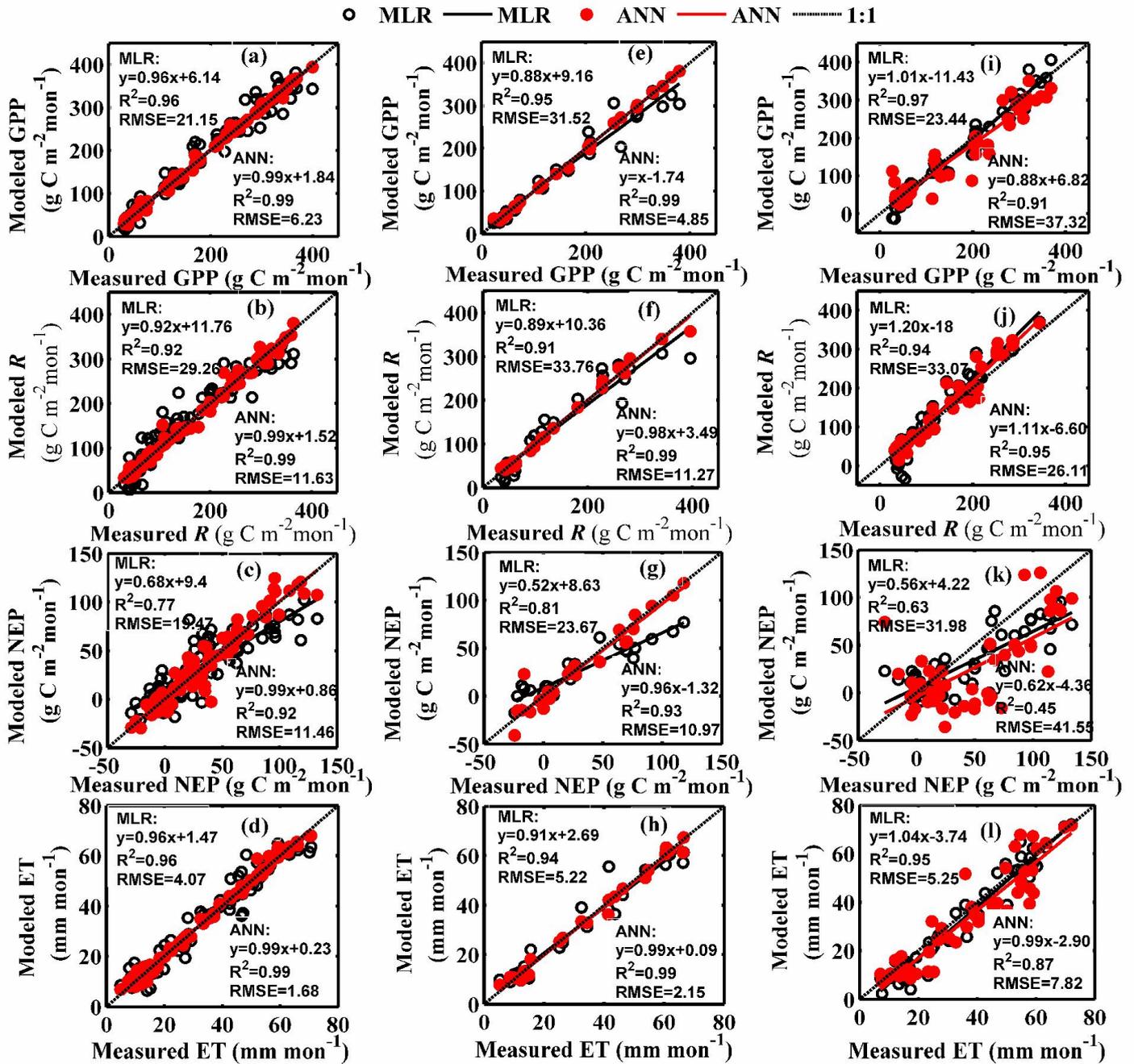


Fig. 5. Comparisons of ANN, MLR simulated and EC-measured monthly C component fluxes and evapotranspiration (ET) at DF49 from 1998 to 2010. (a)–(d) for the ANN and MLR calibration years 1998–2004, (e)–(h) for the validation years 2005–2006, and (i)–(l) for the fertilized years from 2007 to 2010.

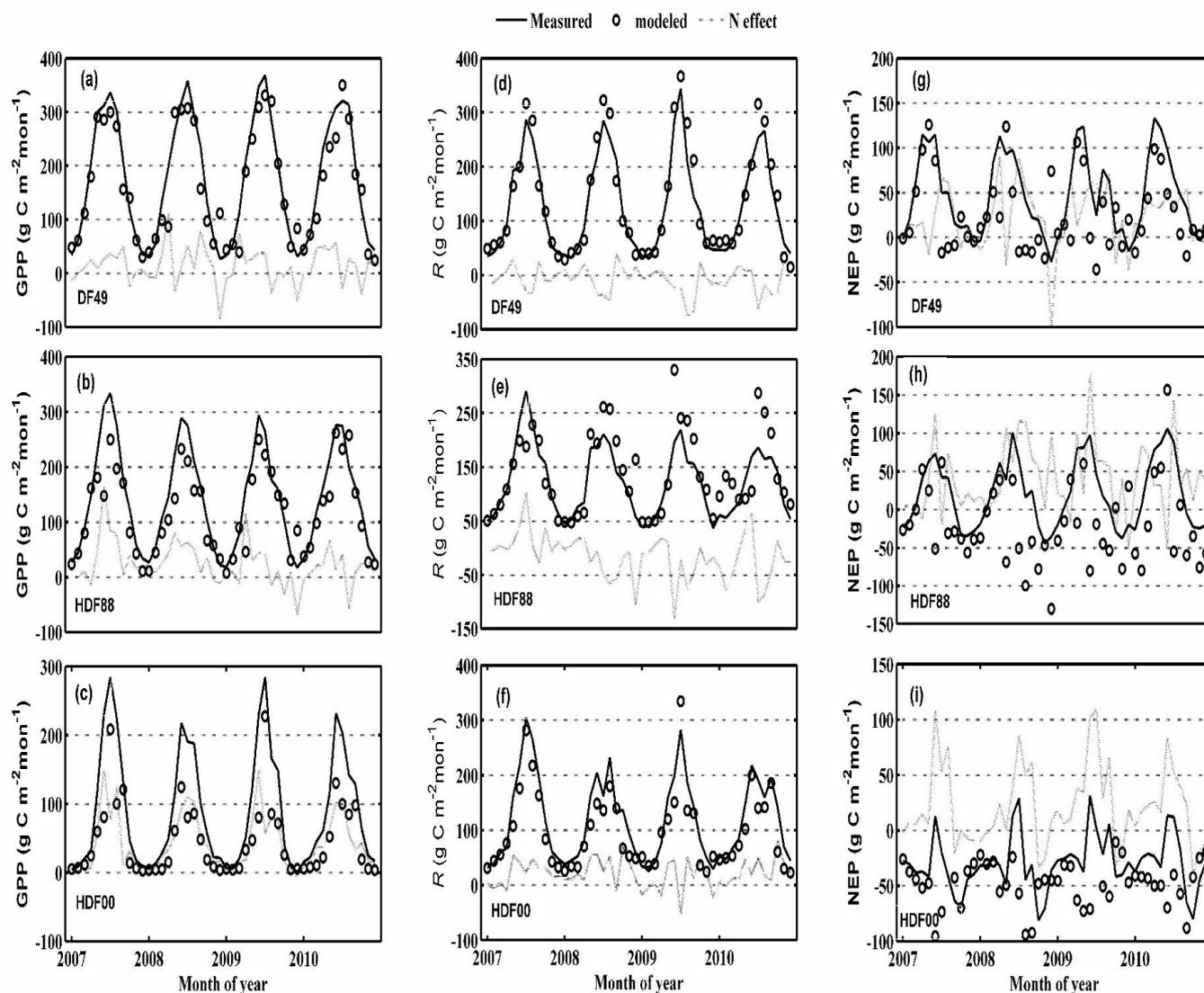


Fig. 6. ANN simulated and EC-measured monthly C component fluxes in all three stands for the post-fertilization four years 2007–2010. (a)–(c) for GPP, (d)–(f) for *R*, and (g)–(i) for NEP. The effects of N fertilization on C component fluxes were estimated as the differences between the measured C component fluxes and their corresponding modeled values, respectively.

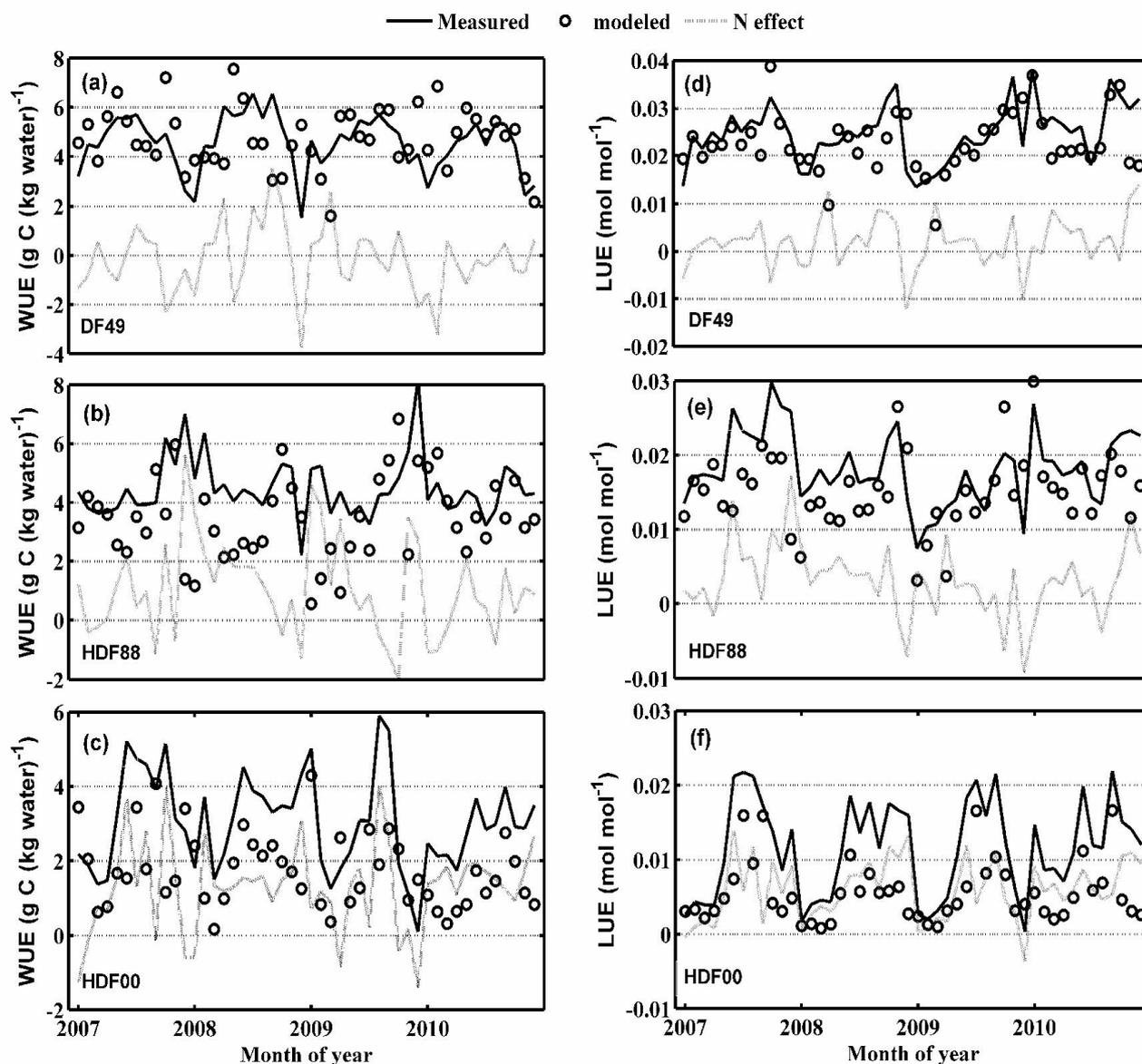


Fig. 7. ANN simulated and EC-measured monthly water-use efficiency (WUE) and light-use efficiency (LUE) in all three stands for the post-fertilization four years 2007–2010. (a)–(c) for WUE and (d)–(f) for LUE. The effects of N fertilization on WUE and LUE were estimated as the differences between the measured WUE and LUE and their corresponding modeled values.

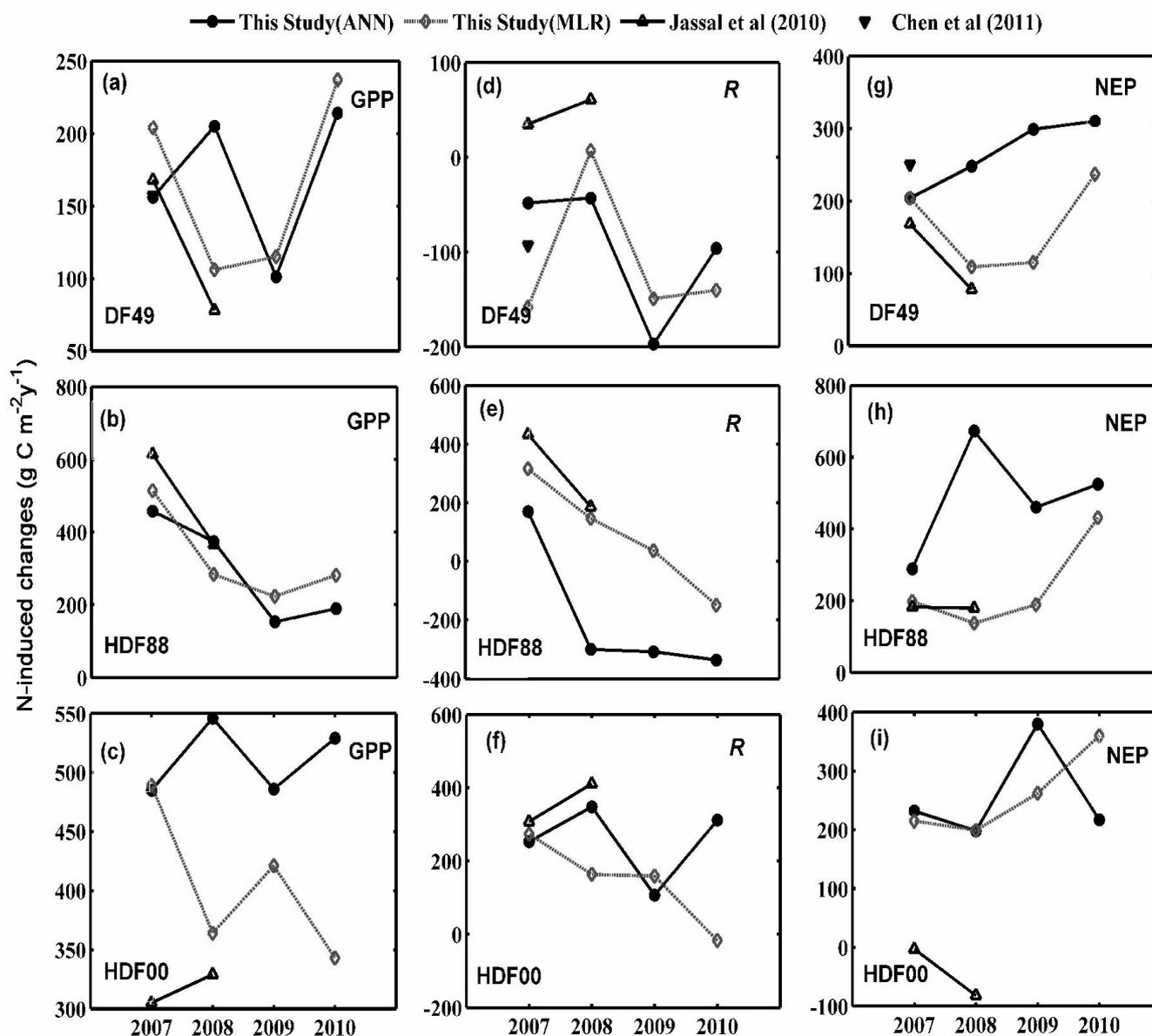


Fig. 8. N-induced changes in C component fluxes from different study results in the three West Coast Douglas-fir stands during 2007 to 2010. (a)–(c) for gross primary productivity (GPP), (d)–(f) for ecosystem respiration (*R*), and (g)–(i) for net ecosystem productivity (NEP).