

Impact of nitrogen fertilization on carbon and water fluxes

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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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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, HDF11 and HDF00, respectively). An 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 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, $p < 0.000$) 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 (for fertilized conditions) to quantify the effects of fertilization during the post-fertilization four years. Results showed that N fertilization increased gross primary productivity of all three stands in all four years with the largest absolute increases in the 10 yr-old stand (HDF00) followed by the 22 yr-old stand (HDF11). Ecosystem respiration increased in all four years at HDF00, but decreased over the last three years at HDF88, and over all 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 HDF88 followed by DF49. In addition, fertilization caused a small increase in annual ET in all four years at DF49; a small increase in the first year and a decrease in the next three years at HDF88; and no consistent effect at 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 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., 2009; Tian et al., 2011). In terrestrial ecosystems, the important processes 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., 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 forest ecosystems, but it remains uncertain as to how 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 annual anthropogenic C emissions could be sequestered due to the increased atmospheric N deposition in future decades. Magnani et al. (2007) found C sequestration is highly correlated with N deposition in forest ecosystems. However, there is still some controversy over the magnitude and sustainability of C sequestration resulting from N enrichment and its potential mechanisms (de Vries et al., 2008; Sutton et al., 2008; 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 (Körner, 2000) or a negative effect by altering plant and microbial communities, includ-

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ing threatened and endangered species (Fenn et al., 2003). Nadelhoffer et al. (1999) pointed out that the influence of N enrichment on C storage should be minor in forest ecosystems in the northern temperate zone. Currie et al. (2004) found indicated that small increases in C storage occurring primarily in living and dead wood might result from elevated N deposition over the next few decades. Using meta-analysis of data from global N addition experiments, Liu and Greaver (2010) concluded that the increases in short-term C sequestration below ground caused by N enrichment is due to increased C storage in the surface organic layer. Harpole et al. (2007) reported that the ability of grassland ecosystems to sequester C late in the 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 are also associated with evapotranspiration (ET) in forest and other ecosystems (Felzer et al., 2011). Water use 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 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 effect (Mitchell and Hinckley, 1993; Jassal et al., 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 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.

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Using multiple-year EC measurements in the same three stands 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 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 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.

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 motivation of this study is to try to resolve the slightly different findings in the two previous studies (Jassal et al., 2010a and Chen et al., 2011) by using the ANN approach based on the measurement of pre- and post-fertilization fluxes. The responses of C fluxes, ET, WUE and LUE to N fertilization were first investigated, and the differences among the three stands during the four years after fertilization were compared.

2 Materials and methods

2.1 Site descriptions

The 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 10yr old, which spanned the typical ages of stands in this area. The three stands were dominated by Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) with

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relatively similar stand density, soil, topography, elevation, and biogeoclimatic classification (Humphreys et al., 2006). On the whole, stand age and the 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, 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 comprised 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).

2.2 Stand fertilization

Urea fertilizer was spread aerially at 200 kgNha⁻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 and 2007, respectively. Because of the young age of the planted trees and the competing understory at HDF00, 80 g urea per tree (~ 60 kgNha⁻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 was used to continuously measure half-hourly CO₂ and water vapour fluxes since 1997, 2001 and 2000 at DF49, HDF88 and HDF00, respectively. Weather variables that were also measured included precipitation, solar and net radiation, PAR, windspeed, air temperature and humidity, soil temperature and moisture content. Details on EC instrument characteristics, measurements and calculation procedures for these stands can be found 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 details on the methodologies of gap-filling for EC-measured NEP and its separation into GPP and *R* can be found in Chen et al. (2009b).

2.4 Artificial neural network

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 ANN is conducted by 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 the 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 exchange and

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synthesis, respiration and ET, were selected to train the 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 CO_2 fluxes (GPP and R) 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 multiple linear regression (MLR) to simulate C fluxes and ET with the same variables to assess the advantages of the ANN approach over traditional regression modeling techniques. The performances of the ANN and MLR approaches were mainly evaluated by comparing predicted with measured values in the calibration and validation periods. In this study, computer programming and data analysis were performed using MATLAB (version 7.13, R2011b).

Both the ANN and MLR models were applied to simulate GPP, R and ET in all three stands. To assess the accuracy of the two models, they were calibrated using data obtained before 2005 and verified using those made during 2005–2006. We used the validated models to predict C fluxes and ET using post-fertilization climatic variables from 2007 to 2010 assuming the sites were not fertilized. Finally, we quantified the responses of the C fluxes and ET to fertilization using the differences between observed (for fertilized stands) and predicted values (for unfertilized stands).

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3 Results

Since all three stands were exposed to similar weather conditions (warm dry summers and wet cool winters), we first describe the variations of environmental variables and C fluxes and ET at DF49 at seasonal and interannual scales in the pre- and post-fertilization periods. We then quantify the effects of stand age on measured C fluxes, ET, WUE and LUE in the chronosequence. Finally, we determined the impacts of fertilization on the C fluxes (i.e., GPP and R), ET, WUE and LUE using the two modeling approaches.

3.1 Comparisons of observed environmental variables before and after fertilization at DF49

Figure 1 shows that the seasonal patterns of the environmental variables, T_a , T_s , θ , D , Q and R_n , at DF49 during 2007–2010 were similar to those measured before fertilization from 1998 to 2006. Compared with the 9 yr 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 were 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 0.23, 0.20, 0.19 and 0.20 m³ m⁻³, respectively, which were higher than the pre-fertilization 9 yr mean (0.16 m³ m⁻³), especially in 2007 which had a noticeably wet growing season (Fig. 1c).

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

Figure 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 yr were similar to the means during pre-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 mean values for March–December, especially in 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 mean 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, August and September (Fig. 2k). The monthly GPP values during 2007–2010 at HDF88 and HDF00 were higher than the mean values for the pre-fertilization periods (Fig. 2e and i), partly because these two stands were at the stage of rapid growth in their 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 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 in all three stands exhibited similar seasonal and interannual variability as GPP suggesting a strong correlation between GPP and ET irrespective of N fertilization (Fig. 2a, d, e, h, i and l).

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3.3 Variations of observed C exchange fluxes, WUE and LUE in the chronosequence

Figure 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 at DF49 remained stable over the post-fertilization period (2007–2010), and increased by about 200 g C m⁻² yr⁻¹, despite this period being cooler and wetter than pre-fertilization years (Fig. 1a and c). However, at HDF88, annual NEP consistently increased with increasing stand age (Fig. 3c).

An examination of the inter-annual variability of GPP and *R* at DF49 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 unusual climatic conditions alternating between warm and wet seasons in both years (data not shown), which may be challenging for the ANN training.

Annual LUE 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 at DF49, it remained relatively high with values ranging from 0.0208 to 0.0282 mol C mol⁻¹ photons (Fig. 3f). We found that LUE followed trends similar to GPP in all three stands between pre- and post-fertilization years regardless of the PAR level. Annual ET during the post-fertilization period was not apparently different from the previous 9 yr means in all three stands (Fig. 3d). The magnitude of annual WUE 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.

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3.4 Influences of fertilization on C fluxes

Figures 4a and 5a–c illustrate the differences between EC-measured, ANN and MLR simulated monthly GPP, R , and NEP for 1998–2004 at DF49, 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 comparing 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 $\text{gCm}^{-2}\text{month}^{-1}$ for GPP, R and NEP, respectively. These results indicate that through the trained network parameters, the ANN model achieved reasonable simulation of monthly GPP and R . Figure 4b and 5d–f illustrate the differences between EC-measured, ANN- and MLR- modeled monthly GPP, R , and NEP for the 2005–2006 validation period at DF49. The linear regression analysis comparing ANN-modeled and measured values in Fig. 5d–f shows that $R^2 = 0.99, 0.99$ and 0.93 ; RMSE = 5, 11 and 11 $\text{gCm}^{-2}\text{month}^{-1}$ for GPP, R , and NEP, respectively, which are similar to the assessment of model performance during the model calibration period 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 the values for the 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 $\text{gCm}^{-2}\text{month}^{-1}$ 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 comparing MLR-calculated and observed values in Fig. 5d–f shows that RMSE = 32, 34 and 24 $\text{gCm}^{-2}\text{month}^{-1}$ 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

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calibration period, indicating that it is difficult for the MLR model to reveal the actual interactions of C fluxes and climatic variables.

Furthermore, for the two younger stands, results of 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 the ANN model was clearly superior to the MLR model, in the rest of this section we discuss only use the ANN approach together with EC measurements to ascertain the responses of C component fluxes and ET and to N fertilization.

For the post-fertilization period, ANN modeled monthly values of GPP were lower than measurements at DF49 (Fig. 5g, $R^2 = 0.91$), but were higher for R (Fig. 5h, $R^2 = 0.95$), and consequently, predicted monthly values of NEP were lower than observed values (Fig. 5i, $R^2 = 0.45$). Table 2 shows the responses of C fluxes 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 at DF49 by 8% (from 1939 to 2095 $\text{gCm}^{-2}\text{yr}^{-1}$), 11% (from 1906 to 2111 $\text{gCm}^{-2}\text{yr}^{-1}$), 5% (from 2002 to 2103 $\text{gCm}^{-2}\text{yr}^{-1}$) and 11% (from 1924 to 2138 $\text{gCm}^{-2}\text{yr}^{-1}$) for 2007 to 2010, respectively, and decreased annual R by about 3% (from 1591 to 1543 $\text{gCm}^{-2}\text{yr}^{-1}$), 3% (from 1623 to 1580 $\text{gCm}^{-2}\text{yr}^{-1}$), 11% (from 1755 to 1558 $\text{gCm}^{-2}\text{yr}^{-1}$) and 6% (from 1616 to 1520 $\text{gCm}^{-2}\text{yr}^{-1}$), respectively. As a result, N-addition increased annual NEP by about 59% (from 348 to 552 $\text{gCm}^{-2}\text{yr}^{-1}$), 88% (from 283 to 531 $\text{gCm}^{-2}\text{yr}^{-1}$), 122% (from 246 to 545 $\text{gCm}^{-2}\text{yr}^{-1}$) and 101% (from 308 to 618 $\text{gCm}^{-2}\text{yr}^{-1}$) for 2007 to 2010, respectively, and showed a steady increase in annual NEP from 2007 to 2010.

Furthermore, at HDF88, fertilization resulted in greater relative increases in annual GPP than at DF49 with values of 32.9% (from 1393 to 1851 $\text{gCm}^{-2}\text{yr}^{-1}$), 29% (from 1302 to 1675 $\text{gCm}^{-2}\text{yr}^{-1}$), 11% (from 1419 to 1572 $\text{gCm}^{-2}\text{yr}^{-1}$) and 12% (from 1527 to 1716 $\text{gCm}^{-2}\text{yr}^{-1}$) for 2007 to 2010, respectively (Table 2). Annual R at HDF88 increased by 11% (from 1544 to 1714 $\text{gCm}^{-2}\text{yr}^{-1}$) in 2007, but decreased by 17% (from

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1757 to 1457 $\text{gCm}^{-2}\text{yr}^{-1}$), 19 % (from 1634 to 1326 $\text{gCm}^{-2}\text{yr}^{-1}$) and 20 % (from 1701 to 1365 $\text{gCm}^{-2}\text{yr}^{-1}$) for 2008 to 2010, respectively. This caused annual NEP increases from -151 to 137 $\text{gCm}^{-2}\text{yr}^{-1}$, -455 to 218 $\text{gCm}^{-2}\text{yr}^{-1}$, -215 to 246 $\text{gCm}^{-2}\text{yr}^{-1}$ and -174 to 351 $\text{gCm}^{-2}\text{yr}^{-1}$ for 2007 to 2010, respectively.

5 For 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 $\text{gCm}^{-2}\text{yr}^{-1}$), 34 % (from -589 to -391 $\text{gCm}^{-2}\text{yr}^{-1}$), 62 % (from -610 to -230 $\text{gCm}^{-2}\text{yr}^{-1}$) and 38 % (from -567 to -350 $\text{gCm}^{-2}\text{yr}^{-1}$) for 2007 to 2010, respectively. The results show that N
10 addition led to the greatest increases in GPP at HDF00 during the post-fertilization four years, followed in decreasing order by HDF88 and DF49. However, it caused the greatest increases in NEP at HDF88.

3.5 Influences of N fertilization on ET, WUE and LUE

15 Figures 6 and 7 and Table 1 show the differences between EC-measured, and ANN and MLR simulated monthly ET in the pre- and post-fertilization periods at DF49. During the calibration and validation 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 analysis of C component fluxes, we focused on describing the simulation results of the ANN approach in predicting fertilization effects on ET. Table 3 indicates the overall N effects on annual ET, WUE and LUE in
20 the post-fertilization four years during 2007 to 2010 in the three stands. There were no discernible effects of fertilization on annual ET in the post-fertilization period in all three stands. However, N addition led to substantial increases in annual WUE for HDF88 and HDF00, especially at the youngest stand (HDF00) with increases ranging from 1.14 to
25 1.89 $\text{gC}(\text{kg water})^{-1}$, due to the major impact on GPP compared to the small impact on ET, and remained relatively stable for both stands during the post-fertilization four years. However, N fertilization had little effect in annual WUE at DF49 due to that it

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to fertilization among the three stands was the highest in the youngest stand (HDF00), followed by the 22 yr-old stand (HDF88) (Fig. 9 and Table 2). This agrees with a positive response of LUE to fertilization of 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 HDF88, perhaps resulting from the decreasing trend in LUE (Table 3). This suggests that the response of stand productivity to N fertilization may be temporary (less than 5–10 yr), which is agrees with findings in previous studies (Footen et al., 2009; Högberg et al., 2006; Niu et al., 2009; Stegemoeller and Chappell, 1991).

4.2 Effects of N fertilization on ecosystem respiration

Our results indicate that N fertilization at DF49 reduced R by approximately $48 \text{ gC m}^{-2} \text{ yr}^{-1}$ (about 3%) in the post-fertilization first year, which is somewhat less than the $93 \text{ gC m}^{-2} \text{ yr}^{-1}$ (6%) decrease calculated by Chen et al. (2011), but disagrees with the $35 \text{ gC m}^{-2} \text{ yr}^{-1}$ increase in R at DF49 found by Jassal et al. (2010a). In addition, during the next three years (2008–2010) after fertilization at DF49, ANN modeled R remained greater than measured R by 43 to $197 \text{ gC m}^{-2} \text{ yr}^{-1}$, implying that N fertilization suppressed R . In this study, however, the increase in R due to N fertilization at 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 HDF00 continued for the remaining two years (Fig. 9 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 .

Jassal et al. (2007) found that soil respiration (R_s) accounted for about 62% R at DF49, which is similar to 67% found by Chen et al., (2011) for DF49 in the first year after 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 CO_2 flux measurements using automated- and manual-chamber systems at DF49, Jassal et al. (2010b) found that N led to a significant increase in R_s over

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about 4 months resulting from the increase in R_a accompanied by a slight decrease in R_h . Olsson et al. (2005) reported 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 in tropical forests has caused R_s to decrease, but it might change depending on rates of atmospheric N deposition. On the basis of weekly R_s measurements, Bowden et al. (2004) reported continuous 13 yr N application reduced R_s by 41 % in the plots with high soil N content during the growing season based on weekly R_s measurements.

4.3 Effects of N fertilization on C sequestration

In this study, N fertilization significantly increased NEP in the four years following fertilization of all three stands, especially for HDF88 and followed by DF49, largely due to increases in GPP and decreases in R (Fig. 9). These results are consistent with the results obtained for DF49 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) at DF49 and HDF88 in the first 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 an increase in GPP rather than R (Fig. 9). In summary, fertilization led to an 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 first three post-fertilization years but decline in the fourth year both at DF49 and HDF00 (Fig. 9 and Table 2). Niu et al. (2010), from results of a 4 yr experimental study in a temperate steppe, reported that N enrichment stimulated NEE with increases of 60 % and 21 % in the first two post-fertilization years, 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 factors such as soil acidification (Niu et al., 2010) and the slight deficiency of potassium (Jassal et al., 2010a).

In addition, in this study, N-use efficiency defined as the increase in the net C sequestration (i.e., NEP) per unit of N application is comparable with that found in previous studies. According to our results, N-use efficiency varied with stand age being the highest (33 to 63 kgC(kgN)⁻¹) for the youngest stand (HDF00), followed by the 22 yr-old stand (HDF88) (14 to 33 kgC(kgN)⁻¹) and the oldest stand (DF49 (10 to 15 kgC(kgN)⁻¹) during the four post-fertilization years. The results are consistent with the findings (about 50–75 kgC(kgN)⁻¹) obtained by Sutton et al. (2008) across 22 European forest sites taking into account the impacts of climatological differences among stands. The results from our study and Sutton et al. (2008) are similar to the findings obtained by de Vries et al. (2008) and Högberg (2007), which are significantly smaller than the assessment by Magnani et al. (2007). Furthermore, for the youngest stand (HDF00), it seems that the 60 kgNha⁻¹ application to individual trees was more efficient with respect to C sequestration than the larger application(200 kgNha⁻¹) for the two older stands, consistent with the studies by Hyvönen et al. (2008).

4.4 Effects of N fertilization on ET, WUE and LUE

Our study demonstrated that N application had no significant effect on annual ET at DF49. N fertilization decreased ET at HDF88 in the last three years and had no clear effect on ET at HDF00 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 significantly increased WUE at HDF88 and HDF00, 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). 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.

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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 application rates. They also found 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 conditions, resulting in increased WUE and stimulating growth and biomass production, but excessive N application is not advisable. Consistent with the 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 the higher application rate 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 four post-fertilization 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 relationship 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 not significantly correlated with foliar N content during the peak growing season in forest ecosystems. Furthermore,

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Still et al. (2004) suggested that due to the effect of deciduous forests having higher foliage 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 considerable interannual variability of C component fluxes and ET in these stands which to a large extent results from inter-annual 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 experimental methods 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. According to those, however, there remains considerable uncertainty in estimating the effects of fertilization on C and water fluxes, which is shown in Fig. 9. 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 (Figs. 5 and 7). Further improvements in the use of ANNs to model these effects can possibly be made by using weekly, daily or half-hourly data.

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 was associated with stand age. In addition, fertilization decreased R in all four years in

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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 increase in the 22 yr-old stand 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 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 for calibration and verification periods for monthly gross primary productivity (GPP, $\text{gCm}^{-2}\text{yr}^{-1}$), ecosystem respiration (R , $\text{gCm}^{-2}\text{yr}^{-1}$) and evapotranspiration (ET, mmyr^{-1}).

Stand	DF49				HDF88				HDF00			
	MVMLR		MVANN		MVMLR		MVANN		MVMLR		MVANN	
Method Status	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 vs. MLR modeled; MVANN, measured vs. ANN modeled. Period 1 refers to calibration step; Period 2 refers to validation step.

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Table 2. Effects of nitrogen fertilization on annual gross primary productivity (GPP, $\text{gC m}^{-2} \text{yr}^{-1}$), ecosystem respiration (R , $\text{gC m}^{-2} \text{yr}^{-1}$) and net ecosystem productivity (NEP, $\text{gC m}^{-2} \text{yr}^{-1}$).

Stands	Flux	2007			2008			2009			2010		
		<i>a</i>	<i>b</i>	<i>c</i>									
DF49	GPP	2095	1939	+156	2111	1906	+205	2103	2002	+101	2138	1924	+214
	R	1543	1591	-48	1580	1623	-43	1558	1755	-197	1520	1616	-96
	NEP	552	348	+204	531	283	+248	545	246	+299	618	308	+310
HDF88	GPP	1851	1393	+458	1675	1302	+373	1572	1419	+153	1716	1527	+189
	R	1714	1544	+170	1457	1757	-300	1326	1634	-308	1365	1701	-336
	NEP	137	-151	+288	218	-455	+673	246	-215	+461	351	-174	+525
HDF00	GPP	1126	641	+485	1005	459	+546	1083	597	+486	1068	539	+529
	R	1566	1313	+253	1396	1048	+348	1314	1207	+107	1418	1106	+312
	NEP	-440	-672	+232	-391	-589	+198	-230	-610	+380	-350	-567	+217

a Measured with N fertilization;

b ANN modeled without N fertilization;

c N effect ($= a - b$).

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Table 3. Effects of nitrogen fertilization on annual evapotranspiration (ET, mm yr^{-1}), water use efficiency (WUE, $\text{g C (kg water)}^{-1}$) and light use efficiency (LUE, mol C mol^{-1} photons).

Stands	Flux	2007			2008			2009			2010		
		<i>a</i>	<i>b</i>	<i>c</i>									
ET	DF49	421	388	+33	394	377	+17	421	405	+16	472	382	+91
	HDF88	448	428	+20	385	468	-83	397	455	-58	425	443	-18
	HDF00	283	307	-24	288	234	+54	360	320	+40	364	423	-59
WUE	DF49	4.97	5.00	-0.03	5.35	5.06	+0.30	4.99	4.95	+0.04	4.53	5.04	-0.51
	HDF88	4.14	3.26	+0.88	4.36	2.79	+1.57	3.96	3.12	+0.84	4.04	3.45	+0.59
	HDF00	3.97	2.09	+1.89	3.48	1.96	+1.52	3.01	1.87	+1.14	2.93	1.27	+1.66
LUE	DF49	0.0254	0.0235	+0.0019	0.0241	0.0218	+0.0023	0.0216	0.0206	+0.0010	0.0253	0.0228	+0.0025
	HDF88	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	0.0146	0.0083	+0.0063	0.0117	0.0053	+0.0064	0.0129	0.0071	+0.0058	0.0130	0.0066	+0.0064

a Measured with N fertilization;

b ANN modeled without N fertilization;

c N effect (= $a - b$).

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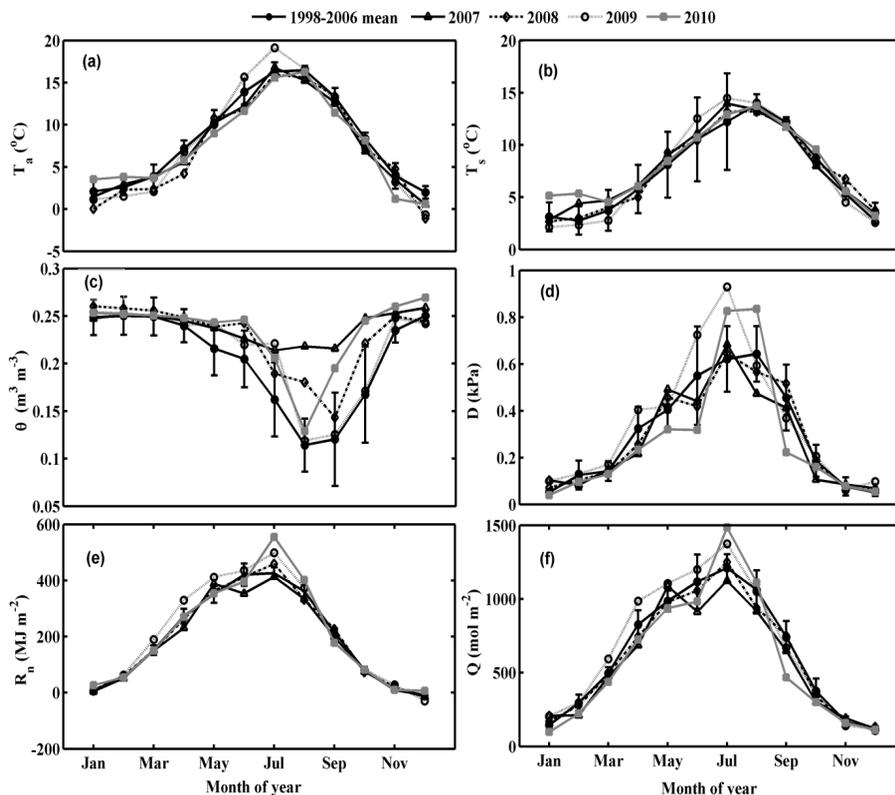


Fig. 1. Comparisons of mean monthly values of environmental variables observed at DF49 for the pre-fertilization means (1998–2006) and for the fertilized years 2007–2010. The error bars are $\pm 1SD$ for all the pre-fertilization 9 yr data. **(a)** Air temperature above the canopy (T_a), **(b)** 5 cm 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).

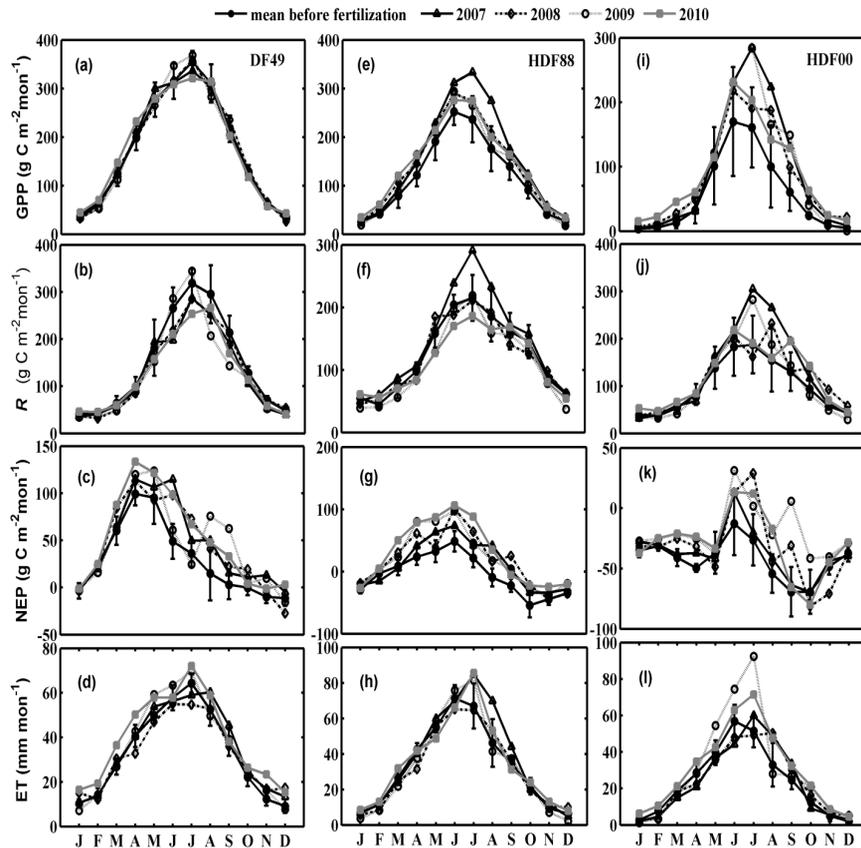


Fig. 2. Comparisons of mean monthly values of EC-measured C fluxes and ET in all three stands for the pre-fertilization means and for the post-fertilization years 2007–2010. The means of EC-measured C component fluxes and ET at DF49, HDF88 and HDF00 are during 1998 to 2006, 2002 to 2006 and 2001 to 2006,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

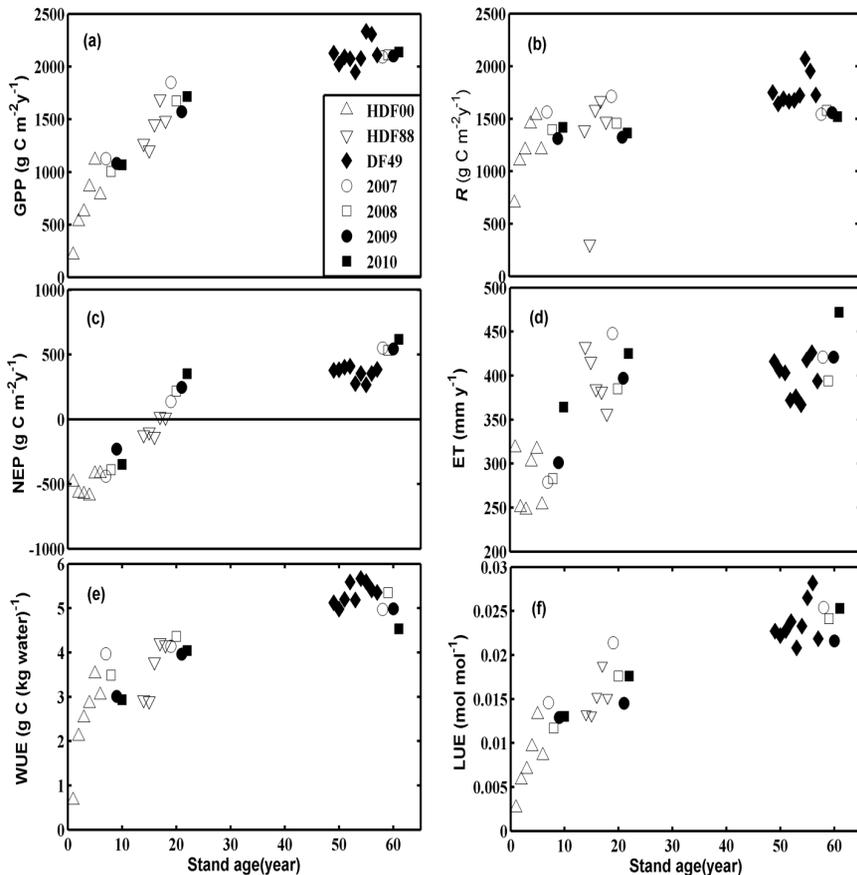


Fig. 3. Effects of N fertilization and stand age on EC-measured annual C fluxes, WUE and LUE in the three West Coast Douglas-fir stands. **(a)** Gross primary productivity (GPP), **(b)** ecosystem respiration (R), **(c)** net ecosystem productivity (NEP), **(d)** evapotranspiration (ET), **(e)** water-use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

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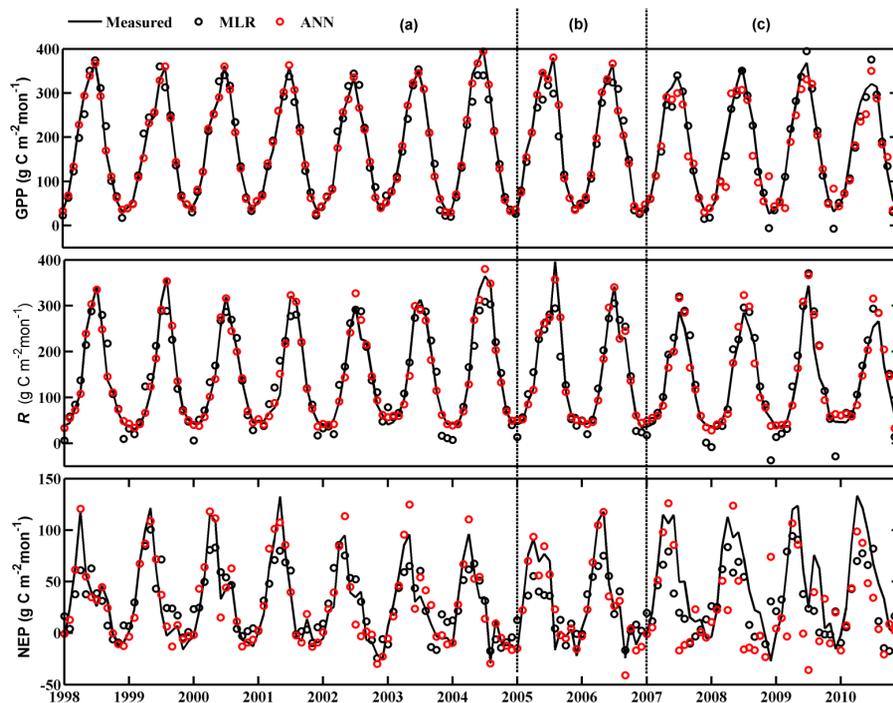


Fig. 4. ANN, MLR simulated and EC-measured monthly C component fluxes at DF49 from 1998 to 2010. **(a)** for the ANN and MLR calibration years 1998–2004, **(b)** for the model validation years 2005–2006, and **(c)** for the post-fertilization four years 2007–2010.

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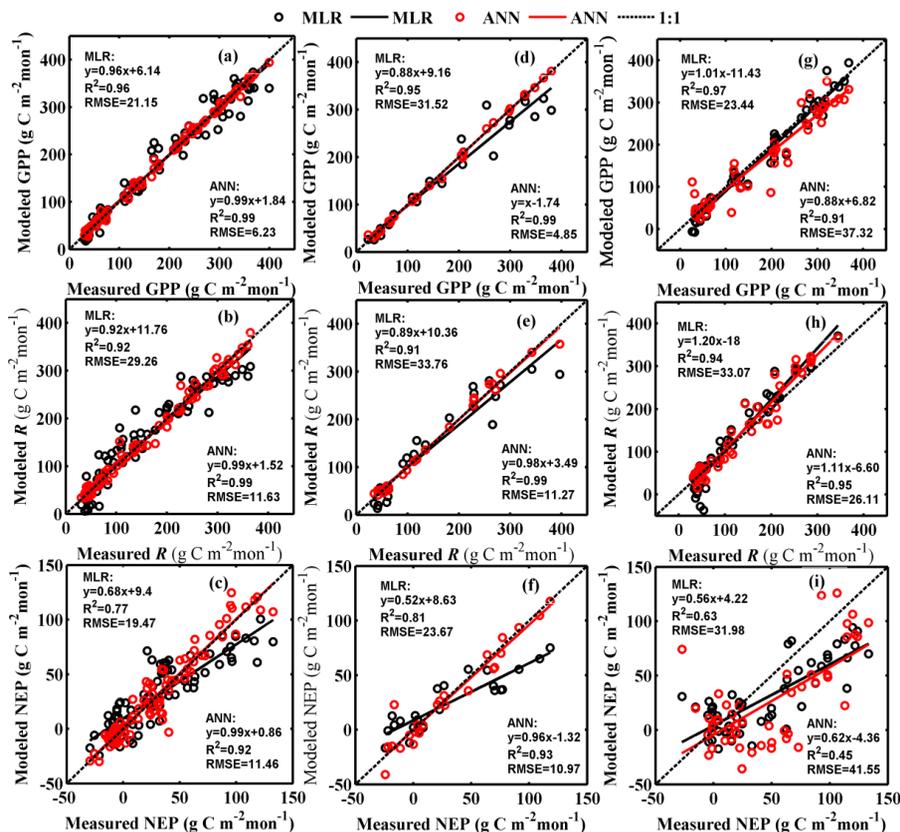


Fig. 5. Comparisons of monthly ANN, MLR simulated and EC-measured C component fluxes at DF49 from 1998 to 2010. **(a–c)** for the ANN and MLR calibration years 1998–2004, **(d–f)** for the validation years 2005–2006, and **(g–i)** for the fertilized years from 2007 to 2010.

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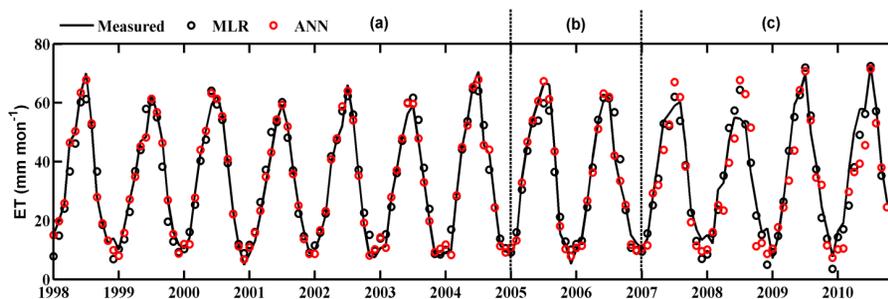


Fig. 6. ANN, MLR simulated and EC-measured monthly evapotranspiration (ET) at DF49 from 1998 to 2010. **(a)** for the ANN and MLR calibration years 1998–2004, and **(b)** for the model validation years 2005–2006, and **(c)** for the post-fertilization four years 2007–2010.

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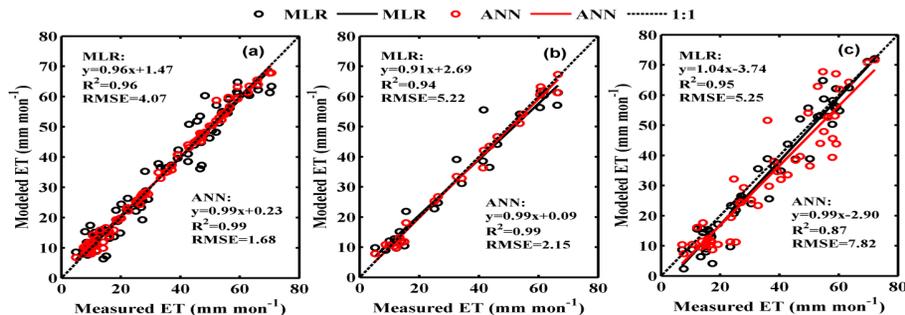


Fig. 7. Comparisons of ANN, MLR simulated and EC-measured monthly evapotranspiration (ET) at DF49 for 1998 to 2010. **(a)** for the ANN and MLR calibration years 1998–2004, **(b)** for the validation years 2005–2006, and **(c)** for the fertilized years 2007–2010.

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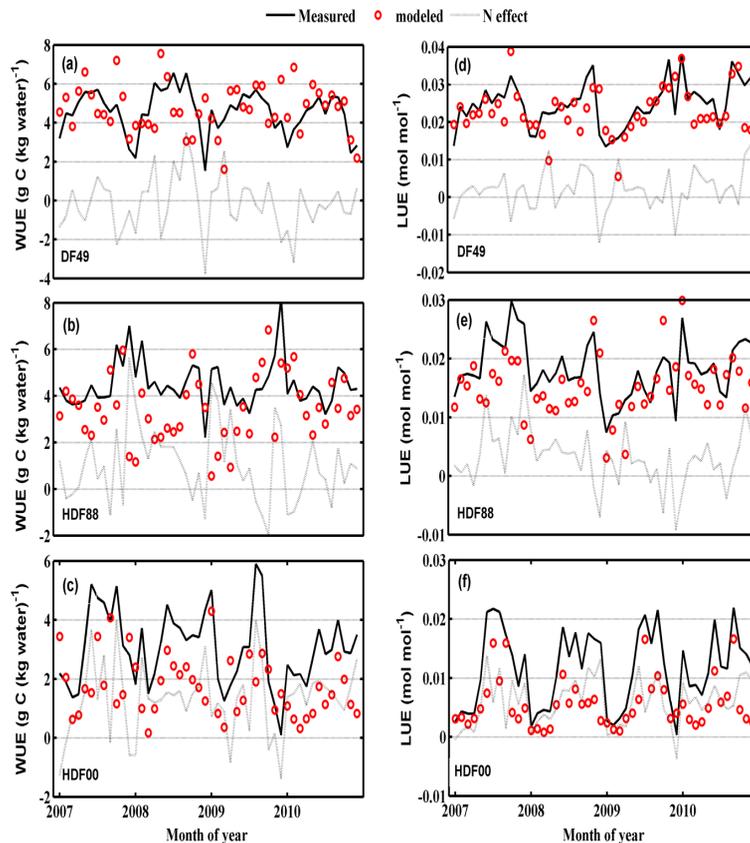


Fig. 8. ANN simulated and EC-measured monthly water-use efficiency (WUE) and light-use efficiency (LUE) in all three stands for the post-fertilization four years 2007–2010. **(a–c)** for WUE and **(d–f)** for LUE. The effects of N fertilization on WUE and LUE were estimated as the differences between the measured WUE and LUE and their corresponding modeled values.

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Interactive Discussion

Impact of nitrogen fertilization on carbon and water fluxes

X. Dou et al.

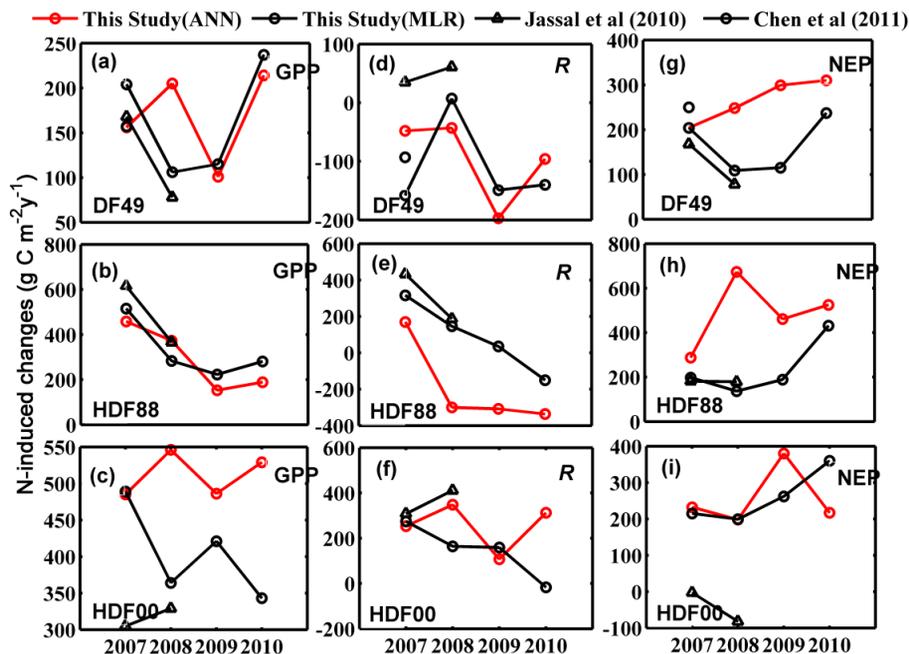


Fig. 9. N-induced changes in C fluxes from different study results in the three West Coast Douglas-fir stands during 2007 to 2010. (a–c) for gross primary productivity (GPP), (d–f) for ecosystem respiration (R), and (g–i) for net ecosystem productivity (NEP).

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