# Investigation of Ozone Stomatal Flux in a Northern Mixed Hardwood Forest during the 1999 to 2004 Growing Seasons

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## Abstract

Hourly ozone stomatal fluxes ($F_{s\_O3}$) were calculated for the University of Michigan's Biological Station (UMBS) forest in northern Michigan from June 15 to September 15 for each year from 1999 to 2004 based on a set of equations derived from a resistance analog model. Measurements key to the calculations include latent and sensible heat flux, wind speed, ambient ozone ($O_3$), and leaf air index. These measurements were taken primarily at the 46 m height of the UMBS Flux Tower on site with the exception of ambient $O_3$, taken at 35 m on the PROPHET Tower. The calculated $F_{s\_O3}$ were used to investigate the effect $O_3$ exposure might have on Net Ecosystem $CO_2$ Exchange (NEE). Analysis of NEE and $F_{s\_O3}$ required consideration of several variables known to significantly affect NEE, including photosynthetic photon flux density (PPFD), vapor pressure deficit (VPD), soil moisture, and air temperature. Statistical analyses based on these variables consistently produced results indicating that a statistically significant positive relationship exists between NEE and $F_{s\_O3}$. These results are unexpected as $F_{s\_O3}$ is expected to be negatively correlated with NEE as a result of pant damage from $O_3$ exposure, but may simply reflect the strong positive relationship known to exist between NEE and stomatal conductance.

## Introduction

There is no doubt in the scientific community that global warming is occurring. The International Panel on Climate Change (IPCC) states "There is a 90-95% chance that human activities are responsible for most of the recent warming of the Earth" (IPCC, 2001). Human activities that are impacting climate change include increased anthropogenic emissions of greenhouse gases including carbon dioxide ($CO_2$), methane ($CH_4$), nitrous oxide ($N_2O$), and chloroflurocarbons (CFCs). These increased emissions are primarily a result of the use of fossil fuels and changes in land use (IPCC, 2007).

Increased anthropogenic emissions are changing the chemistry of the atmosphere. One change of concern is the "hole" in the stratospheric ozone layer that has resulted in increased amounts of harmful ultraviolet (UV) radiation reaching the surface of the Earth (McKenzie *et al.*, 2007). Conversely, scientists have observed increasing levels of ozone ($O_3$) in the troposphere where, ironically, $O_3$ is harmful to animal and plant life (Bell *et. al*, 2007). Ozone is naturally found in the troposphere as a result of both input from the stratosphere and photochemical production through the oxidation of volatile organic compounds (VOCs) and carbon monoxide (CO) in the presence of nitrogen dioxide ($NO_2$) (e.g. Fowler *et al*., 1999). However, increased anthropogenic emissions of $O_3$ precursors such as VOCs, CO, and nitrogen oxides ($NO_x$) has upset the delicate balance between $O_3$ production and consumption that occurs in nature resulting in an overall and, most likely continuing, increase in tropospheric $O_3$.

According to Fowler *et al*., background tropospheric $O_3$ has increased in the mid-Northern latitudes from 10-20 ppbv at the beginning of the 20th century to 20-40 ppbv by the end of the century (1999). Within the past few years, $O_3$ mixing ratios at the University of Michigan Biological Station (UMBS) have ranged from approximately 25 to 80 ppbv (Hogg *et al*., 2007).

Tropospheric $O_3$ is a greenhouse gas. In addition, it is harmful to plant and animal life not only because it is a greenhouse gas but also because it is a key ingredient in smog which is toxic to many living organisms (Bell and Treshow, 2002 p.10). Tropospheric $O_3$ has been a recognized phytotoxin since as early as 1959 when Heggestad and Middleton reported the results of their fumigation studies demonstrating that elevated $O_3$ levels can result in visible foliar injury (Bell and Treshow, 2002 p.10). Today scientists know that acute exposure (120-500 ppbv) to $O_3$, in addition to causing visible foliar injury, can result in changes in cell wall structure and uncontrolled and programmed cell death. Chronic exposure (40-120 ppbv) can result in decreased photosynthesis and productivity. Both acute and chronic exposure can result in accelerated senescence and increased production of $O_3$ detoxification or active oxygen species (AOS) scavengers (Bell and Treshow, 2002 p.69).

Scientists have struggled to quantify exposure in a way that accurately assesses plant response and damage. Initially, scientists used $O_3$ concentration to assess plant response and, as a result, concentration-based damage indices and exposure-based critical levels or thresholds were developed (Musselman *et al.*, 2005). In Europe, for example, the AOT40 index, or the Accumulated exposure Over a Threshold of 40 nmol $mol^{-1}$ (40 ppbv) index, is widely used as an indicator of potential $O_3$ damage to vegetation using 40 ppbv as the threshold at which damage begins to occur (EMPE convention, 2002). However, more recently, scientists have been investigating stomatal flux as a better quantitative assessment of $O_3$ impact and as a better way to establish critical limits. Ozone stomatal flux ($F_{s\_O3}$) may be a better quantification of exposure than concentration because $F_{s\_O3}$ is the entry rate of $O_3$ through the stomata and is, therefore, a measure of $O_3$ uptake. Being able to quantify $O_3$ uptake is desirable and beneficial because damage to vegetation is thought to be primarily a result of $O_3$ uptake into the leaves Karrlson *et al.*, 2007). However, some research suggest that total $F_{s\_O3}$ may not accurately reflect the amount of damage being caused by $O_3$ uptake because some of the $O_3$ taken into the leaves is may be removed or detoxified before damage can be inflicted (Loreto and Fares, 2007). Therefore, it is suggested that perhaps an effective flux instead of a total flux must be calculated in order to better access the amount of damage that actually occurs as a result of $O_3$ uptake.

In the current study, hourly $F_{s\_O3}$ is calculated for the 1999 to 2004 growing seasons (June 15 to September 15) to better understand the impact of $O_3$ exposure on a northern mixed hardwood forest located at the University of Michigan Biological Station in northern Michigan. The relationship between $F_{s\_O3}$ and Net Ecosystem $CO_2$ Exchange (NEE) is investigated to ask the question what impact ozone exposure might have on photosynthesis and respiration in the UMBS forest? The possible effect of $F_{s\_O3}$ on NEE is complicated by several variables that are know to influence NEE. These variables include air temperature, soil moisture, vapor pressure deficit, and photosynthetic photon flux density (PPFD) or light. Therefore, the influence of these variables on NEE must also be considered before any conclusion may be drawn about the effect of $F_{s\_O3}$ on NEE. After the effects of the confounding variables are removed it is predicted that during times of increased $F_{s\_O3}$, NEE will become less negative as a result of increased $O_3$ damage in the leaves causing decreased photosynthesis and productivity.

## Material and Methods

### *Site Description*

The UMBS site in northern Michigan (45°30'N, 84°42'W) is a "mixed" or "transition" forest dominated by aspen but also contains appreciable amounts of white pine, red oak, red maple, and paper birch. The forest is located on an outwash plain with an approximate elevation of 238 m and an average canopy height of 22 m (Hogg *et al*., 2007). The mean annual temperature (1942-2003) for the site is 5.5°C with an average annual rainfall of 817 mm (Curtis *et al*., 2005). Nearby sources of substantial pollution and possible ozone-forming emissions include Chicago (over 400 km to the southwest, 2000 metro area population 9,157,540), Detroit (~ 350 km to the southeast, 2000 metro area population 5,456,428) (U.S. Census Bureau, 2001), Toronto, Ontario (over 400 km to the east-southeast, 2001 metro population 4,682,897) and Sault St. Marie, Ontario (~ 130 km to the north, 2001 metro population of 78,908) (Statistics Canada, 2002). For more information on the UMBS site please refer to Curtis *et al*. (2005) and Carroll *et al*. (2001).

### *Instrumental Methods*

The primary measurements taken for the calculations in this experiment were performed at the 50 m tall UMBS flux tower. At this tower, measurements are taken year-round at a height of 46 m or approximately twice the height of the canopy. The UMBS flux tower instruments include a sonic anemometer (model CSAT-3, Campbell Scientific) to measure three-dimensional turbulent velocities, a closed path infrared gas analyzer (IRGA model Li-6262, LiCor) to measure $CO_2$ concentrations, and a pressure transducer (Vaisala PTB101B) for air pressure measurements (FLUXNET, 2007).

Ambient ozone mixing ratio ($\chi$) measurements used in the calculations were made at the PROPHET tower approximately 132 m south-southwest of the UMBS flux tower. The ozone detector at the PROPHET tower (TEI 49C, Thermo Environmental Instruments, Inc., USA) sampled ambient ozone levels at 35 m (Hogg *et al*., 2007).

*Calculation of Ozone Stomatal Flux*

To begin, the hourly stomatal conductance of water vapor in the canopy was calculated based on a simple resistance analog model for gas transfer and uptake demonstrated in the diagram below.

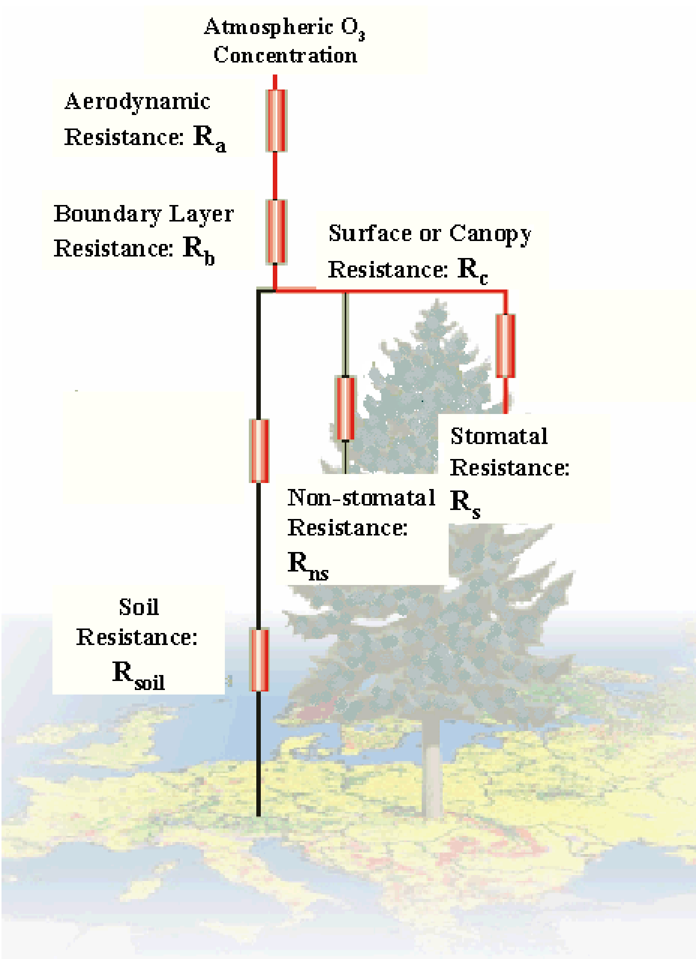


**Diagram 1:** The resistance analog model of gas transfer differentiates between the types of resistance ozone and other molecules or particles experience in the atmosphere and at the surface.

Using the resistance analog model, the total resistance ($R_{tot}$) associated with the canopy is divided into three main sections: aerodynamic or turbulent layer resistance ($R_a$), the boundary layer resistance ($R_b$), and the canopy or surface resistance ($R_c$). As seen in Diagram 1, $R_c$ consist of both the stomatal ($R_s$)

and non-stomatal ($R_{ns}$) resistances in parallel and the total resistance then becomes the sum of three resistances main resistances:

$R_{tot} = R_a + R_b + R_c$ (1)

The inverse of the total canopy resistance (Equ.1) is equal to the total conductance of the canopy ($g_{tot}$) as follows:

$$g_{tot} = \frac{1}{R_{tot}} = \frac{1}{R_a + R_b + R_c} \qquad (2)$$

and the total canopy conductance is equal to the sum of the turbulent layer conductance ($g_a$), the boundary layer conductance ($g_b$), and the surface conductance ($g_c$).

$g_{tot} = g_a + g_b + g_c$ (3)

The $g_a$ was calculated from wind speed (u(z) ) and friction velocity (u*) at the measurement height, z.

$$g_a = \frac{1}{R_a} = \frac{(u^*)^2}{u(z)} \qquad (4)$$

Filters were applied and fluxes were not calculated when u(z) and u* were less than 0.3 m/s.

Next, the $g_b$ is calculated from the following equation

$$g_b = \left(\frac{2a}{\alpha}\right)\sqrt{\left(\frac{u(h)}{0.7w}\right)}\left[1 - \exp\left(\frac{-\alpha}{2}\right)\right] \bullet LAI \qquad (5)$$

where $a$ = 0.206 mol $m^{-2}$ $s^{-1}$ for water and $a$ = 0.378 for heat, u(h) calculated the wind speed at the top of the canopy, $w$ = 0.08 m is average leaf width, $\alpha$ = 2.5 is the dimensionless attenuation coefficient for wind speed in the canopy, and LAI is the dimensionless leaf air index estimated from litter traps. LAI estimates were typically made by the UMBS flux team every three to four weeks from the beginning to the end to of the growing season but no daily. Therefore, the LAI data was interpolated to provide LAI values for the days between direct estimations (See Appendix Figure A1).

Following the calculation of $g_b$, the bulk leaf or canopy temperature ($T_c$) was estimated from sensible heat (H) and the air temperature at the measurement height ($T_a$) to be used to calculate $g_{tot}$.

$$T_l = \frac{H}{c_p}\left(\frac{g_a + g_b}{g_a g_b}\right) + T_a \quad (6)$$

$$g_{tot-H_2O} = \frac{Ep_a}{VPD} \quad (7)$$

where $E$ is the latent heat flux, $p_a$ is the atmospheric pressure, and VPD is the vapor pressure deficit calculated from $T_a$, $T_l$, and relative humidity.

Next, assuming the canopy is dry, $g_c$ can be equated to water stomatal conductance ($g_{s\_H2O}$) because other conductance sources are considered negligible and $g_{s\_H2O}$ can be solved from:

$$g_{s\_H2O} = g_{tot} - g_a - g_b \quad (8)$$

A filter was applied to remove data when measurements were taken with a relative humidity greater than 90% and the canopy was no longer considered dry.

Once the $g_{s\_H2O}$ values were calculated, they were converted to ozone stomatal conductance ($g_{s\_O3}$) using the ratio of ozone's and water's molecular diffusivities. The $g_{s\_O3}$ were then used to with ozone mixing ratios, $\chi_{(z)}$, to calculate ozone stomatal flux ($F_{s\_O3}$):

$$F_{s\_O3} = g_{s\_O3} \cdot \chi_{(z)} \quad (9)$$

All calculations were performed in Igor 6.0 and Microsoft Excel and after the calculations were complete, the relationships between ozone stomatal flux and NEE, VPD, ambient ozone, and flow regime were explored using Igor 6.0 software.

*Data Analysis*

Multiple linear regressions were performed with NEE as the dependent variable and PPFD, VPD, air temperature, soil moisture (SM), and $F_{s\_O3}$ as independent variables. Four different procedures were used on each of the six years individually (1999-2004) and for all six years combined to produce a total of 28 separate analyses. In Procedure 1 and 2, the order in which the five independent variables were considered was not set and the SPSS statistical package ordered the independent variables based on their significance in order from the most significant to the least significant. If SPSS found no significance between NEE and an independent variable, the independent variable would be eliminated entirely from consideration. In Procedure 3 and 4, $F_{s\_O3}$ was forced to look at its relationship with NEE only after the other four independent variables had been considered Finally, Procedures 2 and 4 were distinct from Procedures 1 and 3 because two and four used PPFD data filtered to include only PPFD values greater than 500 $\mu$mol m$^{-2}$ s$^{-1}$ and Procedure 1 and 3 used unfiltered PPFD.

Igor 6.0 was used to produce an exponential fit for the NEE versus the unfiltered PPFD data. The residuals of this fit were graphed versus VPD, soil moisture, air temperature, $F_{s\_O3}$, $g_{s\_O3}$, and $g_{s\_H2O}$. Linear regressions were performed on each of the residual versus independent variable graphs. This was done for each of the years separately and for all six years combined.

**Results**

*Data*

The calculated $g_{s\_H2O}$, $g_{s\_O3}$, and $F_{s\_O3}$ versus time are plotted for June 15 to September 15 from 1999 to 2004 in Figure 2, Figure 3, and Figure 4, respectively.

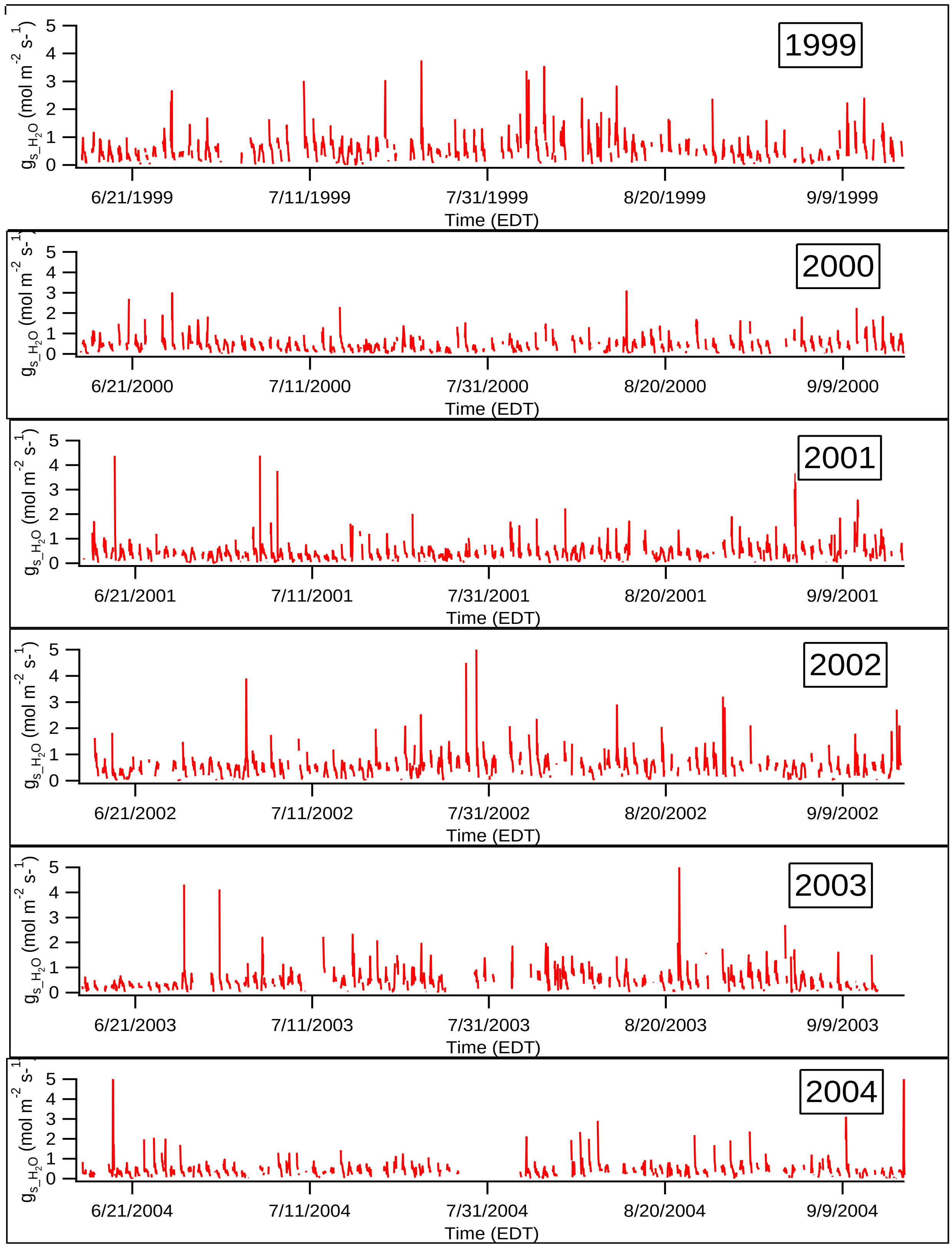


**Figure 2:** The calculated water vapor stomatal conductance ($g_{s\_H2O}$) values versus time for each year from June 15 to September 15.

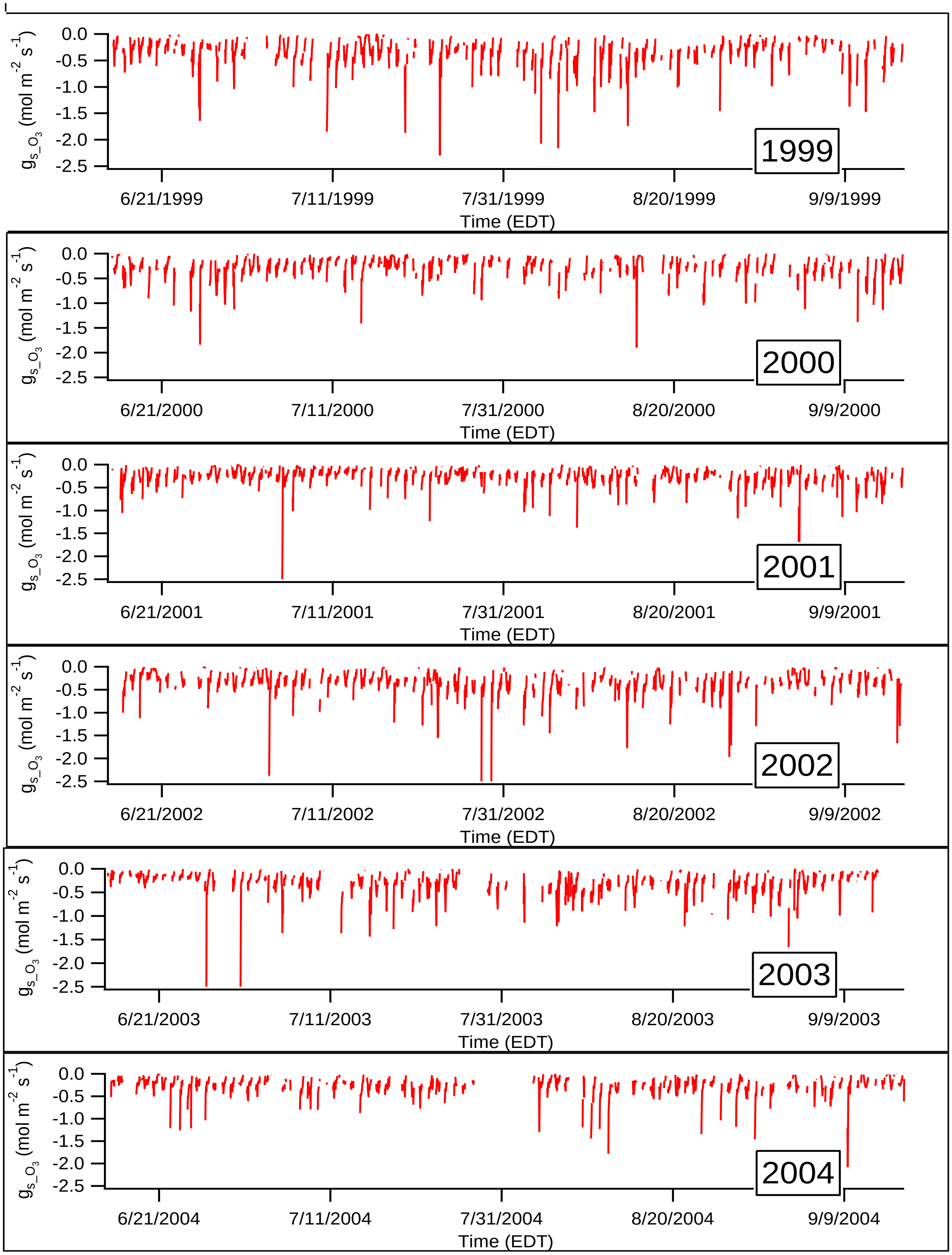

**Figure 3:** The calculated ozone stomatal conductance ($g_{s\_O3}$) values versus time for each year from June 15 to September 15.

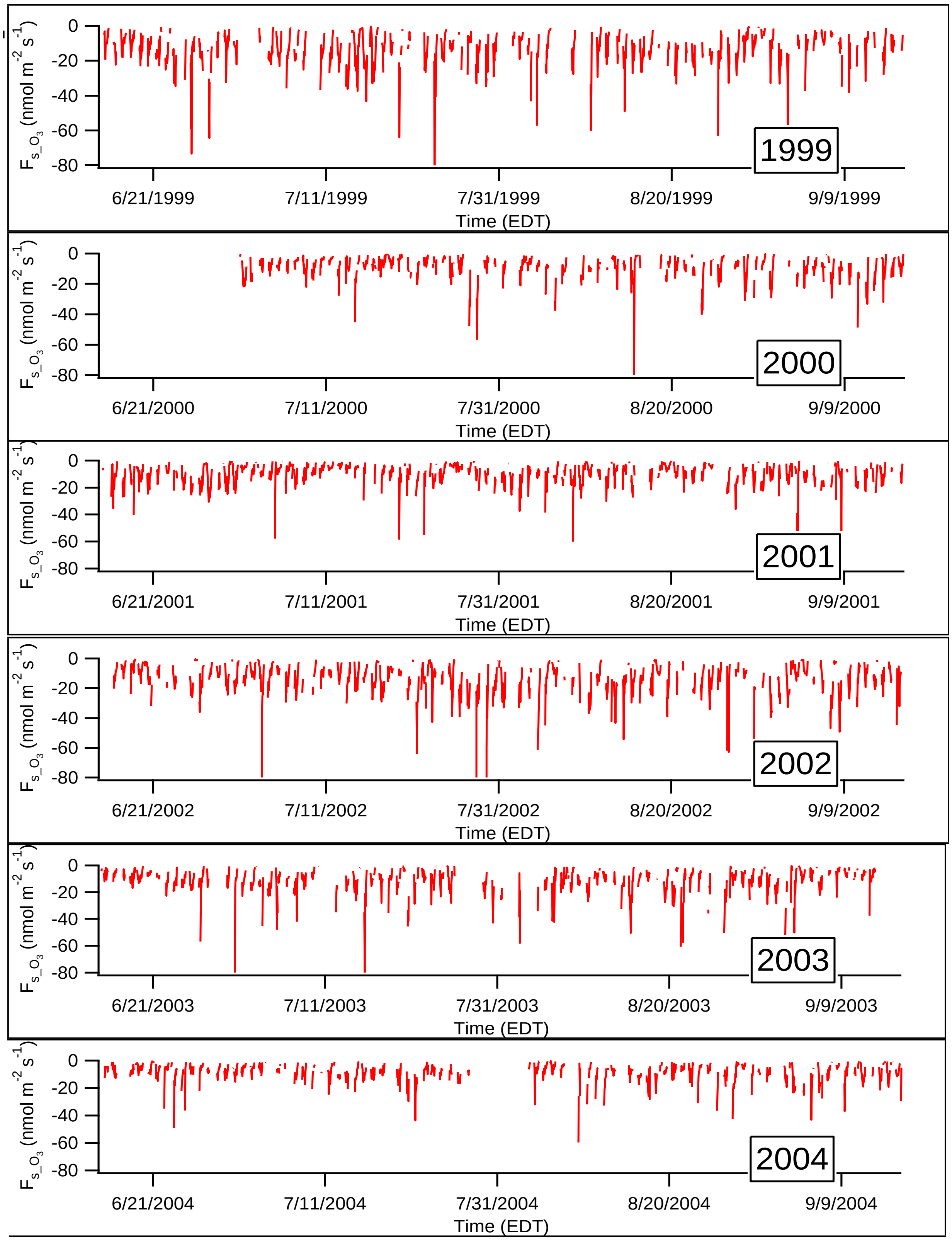


**Figure 4:** The calculated ozone stomatal flux ($F_{s\_O3}$) values versus time for each year from June 15 to September 15.

*SPSS*

When Procedure 1 was run in SPSS with no set order for any of the independent variables, $F_{s\_O3}$ was significant in each of the six years and for the combined year as seen in Table 1.

| Procedure 1: No Set Order (unfiltered PPFD) | | | | | |
|---|---|---|---|---|---|
| **Year (6/15 to 9/15)** | **N** | **$F_{s\_O3}$ Slope** | **Excluded Variables** | **$F_{s\_O3}$ Placement** | **Order of Variables** |
| 1999 | 863 | 0.101 | SM, Air Temp | 3rd | PPFD, VPD, $F_{s\_O3}$ |
| 2000 | 735 | 0.324 | Air Temp | 2nd | PPFD, $F_{s\_O3}$, VPD, SM |
| 2001 | 990 | 0.127 | SM, Air Temp | 2nd | PPFD, $F_{s\_O3}$, VPD |
| 2002 | 993 | 0.159 | SM | 2nd | PPFD, $F_{s\_O3}$, VPD, Air Temp |
| 2003 | 857 | 0.137 | SM | 2nd | PPFD, $F_{s\_O3}$, VPD, Air Temp |
| 2004 | 893 | 0.378 | none | 2nd | PPFD, $F_{s\_O3}$, VPD, Air Temp, SM |
| 1999-2004 | 5336 | 0.179 | VPD | 2nd | PPFD, $F_{s\_O3}$, SM, Air Temp |

**Table 1:** Summary of SPSS output from Procedure 1 when no set order was placed on the independent variables and PPFD was unfiltered.

In each year and in the combined years, $F_{s\_O3}$ was significant ($p<0.5$) and in six of the seven analyses $F_{s\_O3}$ was considered the second most significant independent variable. The slope of the linear regression fit line was small and positive in all seven analyses using Procedure 1 (see Table 1). Procedure 2 with PPFD>500, produced results similar to Procedure 1 as seen in Table 2.

| Procedure 2: No Set Order (PPFD >500) | | | | | |
|---|---|---|---|---|---|
| **Year (6/15 to 9/15)** | **N** | **$F_{s\_O3}$ Slope** | **Excluded Variables** | **$F_{s\_O3}$ Placement** | **Order of Variables** |
| 1999 | 660 | 0.063 | SM, Air Temp | 3rd | PPFD, VPD, $F_{s\_O3}$ |
| 2000 | 522 | 0.089 | SM, Air Temp | 2nd | PPFD, $F_{s\_O3}$, VPD |
| 2001 | 630 | 0.097 | SM, Air Temp, VPD | 2nd | PPFD, $F_{s\_O3}$ |
| 2002 | 676 | 0.104 | SM, Air Temp, VPD | 2nd | PPFD, $F_{s\_O3}$ |
| 2003 | 590 | 0.085 | SM | 3rd | PPFD, VPD, $F_{s\_O3}$, Air Temp |
| 2004 | 532 | 0.203 | none | 2nd | PPFD, $F_{s\_O3}$, Air Temp, VPD, SM |
| 1999-2004 | 3615 | 0.123 | none | 2nd | PPFD, $F_{s\_O3}$, SM, VPD, Air Temp |

**Table 2:** Summary of SPSS output from Procedure 2 when no set order was placed on the independent variables and PPFD>500 was used.

Again $F_{s\_O3}$ was significant in all seven cases and the linear fit was small and positive. However, Procedure 2 analyses placed $F_{s\_O3}$ as the second most significant variable in five of the seven cases. Procedure 3 and 4 analyses (Table 3 and 4) with the $F_{s\_O3}$ considered last also indicated that $F_{s\_O3}$ is significant with a slight positive slope in all fourteen analyses.

| Procedure 3: Ozone Stomatal Flux Forced Last (unfiltered PPFD) | | | | |
|---|---|---|---|---|
| **Year(s) (6/15 to 9/15)** | **N** | **$F_{s\_O3}$ Slope** | **Excluded Variables** | **Order of Variables** |
| 1999 | 863 | 0.098 | SM | PPFD, VPD, Air Temp, $F_{s\ O3}$ |
| 2000 | 735 | 0.250 | SM | PPFD, VPD, Air Temp, $F_{s\ O3}$ |
| 2001 | 990 | 0.111 | Air Temp | PPFD, VPD, SM, $F_{s\ O3}$ |
| 2002 | 993 | 0.135 | SM | PPFD, VPD, Air Temp, $F_{s\ O3}$ |
| 2003 | 857 | 0.106 | SM | PPFD, VPD, Air Temp, $F_{s\ O3}$ |
| 2004 | 893 | 0.464 | none | PPFD, VPD, SM, Air Temp, $F_{s\ O3,}$ |
| 1999-2004 | 5336 | 0.167 | Air Temp | PPFD, SM, VPD, $F_{s\ O3}$ |

**Table 3:** Summary of SPSS output from Procedure 3 with $F_{s\_O3}$ forced to be considered last and PPFD unfiltered.

| Procedure 4: Ozone Stomatal Flux Forced Last (PPFD > 500) | | | | |
|---|---|---|---|---|
| **Year(s) (6/15 to 9/15)** | **N** | **$F_{s\_O3}$ Slope** | **Excluded Variables** | **Order of Variables** |
| 1999 | 660 | 0.057 | none | PPFD, VPD, SM, Air Temp, $F_{s\ O3}$ |
| 2000 | 522 | 0.150 | SM; Air Temp | PPFD, VPD, $F_{s\_O3}$ |
| 2001 | 630 | 0.097 | SM, Air Temp, VPD | PPFD, $F_{s\ O3}$ |
| 2002 | 676 | 0.091 | SM | PPFD, Air Temp, VPD, $F_{s\_O3}$ |
| 2003 | 590 | 0.061 | SM | PPFD, VPD, Air Temp, $F_{s\ O3}$ |
| 2004 | 532 | 0.378 | none | PPFD, VPD, Air Temp, SM, $F_{s\_O3,}$ |
| 1999-2004 | 3615 | 0.118 | none | PPFD, SM, VPD, Air Temp, $F_{s\_O3}$ |

**Table 4:** Summary of SPSS output from Procedure 4 with $F_{s\_O3}$ forced to be considered last and PPFD>500.

In a separate test, the $F_{s\_O3}$ was binned and analyzed separately for values greater then -40 nmol $m^{-2}$ $s^{-1}$ and lower than -40 nmol $m^{-2}$ $s^{-1}$. No significant correlation existed between NEE and $F_{s\_O3}$>-40 nmol $m^{-2}$ $s^{-1}$ or $F_{s\_O3}$ >-40 nmol $m^{-2}$ $s^{-1}$.

*Igor*

The residual versus independent variable plots in Igor confirmed the SPSS results. The plots of the residuals versus $F_{s\_O3}$ consistently produced fits that had small positive slopes. The plots of the residuals versus $g_{s\_H2O}$ and $g_{s\_O3}$ appeared similar to the $F_{s\_O3}$ plots with the $g_{s\_O3}$ fits producing small positive slopes and $g_{s\_H2O}$ producing small negative slopes.

*Cumulative $O_3$ Burden*

The cumulative $O_3$ burden and cumulative NEE were calculated yearly for the period of June 15 to September 15 in 1999 and 2001-2004 and are seen in Table 5. The averages over the five year are

-19.1 ± 3.7 kg $O_3$ $ha^{-1}$ and -4.41 ± 0.29 Mg C $ha^{-1}$ and total accumulations are -95.4 kg $O_3$ $ha^{-1}$and -22.0 Mg C $ha^{-1}$. Cumulative $O_3$ burden and NEE for 2000 are not included because ambient $O_3$ measurements for June 15 to June 30 were not available making it impossible to calculate $F_{s\_O3}$ for this period of time.

| Year (6/15 to 9/15) | Cumulative $O_3$ Burden (kg $O_3$ $ha^{-1}$) | Cumulative NEE w/ Burden (Mg C $ha^{-1}$) | N | Annual $NEP_B$ (Mg C ha-1 yr-1) | Annual $NEP_M$ (Mg C $ha^{-1}$ $yr^{-1}$) |
|---|---|---|---|---|---|
| 1999 | -20.4 | -4.39 | 864 | -0.96 | -1.67 |
| 2001 | -19.4 | -4.13 | 1086 | -1.98 | -0.8 |
| 2002 | -23.7 | -4.88 | 1003 | -1.34 | -1.72 |
| 2003 | -18.3 | -4.35 | 914 | -1.56 | -1.77 |
| 2004 | -13.5 | -4.28 | 894 | not available | not available |
| Average | -19.1 ± 3.7 | -4.41 ± 0.29 | — | -1.46 ± 0.43 | -1.49 ± 0.46 |
| Total | -95.4 | -22.0 | 4761 | -5.84 | -5.96 |

**Table 5:** Calculated cumulative $O_3$ and NEE (1999; 2001-2004, 6/15-9/15)compared to annual $NEP_B$ and $NEP_M$ values taken from Gough *et al.* (in press).

Cumulative $O_3$ and NEE were also calculated yearly for July 1 to September 15 as seen in Table 6. The averages over this period are -15.1 ± 3.5 kg $O_3$ $ha^{-1}$ and -3.48 ± 0.29 Mg C $ha^{-1}$, and total accumulations are -90.7 kg $O_3$ $ha^{-1}$ and -20.9 Mg C $ha^{-1}$. The plot of cumulative NEE versus cumulative $O_3$ showed no significant correlation.

| Year (7/1 to 9/15) | Cumulative $O_3$ Burden (kg $O_3$ $ha^{-1}$) | Cumulative NEE w/ Burden (Mg C $ha^{-1}$) | N | Annual $NEP_B$ (Mg C $ha^{-1}$ $yr^{-1}$) | Annual $NEP_M$ (Mg C $ha^{-1}$ $yr^{-1}$) |
|---|---|---|---|---|---|
| 1999 | -16.5 | -3.39 | 691 | -0.96 | -1.67 |
| 2000 | -11.9 | -3.34 | 763 | -1.79 | -1.58 |
| 2001 | -15.3 | -3.27 | 896 | -1.98 | -0.8 |
| 2002 | -20.7 | -4.03 | 833 | -1.34 | -1.72 |
| 2003 | -15.4 | -3.54 | 744 | -1.56 | -1.77 |
| 2004 | -11.0 | -3.27 | 699 | not available | not available |
| Average | -15.1 ± 3.5 | -3.48 ± 0.29 | — | -1.53 ± 0.40 | -1.51 ± 0.40 |
| Total | -90.7 | -20.9 | 4626 | -7.63 | -7.54 |

**Table 6:** Calculated cumulative $O_3$ and NEE (1999-2004, 7/1-915) compared to annual $NEP_B$ and $NEP_M$ values taken from Gough *et al.* (in press).

The peak growing season was also broken into two halves with the early growing season represented by June 16 to July 31 and the later growing season running from August 1 to September 15. The early growing season data in Table 7 have an average cumulative $O_3$ burden of -10.0 ± 2.4 kg $O_3$ $ha^{-1}$ and NEE accumulation of -2.5 ± 0.1 Mg C $ha^{-1}$. Total accumulations for the early growing season are -50.0 kg $O_3$ $ha^{-1}$ and -12.47 Mg C $ha^{-1}$ over five years again excluding 2000. The late growing season (Table 8) average $O_3$ burden is -8.8 kg $O_3$ $ha^{-1}$and the average NEE accumulation is -1.87 Mg C $ha^{-1}$. Late growing season total accumulations are -52.5 kg $O_3$ $ha^{-1}$and -11.23 Mg C $ha^{-1}$.

| Year (6/15 to 7/31) | Cumulative $O_3$ Burden (kg $O_3$ $ha^{-1}$) | Cumulative NEE w/ Burden (Mg C $ha^{-1}$) | N |
|---|---|---|---|
| 1999 | -11.8 | -2.56 | 485 |
| 2001 | -10.1 | -2.31 | 580 |
| 2002 | -12.4 | -2.70 | 552 |
| 2003 | -9.3 | -2.42 | 469 |
| 2004 | -6.4 | -2.48 | 449 |
| Average | -10.0 ± 2.4 | -2.5 ± 0.1 | —— |
| Total | -50.0 | -12.47 | 2535 |

**Table 7:** Calculated cumulative $O_3$ and NEE (1999; 2001-2004, 6/15-7/31).

| Year (8/1 to 9/15) | Cumulative $O_3$ Burden (kg O3 ha-1) | Cumulative NEE w/ Burden (Mg C ha-1 yr-1) | N |
|---|---|---|---|
| 1999 | -8.6 | -1.83 | 379 |
| 2000 | -7.1 | -1.68 | 415 |
| 2001 | -9.3 | -1.82 | 506 |
| 2002 | -11.3 | -2.19 | 451 |
| 2003 | -9.0 | -1.93 | 445 |
| 2004 | -7.1 | -1.80 | 445 |
| Average | -8.8 ± 1.6 | -1.87 ± 0.17 | —— |
| Total | -52.5 | -11.23 | 2641 |

**Table 8:** Calculated cumulative $O_3$ and NEE (1999-2004, 8/1-9/15)

Plots of average cumulative NEE versus average $O_3$ burden were created from the data in Tables 5, 6, 7, and 8. There was no correlation between NEE and $O_3$ burden during the early growing season, the entire peak growing season or the peak growing season beginning on July 1. However, the linear regression for average NEE and $O_3$ burden during the late growing season in Figure 5 is significant, with a p-value less than 0.05.

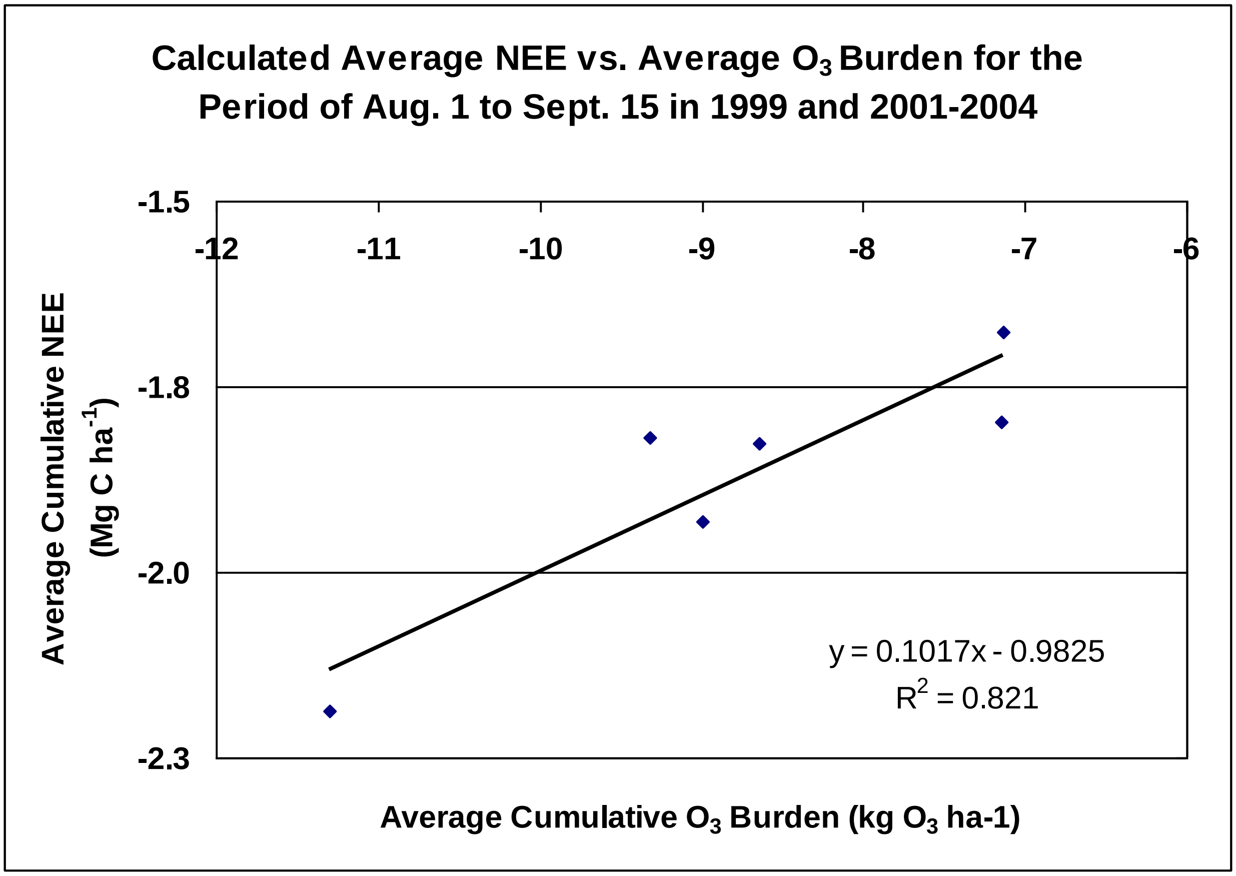


**Figure 5:** The average NEE and cumulative $O_3$ burden were calculated from hourly averaged $F_{s\_O3}$ and NEE. The best fit line of the linear regression is present as the black line.

**Discussion**

The $F_{s\_O3}$ were calculated as hourly averages based on the time scale of the available UMBS Flux data. The peak growing season was set for June 15 to September 15 because these dates occur after full leaf-out is achieved and prior to Fall leaf senescence. Therefore, by limiting the data to the peak growing season, influences on NEE due to significant changes in LAI were eliminated (See Appendix Figure A1). The data set was limited further in the SPSS analysis by limiting the PPFD data to times when the measurement was greater than 500 μmol $m^{-2}$ $s^{-1}$. This limitation was investigated because it allowed the data to be examined during periods when light availability was not a limiting factor for photosynthesis and NEE. When PPFD was limited, $F_{s\_O3}$ remained significant but the sample sizes decreased by approximately 32%.

In all 28 tests in SPSS with PPFD, VPD, soil moisture, air temperature, and $F_{s\_O3}$ as independent variables, $F_{s\_O3}$ demonstrated a significant positive correlation with NEE. This result was unexpected because the positive correlation suggests that as the $F_{s\_O3}$ becomes more negative, indicating more $O_3$ is entering the leaves, NEE also become more negative and carbon storage increases. However, as stated earlier, $O_3$ exposure in lab studies has been demonstrated to result in decreased photosynthesis and productivity. Consequently, if any correlation does in fact exist between $F_{s\_O3}$ and NEE, a negative correlation would be expected. Interestingly, when the residuals of the NEE versus PPFD exponential fit were analyzed in conjunction with $F_{s\_O3}$ the SPSS positive correlation was supported without exception.

Another interesting result of the SPSS analysis was that soil moisture was eliminated as a significant contributor to NEE in 16 of 28 test. This result suggest that the forest at the UMBS site was not generally water stressed during the 1999-2004 growing seasons. In contrast, VPD was eliminated in only four of the 28 cases. Finally, air temperature was eliminated in 11 of 14 cases. PPFD, on the other hand was not only significant in all 28 cases but it was the most significant variable in all cases. This, however, is to be expected since PPFD is a proxy for light which is the driving force behind photosynthesis.

One limitation of the SPSS analysis is that it allows only for multiple linear regressions to be performed. This is problematic because a linear regression may not be the best fit for the data in all cases. For example, the relationship of between NEE and PPFD is better fit by an exponential function. This was the main motivation for using Igor to look at the relationship between the residuals of the NEE-PPFD regression and the other independent variables (See Appendix Figures A7-A12). It may be useful in the future to use a more powerful statistical package that has the ability to perform multiple non-linear or mixed regressions to look at the data.

The calculation of cumulative $O_3$ burden and cumulative NEE was also used to explore the NEE and $F_{s\_O3}$ relationship. The values for the cumulative NEE were only for the time when there was a corresponding calculated $F_{s\_O3}$ value. Assuming that the UMBS forest site is approximately 10,000 acres or 4,050 $ha^{-1}$, then an estimated 367 Mg of $O_3$ was taken up by the UMBS forest during the peak growing season over the five years , 1999 and 2001-2004, and 386 Mg $O_3$ over all six years from July 1 to September 15. The total $O_3$ taken up by the forest during the five-year early growing seasons and six-year late growing seasons are 202 Mg $O_3$ and 213 Mg $O_3$, respectively. These estimates represent a lower limit considering that the filters imposed on the data (e.g. RH < 90%) resulted in $F_{s\_O3}$ being calculated for approximately 69-86% of the time that the stomates were expected to be open based on PPFD measurements greater than 50 μmol $m^{-2}$ $s^{-1}$.

The calculated cumulative $O_3$ burden and NEE support the SPSS and Igor results indicating that a positive relationship exists between $F_{s\_O3}$ and NEE. While the linear regression analysis of the cumulative NEE versus $O_3$ burden in three of the four categories indicate no significant relationship exists, the analysis of the late growing season data resulted in a statistically significant positive correlation similar to SPSS and Igor analyses. The positive relationship may be accounted for by the fact that the influence of the other four independent variables was not fully eliminated and is still present in some form when the $F_{s\_O3}$ and NEE connection is examined. Many of the variables that directly influence NEE also influence $g_{s\_H2O}$ from which $F_{s\_O3}$ is derived. It is well established that as the stomatal conductance increases, so does the amount of $CO_2$ that enters the stomates of the plant leading to greater carbon gain and greater negative values of NEE. Thus it is likely the observed positive relationship between NEE and $F_{s\_O3}$ reflects the expected positive relationship between NEE and stomatal conductance. However, it is possible that the strength of the positive relationship between NEE and stomatal conductance has decreased as a $F_{s\_O3}$ levels have risen from their pre-industrial lows. In this way $F_{s\_O3}$ could still be negatively influencing NEE without seeing a direct negative correlation. If this hypothesis is true and $F_{s\_O3}$ continues to rise, the positive slope of relationship between NEE and $F_{s\_O3}$ may, in the future, may actually become clearly negative (See Appendix Figure A14).

**Conclusions**

Given the limitations of the data set and environment at the time of measurement, $F_{s\_O3}$ values were calculated for approximately 69-86 % of the time $F_{s\_O3}$ is believed to have been occurring. With the data available, an estimated lower limit for the average amount of $O_3$ taken into the forest leaves at the 10,000 acre UMBS site during the peak growing season is 77 Mg per season. Statistical analysis using both SPSS and Igor repeatedly indicated that a statistically significant relationship exist between NEE and $F_{s\_O3}$. This positive relationship suggests that current levels of $F_{s\_O3}$ are not harmful to the UMBS forest but it is not known to what extent NEE may have already been reduced by $O_3$ exposure. The analysis is difficult to perform in such a way that the confounding variables that influence both NEE and $F_{s\_O3}$ are effectively eliminated before the relationship between them is fully considered. In addition, if the positive slope demonstrated by the linear relationship has decreased as ambient $O_3$ and, thus $F_{s\_O3}$ levels, have increased then perhaps the harmful influence of $O_3$ on the UMBS forest is being masked and will only be revealed by the increasing tropospheric $O_3$ levels expected over time.

**Recommendations For Future Work**

It is recommended that more powerful statistical analysis or other tools be used to examine the data to eliminate the influences of the confounding variables. The PnET model used to model carbon, nitrogen, and water cycling in forest ecosystems may be helpful in accomplishing this task. It is also suggested that the relationship between NEE and $F_{s\_O3}$ be more closely examined for early versus late growing season by perhaps breaking the data down into weekly or biweekly periods. The use of daily averages in place of hourly averages for analysis may also give provide transparency into the possible relationship between NEE and $F_{s\_O3}$. Finally, looking at the data with respect to flow regimes and how $F_{s\_O3}$ and NEE relate to its changes may provide a better understanding of the relationship that might exist between $F_{s\_O3}$ and NEE.

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**Appendix**

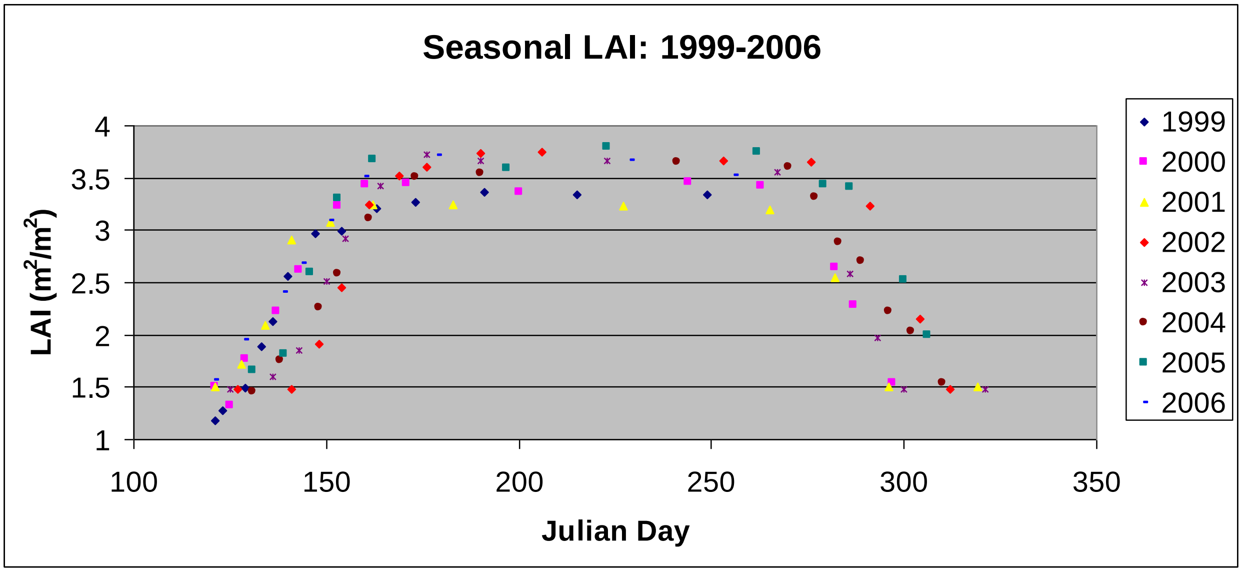


**Figure A1:** Seasonal leaf area index ($m^2/m^2$) measured from UMBS Flux litter traps.

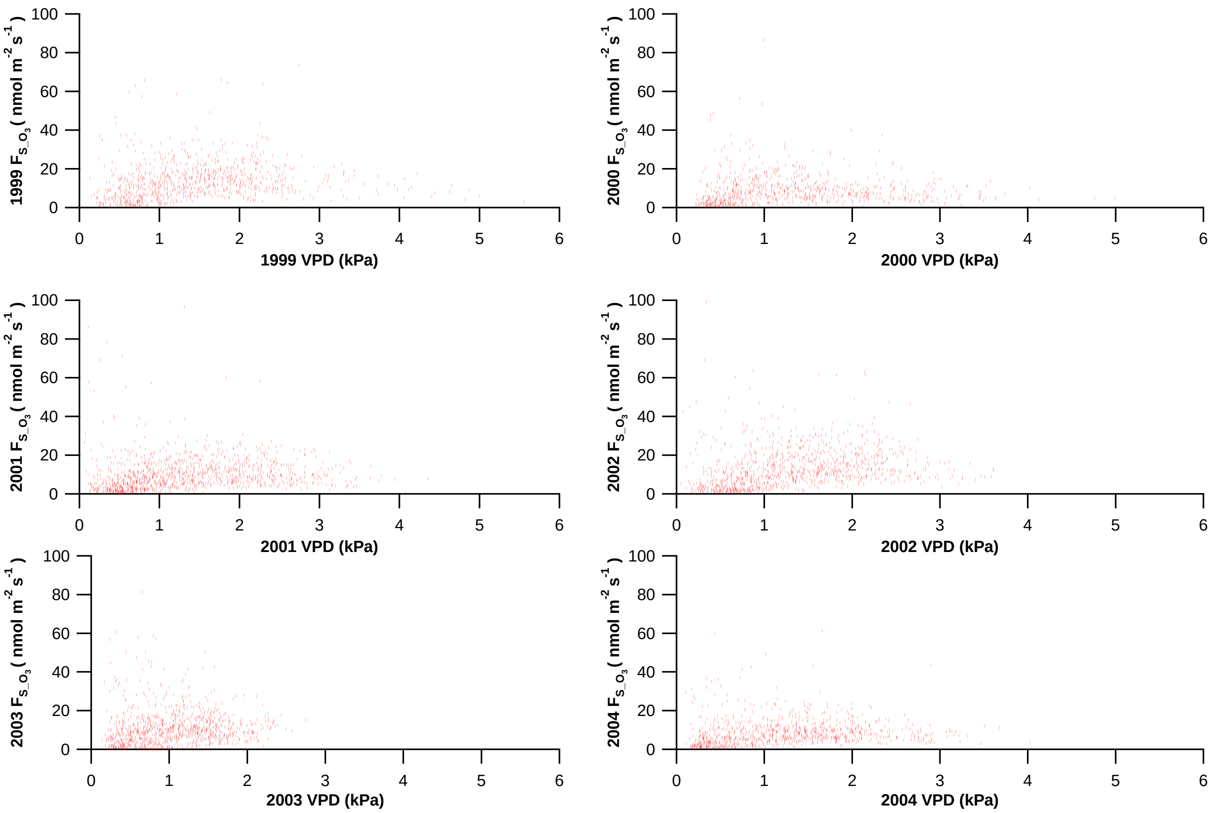


**Figure A2:** The calculated $F_{s\_O3}$ versus VPD for 1999-2004. Graphs were made prior to making $F_{s\_O3}$ negative.

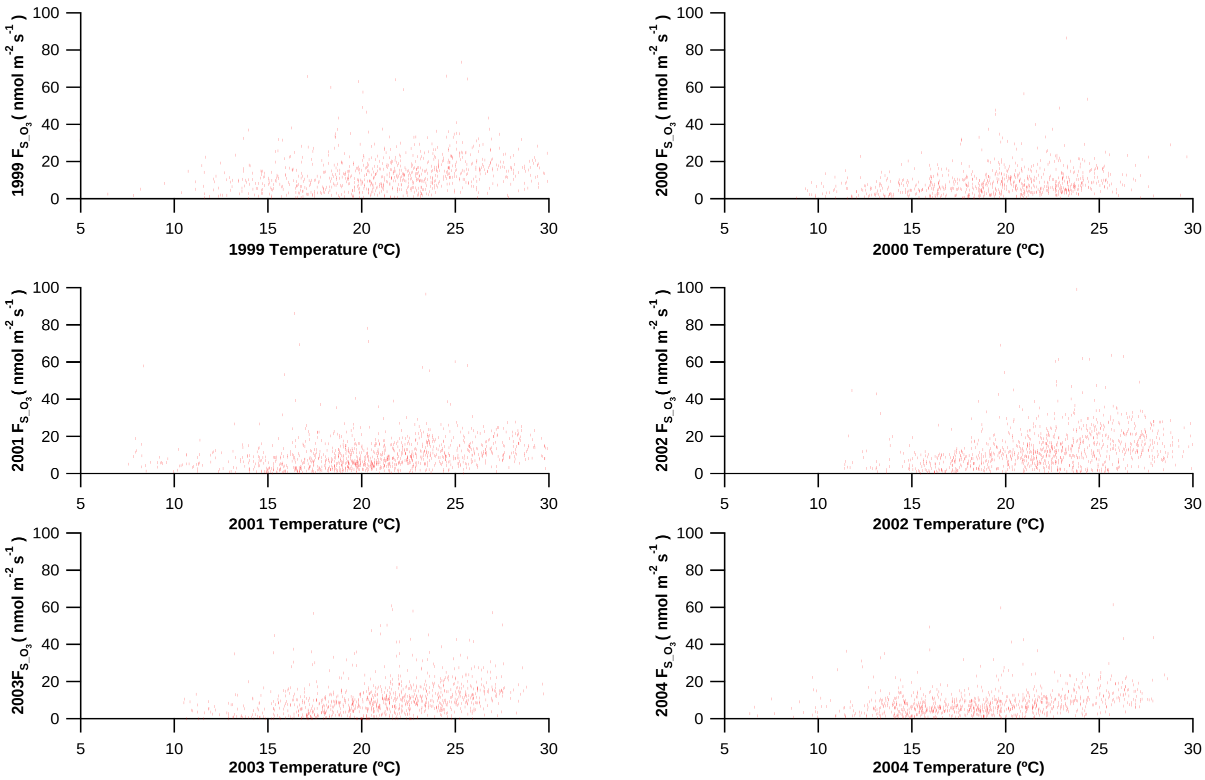


**Figure A3:** The calculated $F_{s\_O3}$ versus air temperature for 1999-2004. Graphs were made prior to making $F_{s\_O3}$ negative.

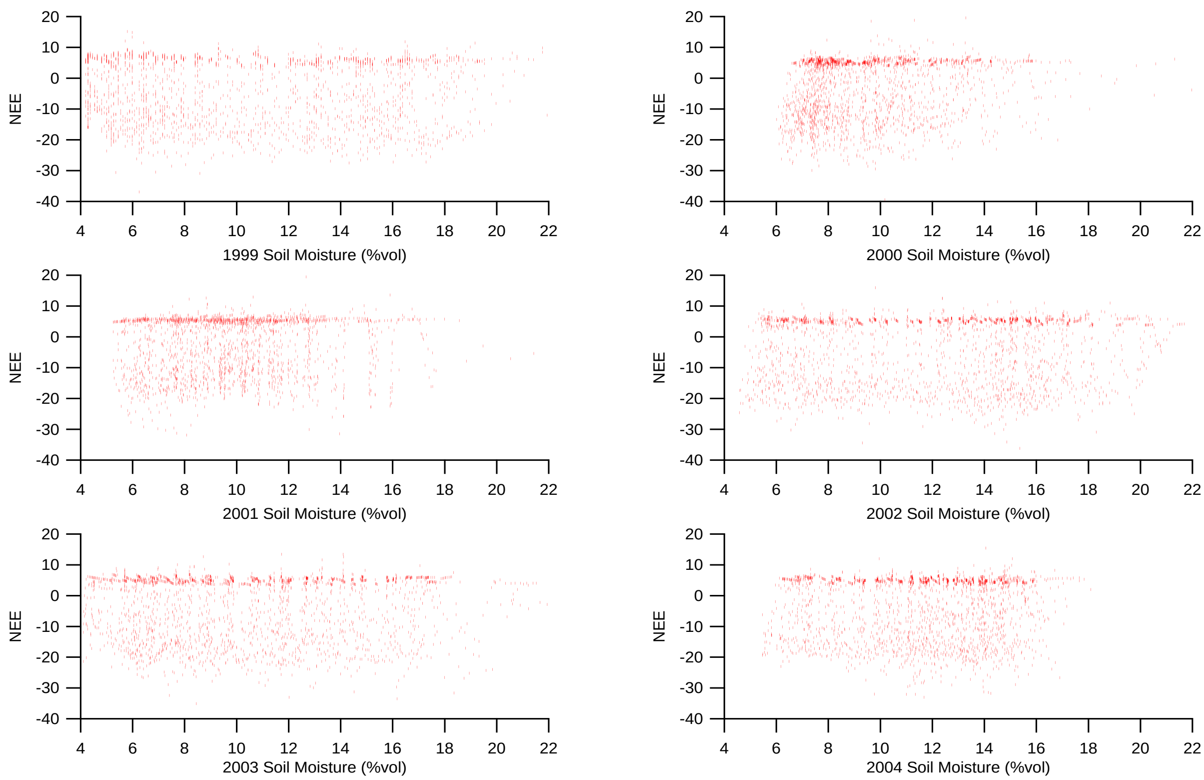


**Figure A4:** The calculated $F_{s\_O3}$ versus soil moisture for 1999-2004. Graphs were made prior to making $F_{s\_O3}$ negative.

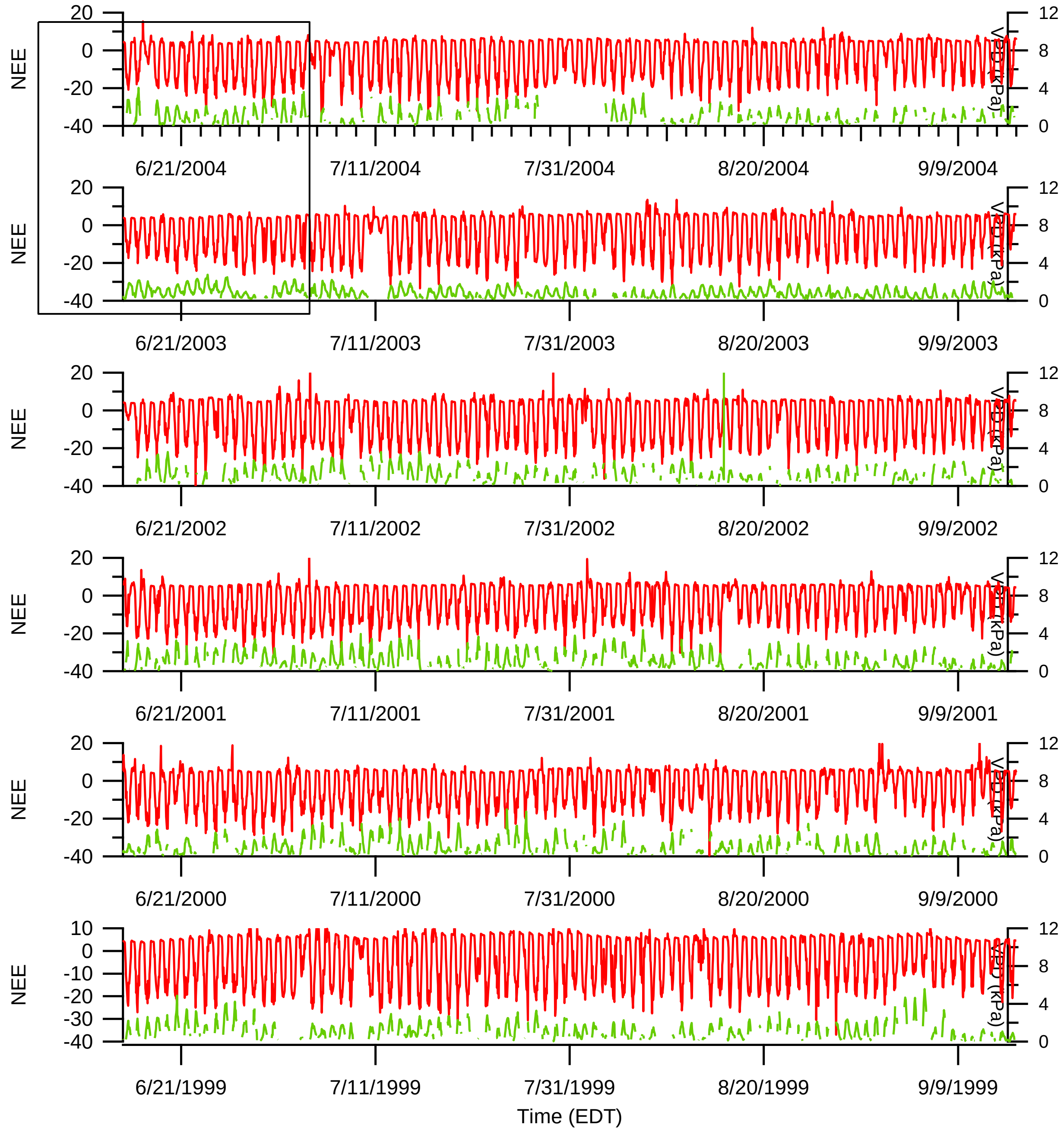


**Figure A5:** Comparison of NEE (red) and VPD (green) from June 15 to September 15.

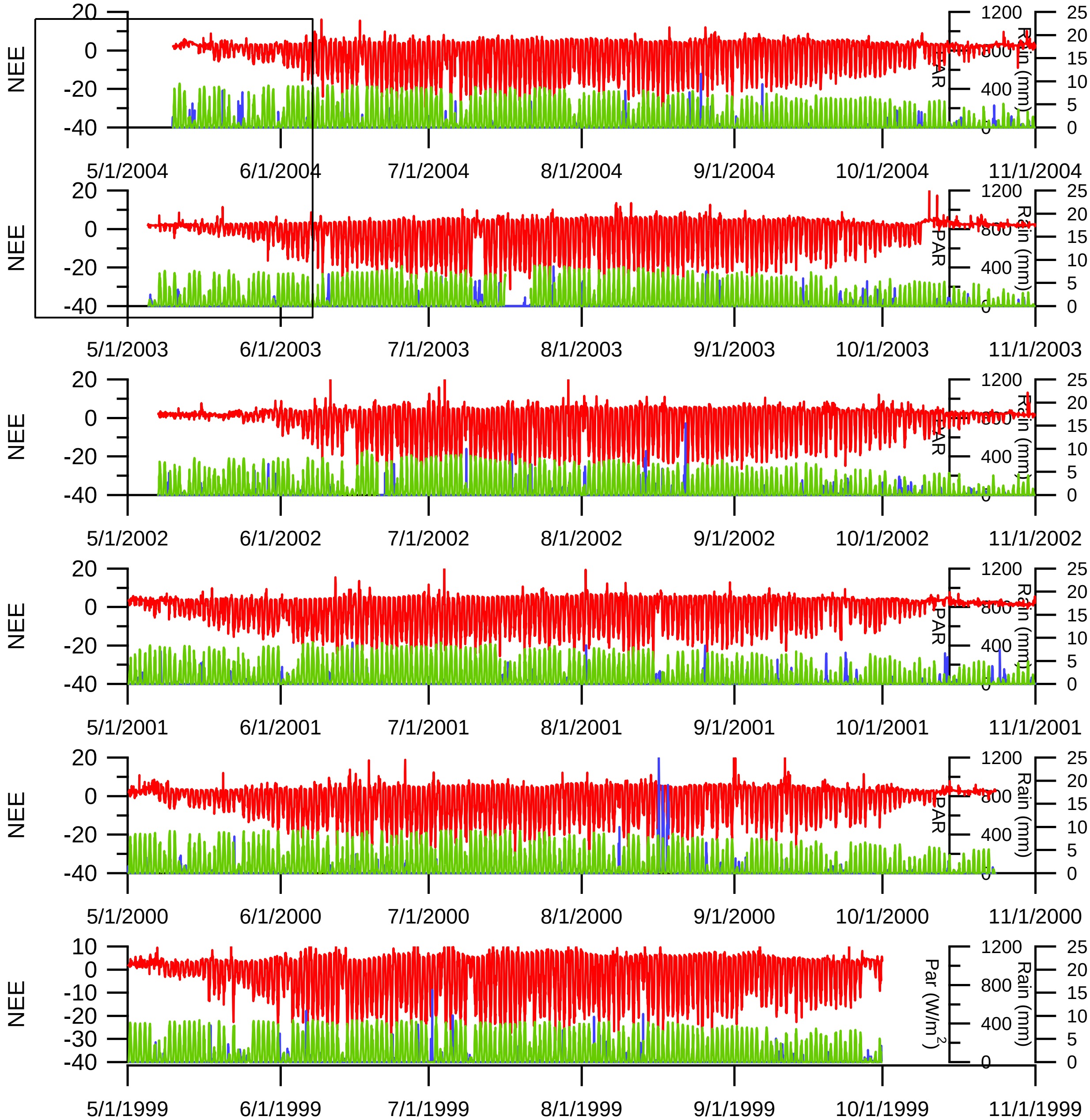


**Figure A6:** Comparison of NEE (red), rain events (blue), and photosynthetic active radiation (PAR) from May 1 to November 1.

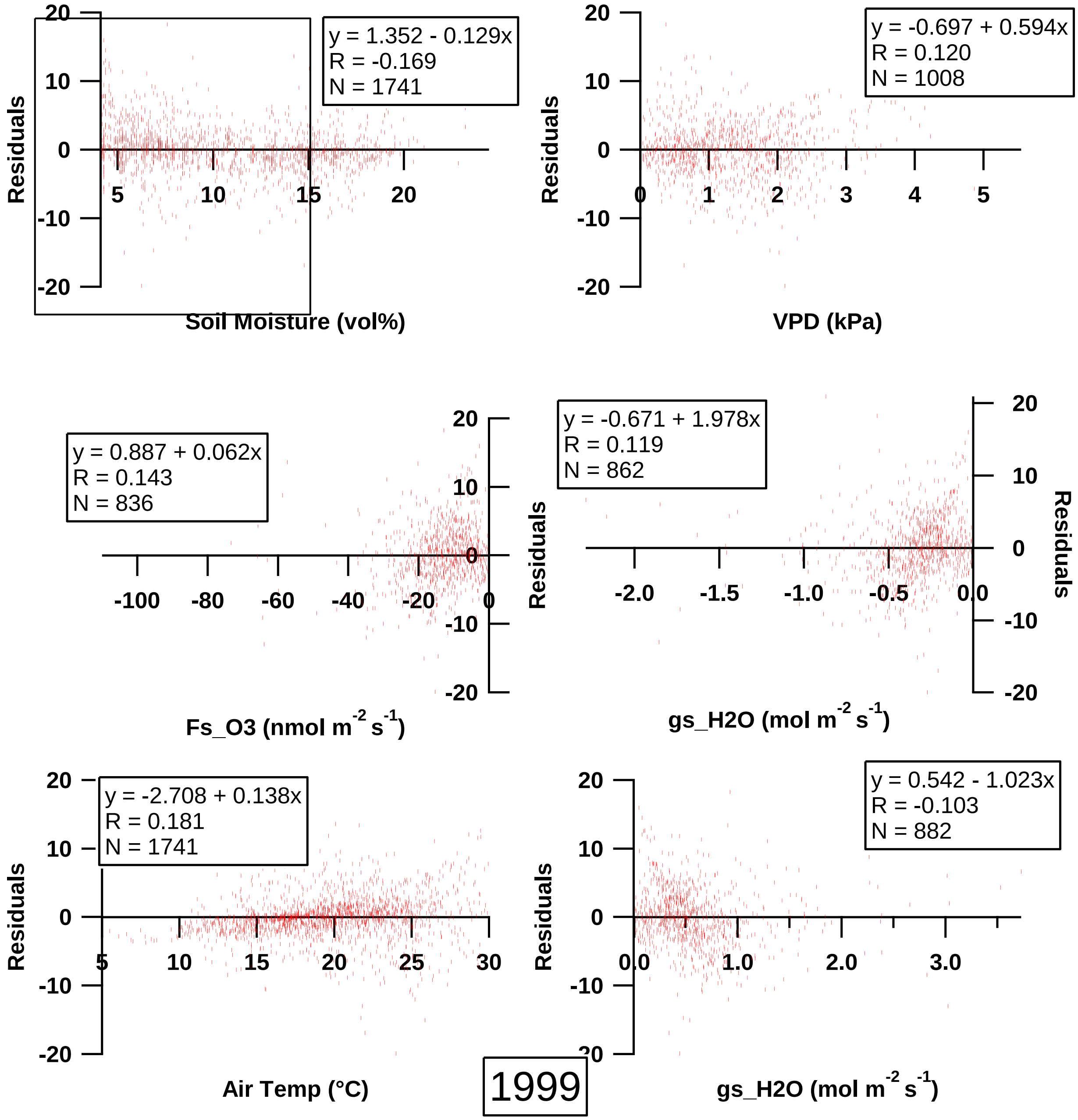


**Figure A7:** Plots of 1999 NEE-PPFD residuals versus remaining independent variables.

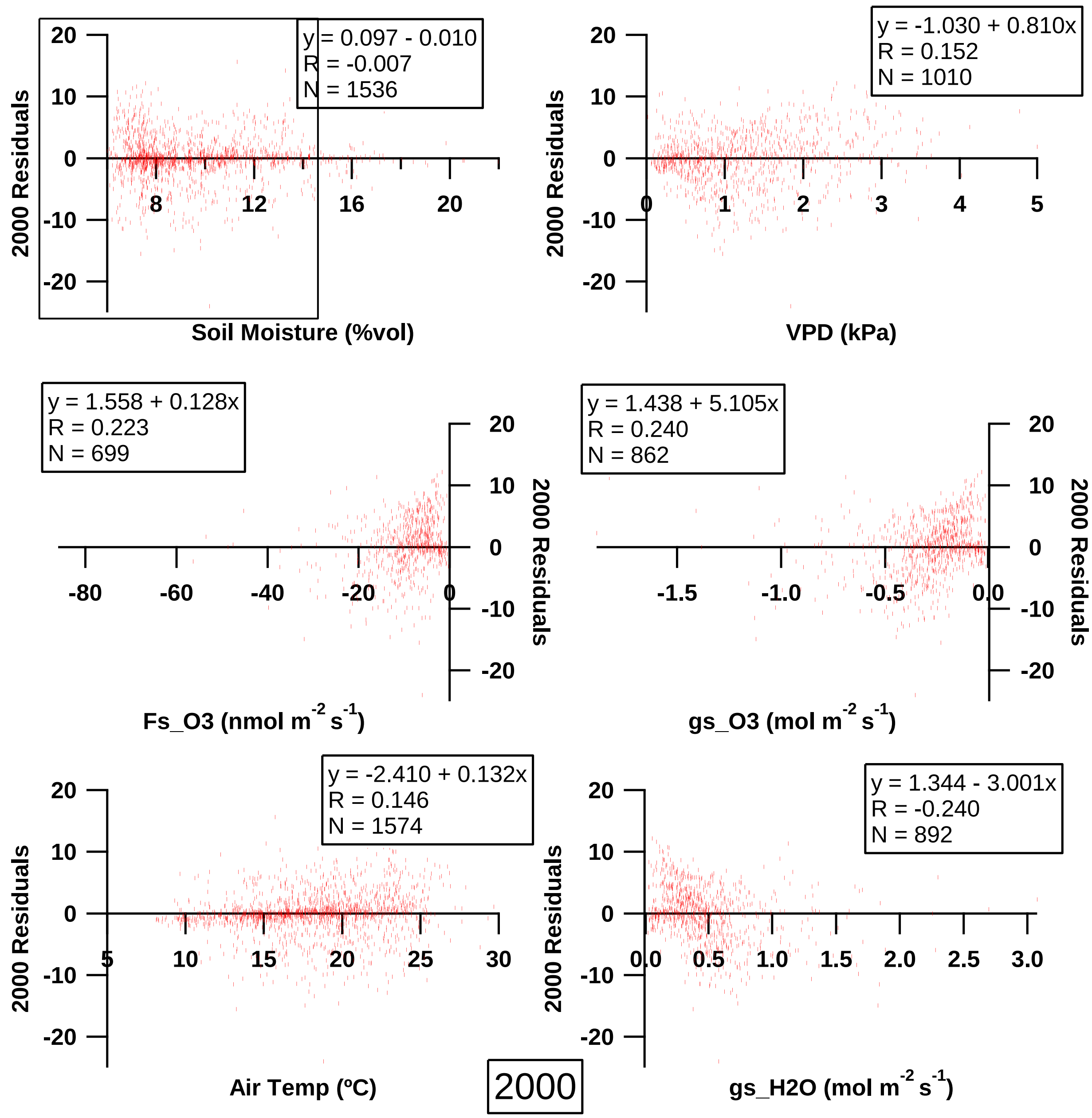


**Figure A8:** Plots of 2000 NEE-PPFD residuals versus remaining independent variables.

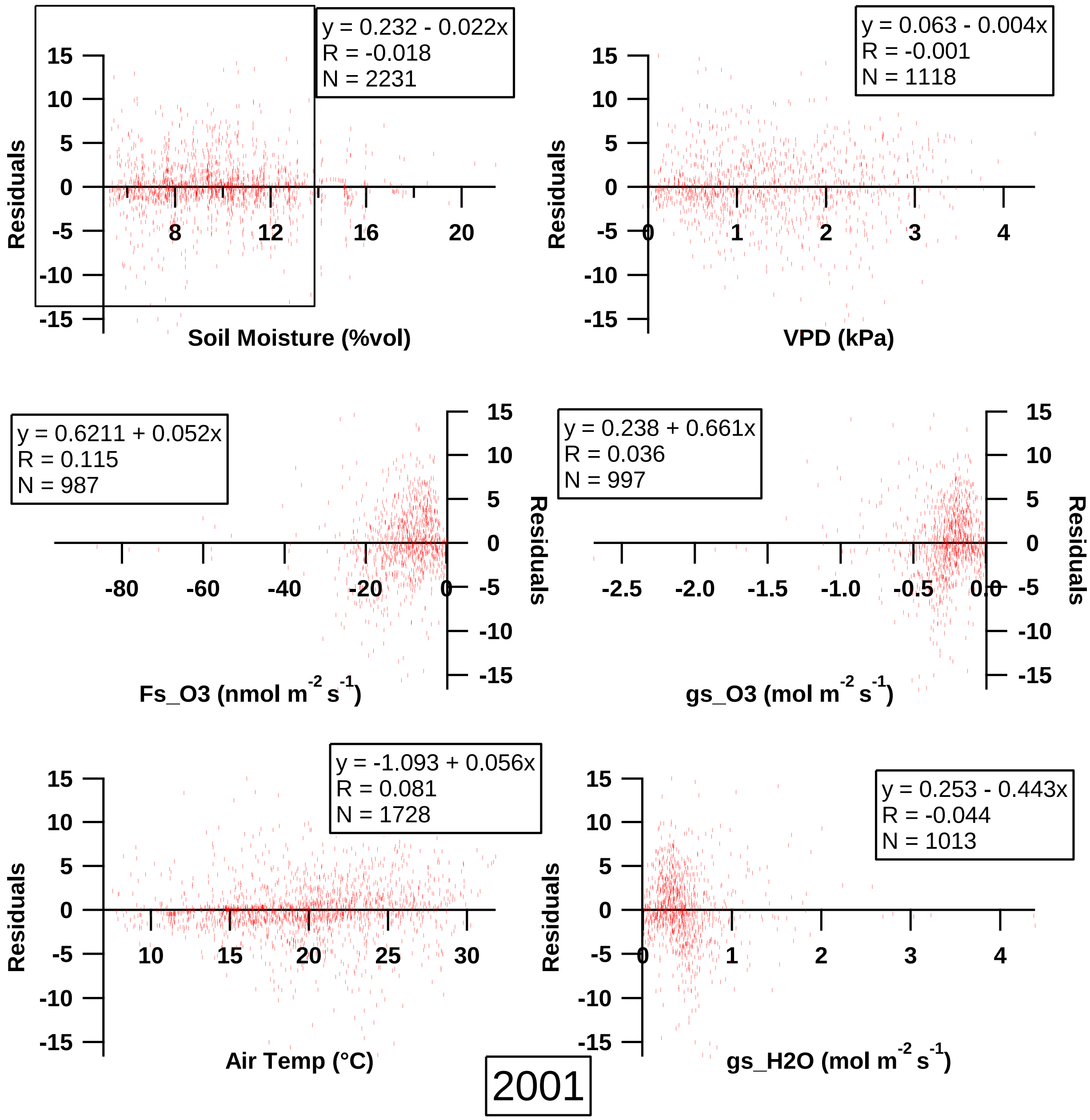


**Figure A9:** Plots of 2001 NEE-PPFD residuals versus remaining independent variables.

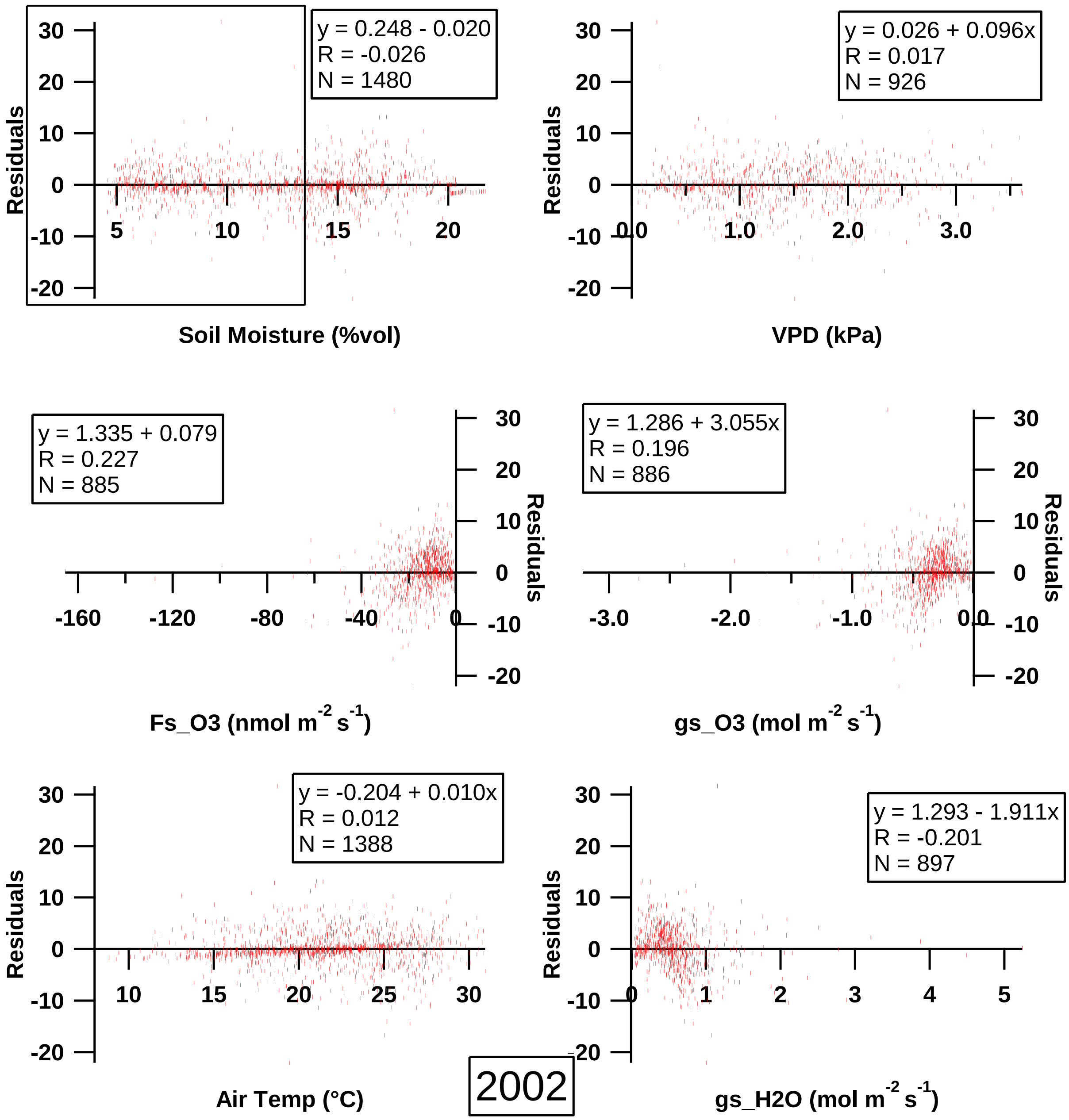


**Figure A10:** Plots of 2002 NEE-PPFD residuals versus remaining independent variables.

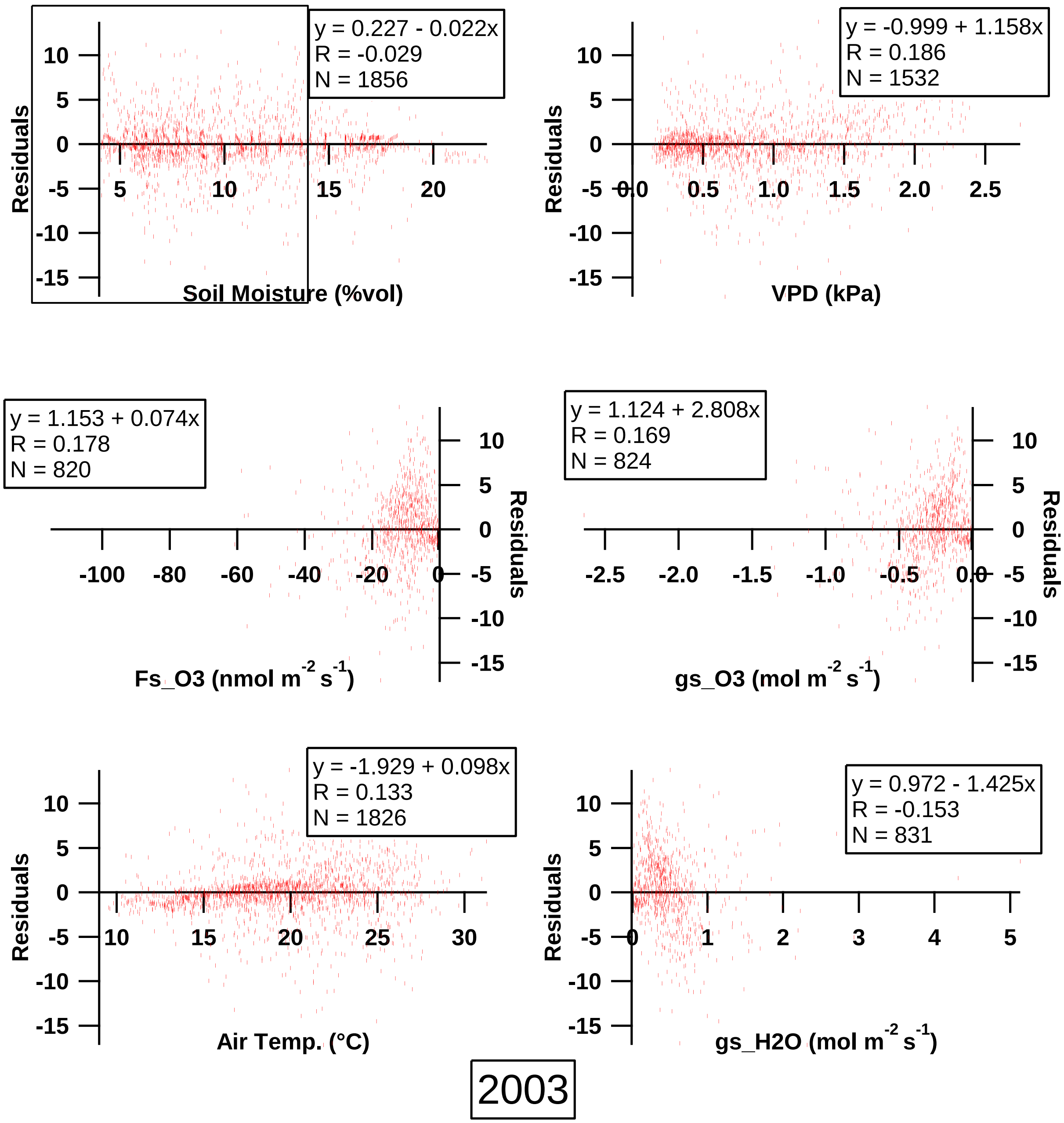


**Figure A11:** Plots of 2003 NEE-PPFD residuals versus remaining independent variables.

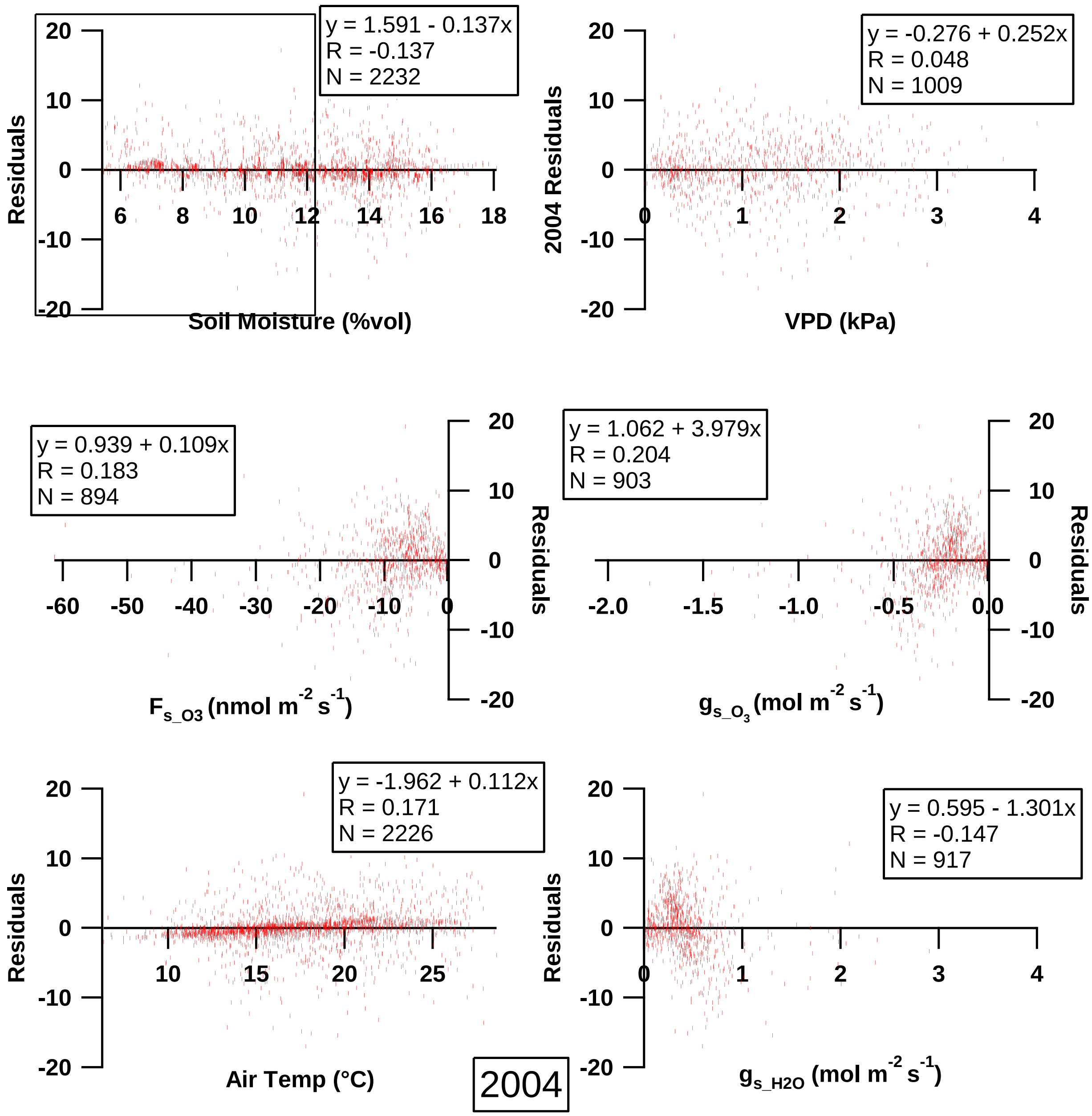


**Figure A12:** Plots of 2004 NEE-PPFD residuals versus remaining independent variables.

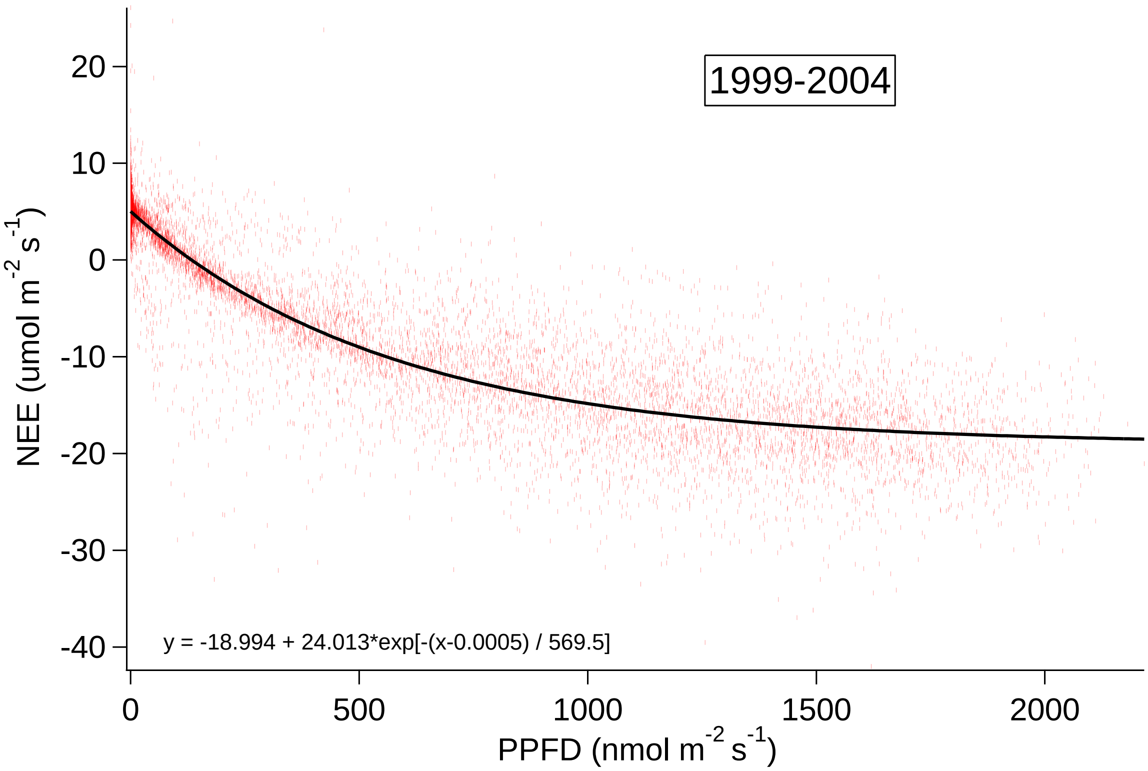

**Figure A13:** Exponential fit for the 1999-2004 NEE versus PPFD plot.

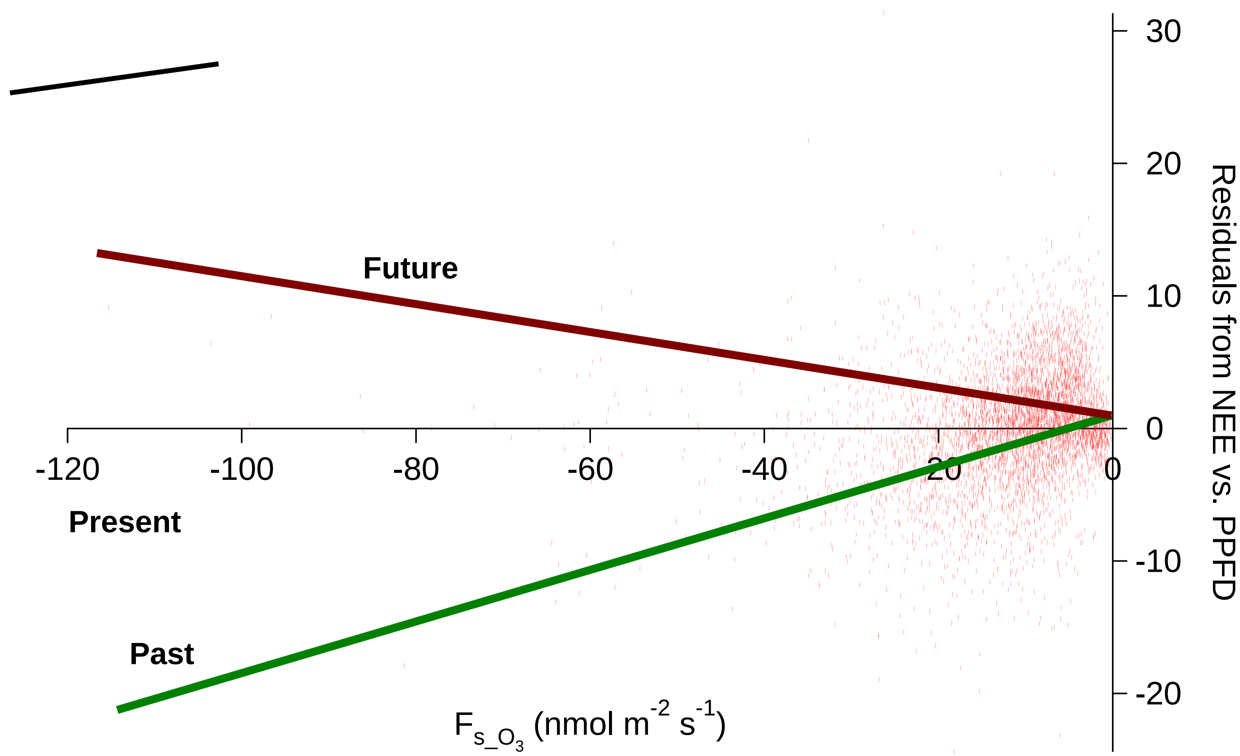


**Figure A14:** Possible transformation of the relationship between NEE and stomatal conductance.