2006

Influence of Nutrient Availability, Stand Age, and Canopy Structure on Isoprene Flux in a Eucalyptus saligna Experimental Forest

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Recommended Citation

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Influence of nutrient availability, stand age, and canopy structure on isoprene flux in a *Eucalyptus saligna* experimental forest

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[1] *Eucalyptus* plantations occupy approximately 10 million ha of land in the tropics and, increasingly, afforestation and reforestation projects are relying on this genus to provide rapid occupation of degraded sites, large quantities of high-quality wood products, and high rates of carbon sequestration. Members of the genus *Eucalyptus* are also very high emitters of isoprene, the dominant volatile organic compound emitted by trees in tropical ecosystems, which significantly influences the oxidative capacity of the atmosphere. While fertilization growth response of these trees has been intensively studied, little is known about how fertilization and tree age alter isoprene production from plantations of these trees. Here we examined the effects of fertilization and tree age on leaf-level isoprene flux from 2- and 6-year-old trees in a *Eucalyptus saligna* experimental forest in Hawaii. Leaf-level emission at a given canopy height did not differ between fertilized and unfertilized 6-year-old trees likely because leaf nitrogen content did not vary with fertilization. Across treatments, however, the standardized emission rate of isoprene (emission at a standard light and temperature) followed patterns of leaf N content and declined with canopy depth. Although leaf nitrogen content was similar between 2-year and 6-year fertilized trees, leaf-level emission rates declined with stand age. Surprisingly, despite differences in stand leaf area and leaf area distribution, modeled canopy-level isoprene flux was similar across stands varying in fertilization and tree age. Model results suggest that leaf area index was high enough in all treatments to absorb most of the light penetrating the canopy, leading to similar canopy flux rates despite the very different sized canopies.

1. Introduction

[2] Isoprene (2-methyl, 1,3-butadiene) is one of over 22,000 isoprenoid compounds produced by plants [*Lichtenthaler et al.*, 1997]. Whereas many isoprenoids are stored for use within plants, isoprene diffuses down a concentration gradient from chloroplasts through stomata into the atmosphere [*Lerdau et al.*, 1997]. The function of isoprene in plants is currently unknown; however, data suggest that isoprene may protect leaves from short episodes of high temperature [*Singsaas and Sharkey*, 1998] and from oxidative damage [*Loreto et al.*, 2001]. Isoprene is the dominant nonmethane hydrocarbon emitted from vegetation

in temperate deciduous and most tropical forest systems, contributing 350–500 Tg C to the atmosphere annually [*Guenther et al.*, 1995; *Poisson et al.*, 2000]. Isoprene is quickly oxidized in the atmosphere, resulting in many direct products such as methyl vinyl ketone and methacrolein, and indirect products such as ozone and organic nitrates (in the presence of high NO_x), which have further effects on atmospheric chemistry (reviewed by *Fuentes et al.* [2000] and *Monson and Holland* [2001]).

[3] It has been suggested that the conversion of agricultural land and natural forests to commercial plantations will have significant impacts on atmospheric chemistry through changes in the composition and quantity of hydrocarbons released by the vegetation [*Lerdau and Slobodkin*, 2002; *Rosenstiel et al.*, 2003]. Because only 30 to 50% of the species present in natural forests typically emit isoprene [*Harley et al.*, 1999; *Geron et al.*, 2002] and crop species (e.g., sugar cane, corn) release low quantities of isoprene, replacement of these plant communities with forest plantations of genera that emit large quantities of isoprene (such as *Eucalyptus* and *Populus*) has the potential to greatly increase local and regional isoprene emission.

[4] In addition to species composition, there are two other important differences between natural forests and planta-

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tions. First, the short rotations typical of commercial plantation forests create landscapes that are dominated by young, rapidly growing trees, which can amplify age-related features of canopy physiology. Second, fertilization is often used to maintain the high productivity of plantation forests of the tropics [*Fisher and Binkley*, 2000], but rarely is applied in unmanaged tropical forests. The impacts of these two differences with respect to terrestrial carbon storage have been discussed [*Vitousek*, 1991], but the consequences for isoprene production and subsequent effects on atmospheric chemistry have not been investigated.

[5] Nitrogen (N) fertilization can alter both leaf- and canopy-level properties. At the leaf level, fertilization often increases leaf N content [e.g., *Fisher and Binkley*, 2000], which directly influences enzyme driven processes, such as photosynthesis [*Field and Mooney*, 1986]. However, fertilization may not always lead to increased leaf N content. Allocation of added N to growth rather than increased leaf N content has been observed in *Eucalyptus globulus* [*Pereira et al.*, 1992], *Populus deltoides* (J. L. Funk et al., manuscript in preparation, 2006), *Metrosideros polymorpha* [*Treseder and Vitousek*, 2001], and many grasses [*Chapin*, 1980; *Hull and Mooney*, 1990; *Williams and Black*, 1996]. Thus N fertilization may also alter canopy structure, including height, biomass [*Leuning et al.*, 1991; *Gower et al.*, 1992; *Albaugh et al.*, 1998; *Fahey et al.*, 1998; *Fisher and Binkley*, 2000], and the distribution of leaf area [*Fahey et al.*, 1998] and N [*Leuning et al.*, 1991].

[6] The effects of fertilization on leaf- and canopy-level properties may translate into changes in leaf- and canopy-level isoprene flux. Two studies examining the effect of N fertilization on isoprene emission from leaves of potted velvet bean [*Harley et al.*, 1994] and aspen and oak [*Litvak et al.*, 1996] report a positive correlation between leaf N content and the leaf-level standardized emission rate of isoprene (isoprene SER; measured at a standard light and temperature). *Litvak et al.* [1996] proposed that N fertilization may increase levels of isoprene synthase, the enzyme responsible for isoprene synthesis [*Silver and Fall*, 1991], thereby increasing isoprene SER. However, these short-term studies of young, potted plants are difficult to extrapolate to stands of older, field-grown trees, particularly where fertilization has been applied for long periods. Fertilization-induced changes in canopy structure may also influence isoprene emission through profiles of light and temperature with canopy depth. Isoprene emission displays a hyperbolic response to increasing light availability and a parabolic response to increasing temperature, increasing exponentially until a temperature optimum is reached above which emission declines [e.g., *Keller and Lerdau*, 1999]. Thus the determination of leaf and light distribution within canopies is essential for accurately integrating leaf-level isoprene emission across all leaf layers in a canopy [*Harley et al.*, 1996b].

[7] The most widely planted tree genus in the tropics is *Eucalyptus*, which constituted 10 of the 43 million ha of tropical plantation forests in 1990 [*Brown et al.*, 1997]. We examined the impacts of fertilization and stand age on isoprene emission in 2- and 6-year-old stands of *Eucalyptus saligna*, a widely planted tree for commercial forestry with typical rotation times of 7 to 10 years [*Fisher and Binkley*, 2000]. Fertilizer use in the management of fast-growing

Eucalyptus plantations is common [*Fisher and Binkley*, 2000], and there have been suggestions that isoprene emission may be higher from young individuals [*Street et al.*, 1997] [see *Geron et al.*, 2001]. Measuring 2- and 6-year-old stands allowed us to survey isoprene flux during peak net primary production (2 years) and during declining primary productivity 4 years after maximal net primary production (6 years).

[8] We designed a study with the following objectives: (1) quantify the effect of fertilization on isoprene SER from leaves of 6-year-old *E. saligna* trees and (2) explore age-related changes in leaf-level isoprene SER from fertilized stands of 2- and 6-year-old trees. We then employed a canopy micrometeorology and physiology model to explore how fertilization- and age-related changes in leaf physiology (e.g., isoprene SER, leaf N), canopy structure, and biomass may interact to influence canopy-level isoprene flux.

2. Materials and Methods

2.1. Overview

[9] This study was conducted in a 2.5-ha experimental forest of fast growing *E. saligna*, 13 km north of Hilo, Hawaii (19°50′N, 155°7′W). The site elevation is 350 m. Air temperature (annual mean, 21°C) and precipitation (annual mean total, 3.5 m) are largely aseasonal. Soils are deep (>2 m) and are moderately acidic, isothermic, Typic Hydrudands. Prior to forest establishment in 1994, the site was cropped with sugarcane beginning in ~1910 [*Binkley and Resh*, 1999].

[10] We examined isoprene flux in nine 30 × 30 m plots with trees planted at a 1 × 1 m spacing. The plots were established and maintained as part of a larger study on fertilizer impacts on physiological and ecological processes (for a complete description, see *Giardina and Ryan* [2002]). Six of the plots were established in 1994, with three maintained as unfertilized controls and three receiving regular fertilizer additions. At the time of planting, all plots received 31 g N/m^2, 13 g P/m^2, 26 g K/m^2, 12.5 g Ca/m^2, 1.2 g Mg/m^2, and 10 g/m^2 balanced micronutrients over two applications to assure successful establishment of the stands [*Binkley and Resh*, 1999]. Three plots continued to receive quarterly additions of 5.6 g N/m^2, 2.4 g P/m^2, 4.6 g K/m^2, and annual additions of 12.5 g Ca/m^2, 5.8 g S/m^2, 2.3 g Mg/m^2, and 10 g/m^2 micronutrients throughout the study (designated 6-year fertilized), while three plots were managed without fertilizer for the remainder of the study period (designated 6-year unfertilized). In 1998, three new 30 × 30 m plots were created (designated 2-year fertilized) at a 1 × 1 m spacing. Unfertilized plots were not created in 1998 (no 2-year unfertilized treatment), which led to an imbalance in our experimental design for fertilization and age.

[11] In May and June of 2000, we intensively examined trees in one randomly selected 6-year fertilized plot (n = 10 trees), one randomly selected 6-year unfertilized plot (n = 15 trees), and two randomly selected 2-year fertilized plots (n = 13 trees collectively). Owing to the logistical and economic constraints of building canopy access scaffold towers in these forests, we were unable to sample leaves from trees in multiple replicate plots of each treatment combination. However, canopy size and structure, leaf and

stem physiological process rates, and stand productivity were generally similar across replicates within each of our treatments [*Giardina et al.*, 2003; *Ryan et al.*, 2004]. At the time of measurement, trees were 20–25 m (6-year fertilized), 15–22 m (6-year unfertilized), and 7–9 m (2-year fertilized) in height.

[12] The CANVEG model was used to calculate canopy-level isoprene fluxes [*Baldocchi and Harley*, 1995; *Baldocchi et al.*, 1999]. A micrometeorological module was used to compute leaf energy exchange, turbulent diffusion, scalar concentration profiles, and radiative transfer through the canopy for 40 canopy layers. Variables generated by the micrometeorological module were then used to drive a physiological module that computes isoprene emission. Input variables include photosynthetic photon flux density, air temperature, wind speed, relative humidity, CO_2 concentration, LAI, leaf angle orientation, a leaf clumping factor, and canopy height. Plant structural variables and environmental variables for a typical sunny (3 May 2000) and cloudy (24 April 2000) day were obtained at the *E. saligna* site (described below). While the model results were not validated with canopy flux measurements at this site, the isoprene module of CANVEG has been validated at other sites, including oak and aspen dominated forests [*Baldocchi et al.*, 1999]. Furthermore, *Baldocchi et al.* [1999] suggest that the model can be used for other forest types if the amount and spatial distribution of isoprene emitting biomass is characterized appropriately.

2.2. Leaf-Level Measurements

[13] Isoprene emission rate and photosynthetic rate (A) were measured with an open system LI-6400 portable infrared gas analyzer (IRGA) with a temperature- and light-controlled cuvette (LI-COR, Lincoln, Nebraska) and a Photovac voyager gas chromatograph (GC) with a photoionization detector (Perkin-Elmer, Norwalk, Connecticut) as described by *Funk et al.* [2003]. Because isoprene emission and photosynthesis are strongly influenced by light environment, we divided the canopy into five layers of equal thickness based on tree height. As stands varied in height, layers varied in thickness among treatments. We conducted gas exchange measurements on representative leaves in each layer. Layer 1, located at the canopy top, consisted of young, expanding leaves that did not emit detectable levels of isoprene. Layer 2 consisted of young but fully expanded leaves. Layer 3 was located below layer 2 in the upper canopy, and consisted entirely of older, fully expanded leaves. Leaves sampled in layers 4 (external) and 5 (internal) were located in the lower canopy and were largely shaded.

[14] Intensive sampling was conducted on layer 3 leaves to assess the effect of fertilization and stand age on isoprene SER and photosynthetic rate. One leaf was sampled on each of 10, 15, and 13 trees in 6-year fertilized, 6-year unfertilized, and 2-year fertilized plots, respectively. Mean light levels in layer 3 likely varied among treatments as the LAI of layers 1 and 2 was greater in fertilized stands. However, across treatments, measured leaves were sunlit for a significant part of the day. Measurements in layers 2, 4, and 5 were conducted on one leaf for each of six trees per plot type. Isoprene SER and photosynthetic rate were measured at 1500 μmol photon m^{-2} s^{-1} and 26°C.

[15] After all gas exchange measurements were completed each day, leaves were clipped and leaf area was measured with a LI-3000 portable leaf area meter (LI-COR, Lincoln, Nebraska). Leaves were then dried at 65°C for 3 days and weighed to determine specific leaf weight (SLW). Leaf material was ground and analyzed for leaf N content (leaf N) with an elemental analyzer (CE Instruments Flash EA 1112, CE Elantech, Lakewood, New Jersey).

2.3. Canopy Characterization

[16] Leaf area index (LAI, m^2 leaf m^{-2} ground) was measured monthly in each plot with paired LAI-2000 plant canopy analyzers (LI-COR, Lincoln, Nebraska), with one analyzer located in an open field adjacent to the forest plots [*Giardina et al.*, 2003]. All estimates of LAI were corrected with an allometric equation that was developed with harvested leaves from the buffer areas of the 18 plots. The distribution of leaf area within the canopy was determined in one plot of each plot type as described by *Hedman and Binkley* [1988].

2.4. Model Parameterization

[17] Isoprene emission rate at a given canopy layer (I_i) was calculated with the following equation, derived from *Guenther et al.* [1993]:

$$I_i = LAI_i * (f_{sun} * (SER_i * C_L * C_T) + f_{shade} * (SER_i * C_L * C_T)), \tag{1}$$

where the modeled standardized emission rate at layer i (SER_i) was corrected for ambient light (C_L) and temperature (C_T), calculated for sunlit (f_{sun}) and shaded leaves ($f_{shade} = 1 - f_{sun}$), and scaled with leaf area index per layer (LAI_i). Each canopy was divided into 40 layers on the basis of height.

[18] Light (n = 21 leaves across canopy layers and treatments) and temperature (n = 16 leaves across canopy layers and treatments) response curves were generated to establish C_L and C_T. For light response curves, leaf temperature was maintained at 26°C while PAR was varied between 300 to 1800 μmol m^{-2} s^{-1}. For temperature response curves, light was maintained at 1500 μmol m^{-2} s^{-1} while leaf temperature was varied between 24 and 34°C. A light correction term was calculated after *Guenther et al.* [1993],

$$C_L = \frac{L_1 * L_2 * Q}{\sqrt{1 + L_1^2 * Q^2}}, \tag{2}$$

where L_1 (0.0015, dimensionless) and L_2 (1.1032, dimensionless) are empirically derived constants for *E. saligna* in this study and Q is PAR (μmol m^{-2} s^{-1}) ($r^2 = 0.91$, $P < 0.01$). Constants (here and elsewhere) were derived by fitting data to a nonlinear regression. The temperature correction term was calculated after *Guenther et al.* [1993] as follows:

$$C_T = \frac{\exp[T_1(T_L - T_S)/RT_S T_L]}{1 + \exp[T_2(T_L - T_3)/RT_S T_L]}, \tag{3}$$

where T_1 (94940 J mol^{-1}), T_2 (350200 J mol^{-1}), and T_3 (312.9 K) are empirically derived constants for *E. saligna* in this study, R is the gas constant (8.314 J K^{-1} mol^{-1}), T_S is
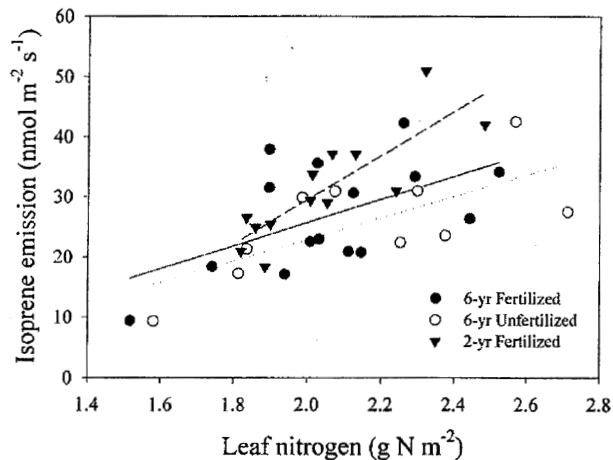
Figure 1. Relationship between the standardized emission rate of isoprene and leaf N on an area basis in *E. saligna*. Regression lines are shown for each plot type (6-year fertilized, n = 10, solid circles, dotted line; 6-year unfertilized, n = 15, open circles, solid line; 2-year fertilized, n = 13, solid triangles, dashed line).

the standard temperature (299.15 K), and T_L is leaf temperature ($r^2 = 0.95$, $P < 0.01$).

[19] The model scales isoprene SER with canopy depth (SER$_i$) with the following equation:

$$SER_i = SER_{top} * \left(0.65 * \frac{h_i}{h_t} + 0.35 \right), \tag{4}$$

where SER$_{top}$ is isoprene SER of layer 2 leaves (maximum isoprene SER), h_i is the height of layer i, and h_t is canopy height.

[20] Ambient light and temperature conditions were monitored at a weather station located in an open field approximately 100 m from the forest plots. Instantaneous measures of air temperature and global photosynthetically active radiation (direct and diffuse GPAR, μmol photon m^{-2} s^{-1}) were collected every 15 s by a CS500 air temperature/relative humidity probe (Campbell Scientific, Logan, Utah) and an LI-190SB quantum sensor (LI-COR, Lincoln, Nebraska), and stored on a CR10X data logger (Campbell Scientific, Logan, Utah). Incident net radiation was calculated from GPAR values with a previously established empirical relationship [*Funk et al.*, 2003]. Average wind speed was determined from data collected at nearby (15 km) Hilo Airport.

[21] V_{cmax} and J_{max}, parameters used to describe carboxylation capacity and electron transport within the photosynthesis module of CANVEG, were 68 and 140 μmol m^{-2} s^{-1}, respectively, as in work by *Warren and Adams* [2004] for *E. grandis*. *E. saligna* and *E. grandis*, both members of the blue gum group, are nearly identical with respect to physiology and morphology [*Food and Agriculture Organization*, 1981]. Parameters used to simulate light interception in the canopy (e.g., variation in leaf inclination angle and leaf clumping factor) have been shown to have a

significant influence on model output [*Baldocchi et al.*, 2002]. Because *E. saligna* leaves were uniformly distributed, a leaf clumping factor of 1.0 was used across plot types. A sensitivity analysis showed that isoprene fluxes increase by approximately 10% per 0.1 decrease in clumping factor. However, the different treatments show consistent behavior when leaf clumping factor is altered. We used a mean leaf angle of 80 degrees and a beta distribution to compute the probability frequency distribution of leaf inclination. Like other *Eucalyptus* species, *E. saligna* leaves display a nearly vertical orientation and an 80 degree leaf angle is a good approximation. However, a sensitivity analysis showed that emission decreases by roughly 25% when a leaf angle of 70 degrees is used. Leaf transmittance (0.03) and reflectance (0.08) were approximated with direct measurements on leaves (n = 3, two sides) from *E. globulus* with a Fiber Optic Spectrometer (Model USB2000, Ocean Optics, Dunedin, Florida).

2.5. Statistical Analysis

[22] Analysis of variance (ANOVA) was used to examine differences in leaf N, SLW, and leaf-level gas exchange among the three plot types. Analysis of covariance (ANCOVA) was employed to factor out the effect of leaf N on these variables. We used tests for parallelism among slopes to identify interactions between main effects (isoprene SER, photosynthesis) and the covariate (leaf N). In only one case (A_{mass} versus leaf N_{mass}) was this interaction significant. Planned post hoc comparisons of leaf-level emission rates were performed with a least-significant-difference approach. Pearson product-moment correlation coefficients were generated to evaluate the linear association between isoprene emission, photosynthesis, and leaf N. Algorithms used for scaling isoprene SER with light and temperature were evaluated with nonlinear regression in the regression module of Sigma Plot v.6.10 (SPSS, Chicago, Illinois). ANCOVA and correlation analyses were conducted with Biomstat 3.30j (Applied Biostatistics, Port Jefferson, New York). All other analyses were performed in Statistica v.5.1 (Statsoft, Tulsa, Oklahoma).

3. Results
3.1. Leaf-Level Gas Exchange

[23] Within and across plots, isoprene emission of layer 3 leaves was positively correlated with leaf N_{area} (6-year fertilized, $r = 0.71$, $P = 0.02$; 6-year unfertilized, $r = 0.55$, $P = 0.03$; 2-year fertilized, $r = 0.83$, $P < 0.01$; Figure 1). Isoprene SER more than tripled over the range of observed leaf N (across plots, $r = 0.60$, $P < 0.01$). A_{mass} and leaf N_{mass} were positively correlated in 6-year trees (6-year fertilized, $r = 0.89$, $P < 0.01$; 6-year unfertilized, $r = 0.81$, $P < 0.01$), while A_{area} and leaf N_{area} were positively correlated in 2-year fertilized trees ($r = 0.58$, $P = 0.04$). The slopes of the relationship between leaf N and isoprene SER or photosynthesis were similar among treatments when expressed on area and mass bases (test of parallelism, $P > 0.10$) with the exception of A_{mass} and leaf N_{mass} ($F = 4.33$, $P = 0.05$). Photosynthetic rate and isoprene emission rate, which are often positively correlated within and across species [e.g., *Funk et al.*, 2003], were not correlated on either a leaf area or mass basis for any plot type.

Table 1. Leaf-Level and Canopy-Level Properties in *E. saligna* From Three Plot Types[a]

	Fertilized 6-Year	Unfertilized 6-Year	Fertilized 2-Year
Leaf-level properties			
Area-based			
Isoprene emission, nmol m^{-2} s^{-1}	25.61(2.85)a	26.97(2.34)a	31.30(2.48)a
Photosynthetic rate, μmol m^{-2} s^{-1}	19.49(1.04)a	20.31(0.69)a	18.70(0.81)a
Leaf nitrogen, g N m^{-2}	2.15(0.11)a	2.06(0.07)a	2.05(0.06)a
Mass-based			
Isoprene emission, μg g^{-1} hr^{-1}	61.58(5.47)a	64.78(5.71)a	80.75(5.03)b
Photosynthetic rate, nmol g^{-1} s^{-1}	204.32(21.05)a	202.99(12.20)a	199.13(8.17)a
Leaf nitrogen, mg N g dry wt^{-1}	2.16(0.09)a	2.03(0.07)a	2.18(0.04)a
Specific leaf weight, g m^{-2}	101.81(8.33)a	102.78(4.04)a	94.22(2.79)a
Canopy-level properties			
Leaf area index (LAI), m^2 leaf m^{-2} ground	8.91(0.42)a	6.02(0.16)b	9.72(0.68)a
Aboveground NPP, kg C m^{-2} yr^{-1}	1.49(0.07)a	0.72(0.02)b	1.98(0.23)a

[a]Leaf-level traits were measured on one leaf from numerous trees per plot (6-year fertilized, n = 10; 6-year unfertilized, n = 15; and 2-year fertilized, n = 13). All data are from layer 3 leaves. LAI data were collected in May 2000, and aboveground NPP data are from July 1999 to June 2000 [*Giardina et al.*, 2003] (n = 3 plots per plot type). Data are means (and standard error). Letters represent significant differences among treatments for a given variable, $P < 0.05$.

[24] The leaf N content of layer 3 leaves did not differ between fertilized and unfertilized 6-year old trees when expressed on either a leaf area or leaf mass basis (Table 1). Because leaf physiological processes are often linked to leaf N, it is not surprising that rates of leaf-level isoprene emission and photosynthesis were similar in leaves of fertilized and unfertilized trees. However, although leaf N content was similar in 2- and 6-year-old trees, isoprene SER was significantly higher in leaves from 2-year fertilized trees compared to 6-year fertilized trees when expressed on a leaf mass basis (Table 1). When we controlled for slight differences in leaf N content between 2- and 6-year-old fertilized individuals, isoprene SER$_{area}$ was also significantly higher in the younger plants (ANCOVA, Table 2).

[25] Isoprene SER, photosynthetic rate, and leaf N varied with leaf position in the canopy (Table 3). Leaves in layers 2 and 3, which comprise roughly the top half of the canopy, displayed higher isoprene SER and photosynthetic rates than leaves in layers 4 and 5. SLW did not change with canopy position; therefore patterns of isoprene SER and photosynthetic were similar when expressed on either a leaf area or mass basis (mass-based data not shown). Because of limited sampling of layers 2, 4, and 5, we pooled isoprene SER, photosynthesis, leaf N, and SLW across plot types. Thus the data presented in Table 3 are means across plot types. Light and temperature response curves for isoprene emission were variable across canopy layers. However, when combined, the data fit selected models (equations

(2) and (3)) well ($r^2 = 0.91$ for light; $r^2 = 0.95$ for temperature, Figure 2).

3.2. Canopy Structure

[26] While fertilization did not affect leaf N or photosynthesis in 6-yr old fertilized individuals relative to unfertilized individuals, fertilization did increase aboveground net primary production [*Giardina et al.*, 2003]. Aboveground net primary production (ANPP) and LAI were 2- and 1.5-fold higher, respectively, in 2- and 6-year fertilized plots compared to unfertilized plots (Table 1). Furthermore, fertilization altered leaf area distribution within the canopy (Figure 3). While roughly 50% of total leaf area in the 6-year fertilized plots resided in the upper half of the canopy, more than 80% of total leaf area in 6-year unfertilized plots resided in these layers. Leaf area distribution in 2-year fertilized plots was between these two extremes, with roughly 66% of total leaf area occurring in the upper half of the canopy.

3.3. Modeled Canopy-Level Isoprene Flux

[27] We simulated canopy isoprene flux over a typical diurnal time course on a sunny (3 May 2000) and cloudy (24 April 2000) day. Despite treatment differences in LAI, leaf area distribution, and leaf-level isoprene SER (2- versus 6-year fertilized plots), canopy-level flux rates were similar (Figure 4). While canopy flux from 2-year stands appeared to be higher than flux from 6-year stands, incorporating the

Table 2. Analysis of Covariance Results for Fertilization (df = 1,22) and Age (df = 1,20) Effects on Area and Mass-Based Isoprene Emission and Photosynthesis in *E. saligna*, Controlling for Leaf N

		Fertilization		Age	
Dependent Variable	Covariate	F	P	F	P
Isoprene emission, nmol m^{-2} s^{-1}	Leaf nitrogen, g N m^{-2}	0.96	0.34	9.15	0.01
Photosynthetic rate, μmol m^{-2} s^{-1}		0.60	0.45	0.15	0.71
Isoprene emission, μg g^{-1} hr^{-1}	Leaf nitrogen, mg N g dry wt^{-1}	0.82	0.38	6.64	0.02
Photosynthetic rate, nmol g^{-1} s^{-1}		2.84	0.11	0.36	0.56[a]

[a]Significant interaction (test of parallelism) between main effect and covariate at $P = 0.05$. The df values are numerator and denominator degrees of freedom, respectively, and P-values reflect the level of F test (ANOVA) significance. Values of the F statistic are given.

Table 3. Physiological Characteristics of Leaves in Four Layers Along a Vertical Gradient in *E. saligna* Canopies[a]

| | Canopy Layer | | | |
	2	3	4	5
Isoprene emission, nmol m^{-2} s^{-1}	31.27(3.86)a	32.14(2.66)a	23.42(4.58)ab	10.28(4.30)b
Photosynthetic rate, µmol m^{-2} s^{-1}	23.43(0.58)a	21.75(0.53)ab	18.75(1.39)b	12.81(2.25)c
Leaf nitrogen, g N m^{-2}	2.26(0.08)a	2.19(0.10)a	2.06(0.09)a	1.69(0.29)b
Specific leaf weight, g m^{-2}	104.90(6.42)a	105.35(6.64)a	110.74(4.53)a	103.80(14.07)a

[a]Here n = one leaf from six individuals per layer. Layer 1 leaves were young, expanding leaves that did not emit isoprene. Data are means (and standard error) across three plot types (6-year fertilized, 6-year unfertilized, and 2-year fertilized). Letters represent significant differences among layers for a given variable, $P < 0.05$.

measured uncertainty in isoprene SER into model projections (denoted as 1 standard error of isoprene SER in Figure 4) led to overlapping flux estimates across the three plot types. In 6-year unfertilized and 2-year fertilized, isoprene was emitted primarily in the top canopy layers (2–6), while isoprene emission was more evenly distributed among upper and middle layers (2–15) in 6-year fertilized stands (Figure 5). Modeled canopy emission was not sensitive to changes in LAI above 7 at high light (sunny day, incident PAR 1682 µmol m^{-2} s^{-1}, Figure 6). Under low light conditions (cloudy day, incident PAR 794 µmol m^{-2} s^{-1}), canopy emission stabilized at an LAI of about 5. To isolate the influence of isoprene SER and canopy structure on canopy flux, the model was run over a diurnal time course on a sunny day (3 May 2000) using a common isoprene SER (32.1 nmol m^{-2} s^{-1}) for all three plot types. Canopy flux rates were nearly identical with a fixed isoprene SER (Figure 7). In both model runs (variable or fixed isoprene SER among treatments), isoprene SER decreased with canopy depth following equation (4).

4. Discussion

4.1. Leaf-Level Emission Patterns

[28] In contrast to results from greenhouse studies, our results indicate that N enrichment may not lead to increased leaf-level isoprene emission in field-grown plants because the enriched plants can allocate additional N to growth rather than leaf N content. Fertilization can result in increased leaf N content when stands are strongly N limited [*Fisher and Binkley*, 2000]. However, we found that fertilized trees maintained leaf N levels that were similar to trees in control plots and, instead, allocated additional N to new biomass. Notably, we fertilized stands with a complete mix of macronutrients and micronutrients so that micronutrient imbalances did not counteract the otherwise positive effects of fertilization with N [e.g., *Fenn et al.*, 1998]. This observed trade-off between growth and leaf N content in fertilized stands agrees with results from other studies [e.g., *Pereira et al.*, 1992]. Because leaf-level emission rates tripled over the range of N observed in this study, our data suggest that leaf-level emission rates will increase should leaf N increase in response to fertilization.

[29] Our observation of slightly higher (~20%) leaf-level isoprene SER in younger individuals agrees qualitatively with the results of *Street et al.* [1997] who found a fivefold increase in mass-based rates of isoprene emission from a 1-year-old *E. globulus* sapling compared to a 7-year-old individual of the same species. In our study, higher

isoprene SER in leaves from young trees relative to older trees occurred despite similar photosynthetic rates in leaves from young and old trees, which indicates that younger plants allocate proportionately more carbon to isoprene production. Analysis of covariance results suggested that higher isoprene SER was not attributable to differences in leaf N content between 2- and 6-year-old trees. It is unclear why younger trees should allocate more carbon to isoprene emission. *Funk et al.* [1999] observed a positive relationship between isoprene SER and the number of actively photosynthesizing source leaves on a plant, suggesting a mechanistic link between isoprene emission and whole-plant carbon dynamics. If isoprene emission is linked to substrate
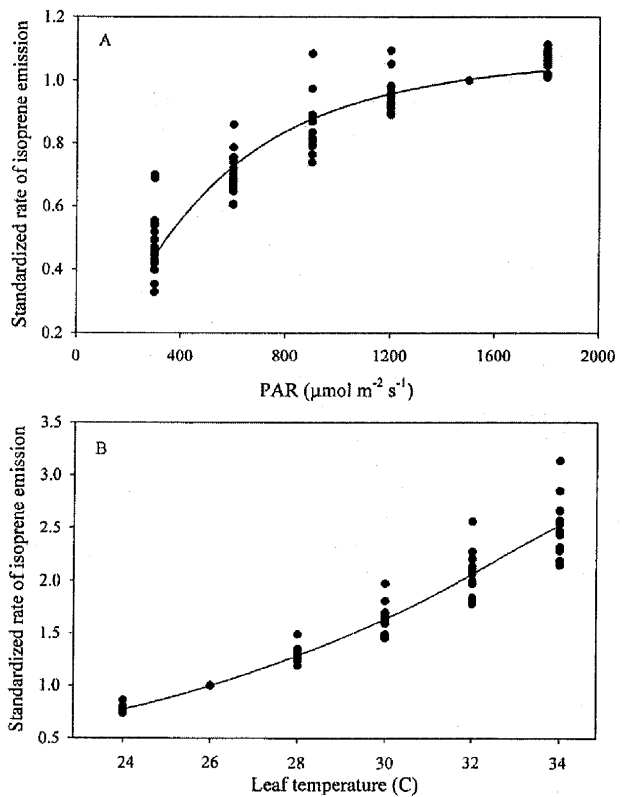
Figure 2. Response of isoprene emission to (a) varying light at standard temperature of 26°C and (b) varying temperature at standard light of 1500 µmol m^{-2} s^{-1} in *E. saligna*. Regression lines are derived from equations (2) and (3).
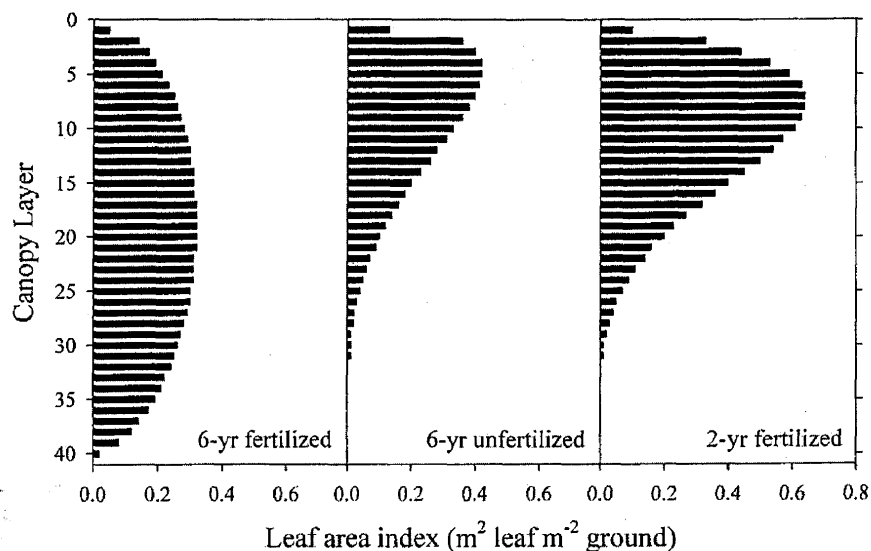
Figure 3. Modeled leaf area distribution in 6-year fertilized, 6-year unfertilized, and 2-year fertilized *E. saligna* canopies. Values were derived from measurements in one plot per treatment [*Hedman and Binkley*, 1988]. Layer 1 is at the canopy top.

supply [*Funk et al.*, 1999; *Karl et al.*, 2002; *Kreuzwieser et al.*, 2002], age-related declines in stand productivity in general [*Gower et al.*, 1996; *Ryan et al.*, 1997] and at this site [*Ryan et al.*, 2004] may result in older trees having less carbon available for isoprene production.

4.2. Leaf- Versus Canopy-Level Flux

[30] By using a canopy flux model, we have been able to explore how fertilization- and age-related effects on canopy structure influence stand isoprene emission. Our flux estimates indicate that changes in canopy-scale emissions, as mediated by fertilization and age, are not significant in forest canopies with high LAI. Simulated canopy emission rates were not different between fertilized and unfertilized plots despite a 1.5-fold difference in LAI. CANVEG calculations showed that 60 to 90% of PAR was absorbed in the first 10 canopy layers, corresponding to an LAI of 4.5. Thus, once the saturation LAI is reached, additional leaf area is likely shaded and, because of the strong sensitivity of isoprene emissions to light intensity, may contribute little to total canopy emission. A sensitivity analysis for LAI on canopy emission in 2-year fertilized stands suggested that canopy-level isoprene flux may saturate at an LAI of 7 at high light and an LAI of 5 under cloudy conditions (Figure 6).

[31] Slightly higher leaf-level emission rates in 2-year fertilized stands did not result in significantly higher modeled canopy flux. However, flux patterns differed across treatments depending on whether a common or variable isoprene SER was used (Figures 4 and 7) suggesting that, even in high LAI stands, increased leaf-level SER could result in higher canopy-level flux. Thus canopy emission could be significantly higher for species that are able to respond to fertilization with increases in leaf N and, consequently, isoprene SER. In lower LAI stands, isoprene SER and canopy structure may interact to produce nonlinear
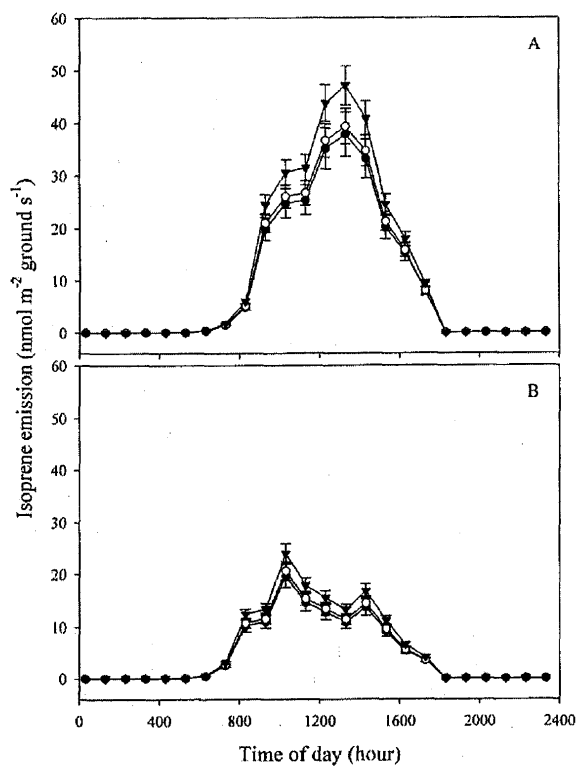
Figure 4. Modeled diurnal canopy isoprene flux from *E. saligna* stands on (a) a sunny day (3 May 2000) and (b) a cloudy day (24 April 2000) using different isoprene SER for each plot type (see values in Table 1). Symbols are flux values from one replicate plot (6-year fertilized, solid circles; 6-year unfertilized, open circles; 2-year fertilized, solid triangles). Error bars represent canopy flux rates ±1 standard error of isoprene SER.
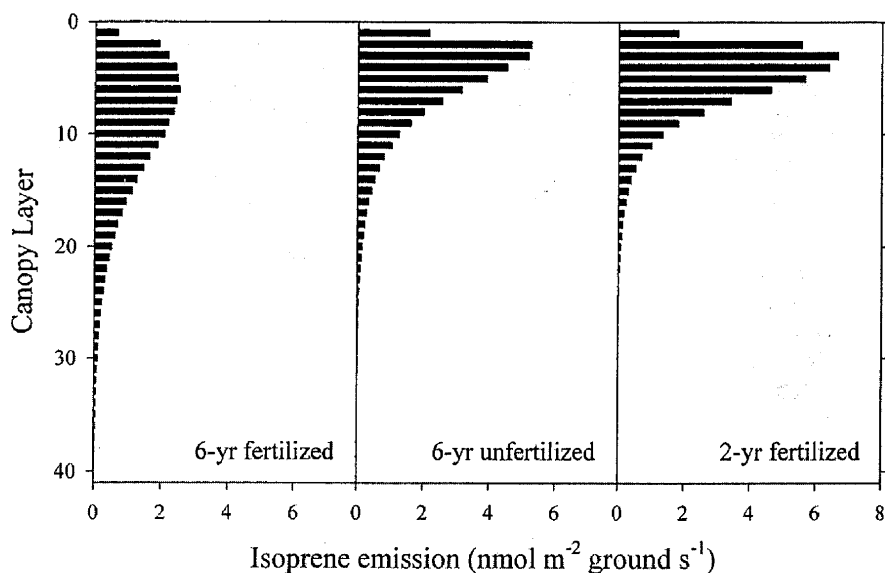
Figure 5. Midday modeled canopy isoprene emission with canopy height for 6-year fertilized, 6-year unfertilized, and 2-year fertilized *E. saligna* stands. Values shown are from one plot per treatment on a sunny day. Layer 1 is at the canopy top. Values from all layers sum to canopy-level flux.

effects on canopy flux. For example, high UV-B did not inhibit leaf-level isoprene SER [*Harley et al.*, 1996a] but may decrease canopy-level emission through reductions in total leaf area. In addition, modeled canopy isoprene flux was either lower or unchanged in response to elevated CO_2, despite increases in stand NPP [*Constable et al.*, 1999]. The authors attributed the lack of isoprene response to increased shading in canopies, which resulted from higher foliar density under elevated CO_2 conditions. These results were independent of biochemical depressions of isoprene emission in response to elevated CO_2 [*Rosenstiel et al.*, 2003], which were not considered in the model.

4.3. Implications for Landscape-Scale Modeling

[32] This study has shown that leaf-level isoprene SER (1) can more than triple over a small range of leaf N (Figure 1), (2) is slightly higher in young plants compared to older plants, and (3) changes markedly with canopy height. These conclusions highlight the need to incorporate spatial and ontogenetic variation in leaf-level emission rates into flux models that are used to make large-scale and long-term predictions. The widely used leaf-level model of *Guenther et al.* [1993], employed here to adjust isoprene SER to light and temperature environment, is based on the short-term response of isoprene emission to light and temperature. Current scaling models are diverse in their treatment of isoprene SER and canopy micrometeorology. While many canopy models do not address the spatial variation in isoprene SER within a canopy [*Lamb et al.*, 1993, 1996; *Guenther et al.*, 1995; *Pier and McDuffie*, 1997], other models adjust isoprene SER through changes in LAI [*Baldocchi et al.*, 1999; *Guenther et al.*, 1999] and specific leaf weight [*Geron et al.*, 1994] with canopy depth.

[33] The robust relationship between leaf N and isoprene SER across canopy layers observed in this study (Figure 1) suggests that leaf N profiles may be used to approximate isoprene SER. However, leaf-level models that incorporate N as a driver of process rates require an understanding of the mechanisms underlying this phenomenon, and interactive canopy effects, such as those between leaf N content and light availability [*Harley et al.*, 1994; *Litvak et al.*, 1996], will complicate these efforts. As demonstrated here, canopy structure is likely to change with increased
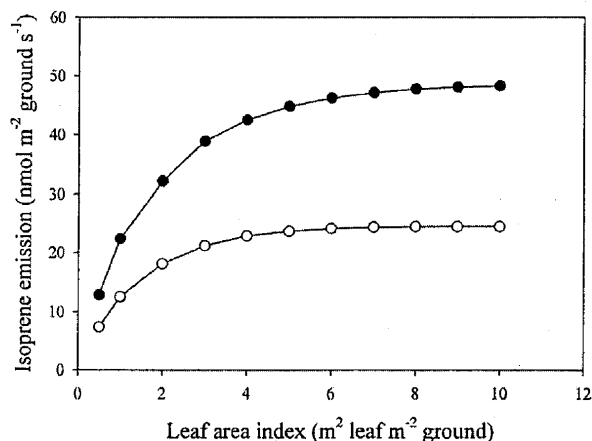
Figure 6. Modeled canopy isoprene flux for varying LAI from 2-year fertilized *E. saligna* stands on a sunny day (incident PAR 1682 μmol m^{-2} s^{-1}, solid circles) and a cloudy day (incident PAR 794 μmol m^{-2} s^{-1}, open circles).
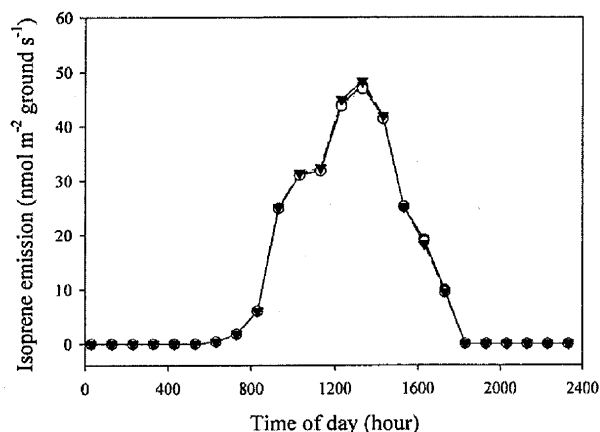
Figure 7. Modeled diurnal canopy isoprene flux from *E. saligna* stands on a sunny day (3 May 2000) using a common isoprene SER (32.1 nmol m^{-2} s^{-1}) for each plot type. Symbols are flux values from one replicate plot (6-year fertilized, solid circles; 6-year unfertilized, open circles; 2-year fertilized, solid triangles).

nutrient supply and stand age [e.g., *Aber*, 1979; *Fahey et al.*, 1998; *Parker et al.*, 2002], which may alter canopy light environment and N and leaf distribution through stand development.

5. Conclusion

[34] The conversion of degraded agricultural land and natural forest into intensively managed plantation forests will likely affect regional atmospheric chemistry through changes in the composition and quantity of hydrocarbons emitted by the vegetation. Increased reliance on chemical fertilizers to manage productivity in these plantations may also further alter the impact of these conversions on regional isoprene budgets. Additionally, anthropogenic activities are doubling the amount of fixed N entering terrestrial systems [*Vitousek et al.*, 1997] and altering the nutrient balance of natural forests. In this study, similar isoprene SER and high LAI across fertilization treatments led to similar modeled canopy flux. However, fertilization may have greater effects on canopy isoprene emission from stands with lower LAI or from tree species that respond to fertilization with increases in leaf N. While leaf- and canopy-level emissions may be higher in young forest canopies (including plantations with short rotations), this study also suggests that total canopy isoprene flux may remain high in older stands. *E. saligna* and *E. grandis* are two of the most widely planted *Eucalyptus* species in the world with successful plantings across the tropics [*Food and Agriculture Organization*, 1981], and our results suggest that nitrogen enrichment may not lead to significant increases in emissions from plantation forests of these species, although different species of *Eucalyptus* may respond differently.

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