



AMAZALERT Delivery Report

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To complete by the Coordinator

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Caxiuana field experiment, 25 September-9 October 2012

Introduction

In September-October 2012 a fieldwork campaign was organised at the Caxiuana experimental site in the eastern Brazilian Amazon. In this campaign researchers from EMBRAPA and ALTERRA were involved. Goal was to perform a set of new physiological measurements that have specific application to model development. These leaf scale parameters, the temperature dependence of maximum rate of carboxylation (Vcmax) and maximum rate of electron transport (Jmax) will be fed into new model formulations, which will then be tested against ecosystem scale measures of NPP and CO_2 exchange. During this campaign we measured the response of these leaf photosynthetic capacity parameters (Vcmax, Jmax) to short-term temperature change. At the same time we installed equipment to heat up and monitor a subset of leaves, to enable the assessment of long-term warming effects. We also evaluated leaf-level water potential, stomatal conductance (determined with porometer), specific leaf area, nutrients and non-structural carbohydrates.

This document serves as a detailed reference to all measurements made during that period. Separately there is a data base in which all data are brought together, quality checked and available for further analysis of parameters.

Materials and Method

Equipment Licor 6400 **Owner:** Thomas Domingues. Calibration: 21-9-2012 (zero and 400ppm) Licor 6400: Owner: Embrapa (Projeto Manflora) Calibration: 21-9-2012 (zero and 400ppm) Licor 6400: Owner: Inpa. Calibration: 21-9-2012 (zero and 400ppm) Settings used for all 3 Licor 6400's: Stomatal ratio = 0.5Flow=250 l/min Area=6 cm² Leaffan=fast DeltaT AP4 Porometer (2x) **Owner:** Alterra LAI2000 (Licor) (2x): Owner: Embrapa Pressure bomb (Plant Water Status Console / Soilmoisture Equipment Corp. USA): **Owner:** Embrapa Other equipment brought to Caxiuana: car batteries (5x), 100ah solar battery (2x), solar panel 100W(2x), boxes, CR10x datalogger, thermocouple wire, solar controllers/power regulators (2x), electrical wire, cylinder CO_2 , cylinder N_2 , scanner, microwave oven, spare parts and tools.

Experimental design

See "Informação Torres - caxiuanã-versao 08102012.xlsx" for a detailed overview of the experiment set up.

In Table 1 a short overview of the leaves selected for measurements.

Table1: Overview of leaves selected for the experiment. A=control plot; B=dry plot; Top=sun exposed leaves; Middle=sub canopy; Shade=shaded leaves; Heated=leaf with a heated element below; Element/no heating=element below the leaf but not heated; No element=reference leaf.

	Co	ontrol plot				Dry plo	t
Code	Specie	level	Treatment	Code	Specie	level	Treatment
A8.1.1	maparajuba	Тор	Heated	B1.1.1	Mouriri	Тор	Heated
A8.1.2	maparajuba	Тор	Element/no heating	B1.1.2	Mouriri	Тор	Element/no heating
A8.1.3	maparajuba	Тор	No element	B1.1.3	Mouriri	Тор	No element
A8.2.1	maparajuba	Тор	Heated	B1.2.1	Mouriri	Тор	Heated
A8.2.2	maparajuba	Тор	Element/no heating	B1.2.2	Mouriri	Тор	Element/no heating
A8.2.3	maparajuba	Тор	No element	B1.2.3	Mouriri	Тор	No element
A8.3.1	maparajuba	Тор	Heated	B2.1.1		Тор	Heated
A8.3.2	maparajuba	Тор	Element/no heating	B2.1.2		Тор	Element/no heating
A8.3.3	maparajuba	Тор	No element	B2.1.3		Тор	No element
A8.4.1	maparajuba	Тор	Heated	B2.2.1		Тор	Heated
A8.4.2	maparajuba	Тор	Element/no heating	B2.2.2		Тор	Element/no heating
A8.4.3	maparajuba	Тор	No element	B2.2.3		Тор	No element
A8.5.1	maparajuba	Тор	Heated	B2.3.1		Тор	Heated
A8.5.2	maparajuba	Тор	Element/no heating	B2.3.2		Тор	Element/no heating
A8.5.3	maparajuba	Тор	No element	B2.3.3		Тор	No element
A8.6.1	maparajuba	Тор	Heated	B2.7.1		Middle	Heated
A8.6.2	maparajuba	Тор	Element/no heating	B2.7.2		Middle	Element/no heating
A8.6.3	maparajuba	Тор	No element	B2.7.3		Middle	No element
A8.7.1	maparajuba	Middle	Heated	B2.8.1		Middle	Heated
A8.7.2	maparajuba	Middle	Element/no heating	B2.8.2		Middle	Element/no heating
A8.7.3	maparajuba	Middle	No element	B2.8.3		Middle	No element
A8.8.1	maparajuba	Middle	Heated	B2.9.1		Middle	Heated
A8.8.2	maparajuba	Middle	Element/no heating	B2.9.2		Middle	Element/no heating
A8.8.3	maparajuba	Middle	No element	B2.9.3		Middle	No element
A8.9.1	maparajuba	Middle	Heated				
A8.9.2	maparajuba	Middle	Element/no heating				
A8.9.3	maparajuba	Middle	No element				
A8.13.1	Duguetia	Shade	Heated				
A8.13.2	Duguetia	Shade	Element/no heating				
A8.13.3	Duguetia	Shade	No element				
A8.14.1	Duguetia	Shade	Heated				
A8.14.2	Duguetia	Shade	Element/no heating				
A8.14.3	Duguetia	Shade	No element				
A8.15.1	Duguetia	Shade	Heated				
A8.15.2	Duguetia	Shade	Element/no heating				
A8.15.3	Duguetia	Shade	No element				
A7.13.1	Licania	Shade	Heated				
A7.13.2	Licania	Shade	Element/no heating				
A7.13.3	Licania	Shade	No element				
A7.14.1	Licania	Shade	Heated				
A7.14.2	Licania	Shade	Element/no heating				
A7.14.3	Licania	Shade	No element				
A7.15.1	Licania	Shade	Heated				
A7.15.2	Licania	Shade	Element/no heating				
A7.15.3	Licania	Shade	No element				

Leaf heating system

We used electric resistance heaters powered by a solar panel (100W) and a 100ah solar battery pack to establish continues warming. Each heater had a 75 cm long, 10 Ω constantan wire folded into a 4x10 cm rectangular iron wire frame. The ends of the constantan wire were soldered to a normal electrical wire which was connected to a voltage converter which on its turn was connected to the battery pack. The frames were closed with aluminium tape and wrapped in aluminium foil. One of the corners of the foil was cut off so the rainwater could flow out if necessary. Three volts were run through the wire (1.2W). The heaters were placed approximately 2 cm below the leave by attaching them to the petiole and branch with iron wire extending from the frame. The connections between power supply, voltage regulators and leaf heater cables were made in small polystyrene boxes (Fig 1).



Fig 1: A resistance heater with folded constantan wire inside (left) and a resistance heater installed below a leaf (middle). Polystyrene boxes with the connections between power supply, voltage regulators and leaf heater cables (right).

At the control and dry plot respectively 3 and 2 tree species were selected. Depending on the distance of the trees to the tower, sun and shaded leaves were selected for measurements. See table 1 for a short description. Together and nearby each heating element an element was installed below a leaf without any heating to measure the influence of the element itself on the leaf. And at the same spot a leaf was selected as a reference (no element installed below). In the control plot 15 heating elements were installed and at the dry plot 8. See "Informação Torres - caxiuanã-versao 08102012.xlsx" for tree numbers, species, heights etc.

Leaf temperature measurements

The heating effect of the leaf heaters was verified by a set of thermocouples attached with Hansaplast sporttape to the lower surface of a subsample of leaves. Only a limited number of leaves was monitored this way because of limited availability of datalogger capacity.

We used two types of sensors: absolute sensors, consisting of single thermocouples, and measuring the absolute leaf temperature, and relative sensors, consisting of two-way thermopiles that compared pairs of heated and non-heated leaves.

We used approximate 0.5 mm thick, copper-constantan type thermocouple wire, twisted and soldered together at the measurement points. Absolute sensors were then connected to a CR10X datalogger using the special thermocouple measurement instruction. Relative sensors consisted of four measurement tips, with the wire alternating between copper-constantan-copper-constantan-copper, and measured as normal differential microvolt signals (Fig 2).



Fig 2: Thermocouple wires installed below a leaf (left) fixed with sporttape to a leaf (right).

Thermocouples (TC) were mounted to leaves at sites A8.7, A8.8 and A8.9, with at each site one absolute TC on leaf *.1 (heated) and one at or near leaf *.3 (control). The differential TC's were mounted to the same or neighbouring leaves (in case of heated leaves, of course still above a leaf heater), with the two 'high' sides underneath the *.1 leaf and the 'low' sides near a *.3 leaf. See table 2 for exact numbering of thermocouples and how they relate to leaves, datalogger input channels and datalogger output fields.

All signal wires were threaded into the same polystyrene box as where the leaf heaters were connected, to connect to a CR10X (Campbell scientific) data logger (Fig 1). The absolute thermocouples were connected as single-ended sensors, and their associated temperatures calculated using specific logger

instructions for Cu/Co Tc wire. The differential TC's were connected as differential sensors to respective hi- and lo poles, and the temperature difference signals calculated using normal differential microvolt voltage instructions, with as multiplier the standard T sensitivity of Cu/Co wire (11.63) multiplied by a factor two, because the two-stage pile. All data were collected at 15 s intervals and averaged over 60 minutes, and stored. Along with the averages, also their standard deviations were collected, as well as panel temperature and power voltage. The datalogger program is listed in appendix A.

Table 7.	Thormocour	la numbarina	1+0	he filled)
Table 2.	тпетносоир	ie numbering	(loc	<i>be nneu)</i>

Input location	Thermocouple	Temperature	Leaf number
	Number	Absolute/differential	
1		Absolute	
2		Absolute	
3		Absolute	
4		Absolute	
5		Absolute	
6		Absolute	
7		Differential	
8		Differential	
9		Differential	

Light and Ci response curves and dark respiration

Leaf gas exchange was measured with a LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA), under different combinations of CO_2 , temperature and light levels on young, fully expanded leaves from 8 am to 4 pm local time. During some days measurements extended until 5-6 pm.

We measured A/PAR and A/Ci curves before we installed the heating elements. We choose to start with the leaves where we planned to install a heating element (numbers 1) and the numbers 3 as a reference.

The protocols have been changed several times, based upon the preliminary results. See Table 3 for the different protocols. We avoided measuring including negative photosynthesis (respiration) in the curves, because in that condition the value of Ci becomes questionable (opposite CO_2 gradient, where diffusion might also take place through epidermis). We aimed to collect enough, but not more than that, numbers along the Ci and PAR responses to be able to:

- 1- fit dark respiration (Rd) and quantum efficiency (at least three points at as low light as possible, and ambient CO_2)
- 2- fit Vcmax (three points at low CO₂, high PAR)
- 3- fit Jmax (two to three points at high (hopefully saturating) CO₂ and high (saturating) PAR).

Initially, we started at ambient CO_2 and high light, but to save time, in a later stage we eliminated double points: we started immediately at low light, going down further, then stepped to high light, while increasing CO_2 , and skipping the CO_2 associated with the light responses (400 ppm). Also, we realised that, in the canopy top of the control plot, photosynthesis was not saturating yet at both PAR=1000 µmol m⁻² s⁻¹ and CO_2 =1000 ppm. Therefore we increased the saturating light value to 1500 µmol m⁻² s⁻¹ and added CO_2 points at 1500 and 2000 ppm. To keep the check whether light saturation really took place at high values only, the last point at this top of canopy sample was taken at PAR=800 µmol m⁻² s⁻¹.

In principle, sampling (logging) was only started when the photosynthesis reading, and (variable per operator) Ci or gs also did not show a trend anymore, while fluctuations were less than about 10% of the reading. We started with logging manual with 5 repetitions at each point of the curve. From 30-9-2012 onwards we used the autolog option logging every 5 seconds for 2-3 minutes.

For dark respiration response to temperature, leaves were covered with black cloth for 30 minutes before measurements. We usually chose a leaf close to one marked for leaf gas exchange. Measurement details are the same for the regular measurements of gas exchange.

Table 3: curve measuring protocols.

Date	28-9-2012)	30-9-2012		3-10-2012		6-10-2012	
Logging	5 loggings at each po	(manual) bint.	Use autolog: every 5 seconds for 3 minutes		Use autolog: every 5 seconds for 2 minutes		Use autolog: every 5 seconds for 2 minutes	
Matching analysers	Ma ev au m ex de		Match was set to every 10 minutes in autolog \rightarrow No matches were done except manual defined		Match analysers by big changes in CO ₂ and/or PAR settings. Preferably after every new setting.		Match analysers by big changes in CO ₂ and/or PAR settings. Preferably after every new setting.	
Light response	curve (A/PA	AR)						
	PAR	CO2	PAR	CO2	PAR	CO2	PAR	CO2
	1000	400	1000	400	100	400	100	400
	900	400	900	400	50	400	50	400
	300	400	300	400	25	400	25	400
	100	400	100	400	1000	400	1000	400
	50	400	50	400			1500	400
			25	400				
Ci response cui	rve (A/Ci)							
	1000	400	1000	400	1000	100	1500	100
	1000	300	1000	300	1000	250	1500	250
	1000	100	1000	100	1000	800 Tower B	1500	800 Tower B
	1000	50	1000	50	1000	1000	1500	1000
	1000	400	1000	400	1000	1500	1500	2000
	1000	800	1000	1000			800	2000
	1000	1000	1000	1500				

We aimed at each leaf to generate curves at leaf temperatures ranging from 25 to 36 degrees (25, 28, 31 and 36°C). Initially we tried to achieve this by fixing the block temperature, as this is the most stable. However, at high radiation load, cooling down the leaf was very difficult, and the leaf temperature was actually variable and correlated with the light intensity. This is a problem, because in fitting the physiological parameters we need the dark respiration (Rd), which is temperature dependent while measured at low radiation loads, and measurements at high light, and hence higher leaf temperature. This makes it difficult to fit the curves for each temperature class. Therefore, we attempted to fix the leaf temperature, but this often resulted in very long equilibration times. In the end, we attempted to bring the leaf temperature as closely as possible to a target, by manually varying the block temperature. This has led to measurements made not always at the pre-defined temperatures. Analysis of the data should therefore account for the measured leaf temperatures, and combine only those data points that had comparable temperatures.

It took a while for the crew to realise what a match is and when it is needed. So early measurements were often not perfectly matched (except for the measurements done with the Licor 6400 owned by EMBRAPA). At a later stage, matching was mainly done if there were large changes in CO_2 or H_2O , because then the analysers are operating in a different concentration range. Also, with larger changes in light, matching was needed, because the 'analysis' analyser changed concentration substantially. Matching should only be done when the CO_2 reading is stable. Finally, changes in temperature also lead to calibration changes, therefore matching is also needed after a step change in temperature. A sign that the match between analysers is not optimal, was often the occurrence of a negative value for Ci, caused by an apparent photosynthesis rate that is too high in comparison with stomatal conductance, or an apparent conductance that is too low. In the case of respiration measurements, however, a negative Ci value often occurs, because photosynthesis is negative while stomatal conductance is still positive (respiration but also transpiration). In that case matching is not needed, and in fact, useless. Ci has no meaning in this case. If negative values for stomatal conductance occur, that is a sign that condensation is taking place. This should be avoided, and should be suppressed by drying the inlet (reference) air.

Porometer and Leaf water potential

Stomatal conductance and leaf transpiration were measured at the leaves selected for the heating experiment using a Delta-T AP4 porometer. At the control plot the measurements were done at the 4th of October 2012 starting at 9, 12 and 15 hour. At the dry plot the measurements were done at the 5th of October starting at 9, 12 and 16 hour. At the same time leaves close to the leaves selected for the heating experiment, were sampled and water potential was measured using a pressure bomb (Soilmoisture Equipment Corp. USA). A predawn water potential measurement was done at 5 o'clock in

the morning at the same days. For the predawn measurements 2-3 leaves were enclosed in aluminium foil in the previous afternoon to guarantee dark conditions during the next day measurements.

LAI

On the 10th of October 2012 we performed measurements of the fraction of sky visible to the sensor and thus also to the leaf (DIFN). For detailed instructions on using the LAI2000 see appendix B.

Leaf sampling NSC

For the non-structural carbohydrates, we sampled leaves from branches close to and under the same environmental conditions as for those leaves selected for the heating experiment. Sampled leaves were immediately stored in zip-lock bags under refrigeration. From 10-15 minutes after sampling leaves were dried in microwave oven for two cycles of 20 seconds. Then samples were stored in paper bags that were oven-dried at ~50-60 °C.

Species	Code	Position	Rep	Date
	A7		А	6-Oct-12
	A7		В	6-0ct-12
	A7		С	6-0ct-12
	A7		D	6-0ct-12
	A7		E	6-0ct-12
Duguetia	A8		А	6-0ct-12
Duguetia	A8		В	6-0ct-12
Duguetia	A8		С	6-0ct-12
Duguetia	A8		D	6-0ct-12
Duguetia	A8		E	6-0ct-12
Duguetia	A8		F	6-0ct-12
Duguetia	A8		G	6-0ct-12
	A8	dossel	А	6-0ct-12
	A8	dossel	В	6-0ct-12
	A8	dossel	С	6-0ct-12
	A8	dossel	D	6-0ct-12
	A8	dossel	Е	6-0ct-12
Maparajuba	A8	subdossel	А	6-0ct-12
Maparajuba	A8	subdossel	В	6-0ct-12
Maparajuba	A8	subdossel	С	6-0ct-12
Maparajuba	A8	subdossel	D	6-0ct-12
Maparajuba	A8	subdossel	E	6-0ct-12
	B1	dossel	А	6-0ct-12
	B1	dossel	В	6-0ct-12
	B1	dossel	С	6-0ct-12
	B1	dossel	D	6-0ct-12
	B2		А	6-0ct-12
	B2		В	6-0ct-12
	B2		С	6-0ct-12
	B2		D	6-0ct-12
	B2		E	6-Oct-12
	B2	dossel	А	6-Oct-12
	B2	dossel	В	6-Oct-12
	B2	dossel	С	6-0ct-12
	B2	dossel	D	6-0ct-12
	B2	dossel	E	6-0ct-12

Table 4: Overview of leaves selected for the NSC measurements.

Dataset organisation

All Licor6400 data were quality checked and an average of the last 5 loggings was made.

Conclusion and progress

In March, 2013, the heating elements and thermocouples were checked in the field. Almost all were still working, but most of the leaves with thermocouples attached had died, so these need to be replaced. As

far as useful, the thermocouple data showed that the heated leaves did indeed heat up with a few degrees.

Data analysis, establishing the parameters, is progressing. And will be ready for reporting soon.

In May 2013 another fieldwork campaign is planned. The Ci response curves and light response curves will be repeated on both the control leaves and the leaves heated since October 2012.

Appendix A: Data logger program

CR10X data logger programme: 'LOGGER PROG THERMOCOUPLES 60 MIN.CSI'

```
;{CR10X}
*Table 1 Program
 01:15
            Execution Interval (seconds)
1: Internal Temperature (P17)
1:30
         Loc [ tref
                      ]
2: Thermocouple Temp (SE) (P13)
1:6
         Reps
2:1
         2.5 mV Slow Range
3:1
         SE Channel
4:1
         Type T (Copper-Constantan)
5:30
         Ref Temp (Deg. C) Loc [ tref
                                        ]
         Loc [ Tabs_1 ]
6:1
         Multiplier
7:1.0
8: 0.0
         Offset
3: Volt (Diff) (P2)
1:3
         Reps
2:1
         2.5 mV Slow Range
3:4
         DIFF Channel
4:7
         Loc [ tdiff_1 ]
5: 11.6279 Multiplier
6: 0.0
         Offset
4: Batt Voltage (P10)
         Loc [ battery ]
1:31
5: If time is (P92)
1: 0000 Minutes (Seconds --) into a
2:60
         Interval (same units as above)
3:10
          Set Output Flag High (Flag 0)
6: Real Time (P77)^16551
          Year, Day, Hour/Minute (midnight = 0000)
1:1110
7: Average (P71)^27583
1:9
         Reps
2:1
         Loc [ Tabs_1 ]
8: Standard Deviation (P82)^252
1:9
         Reps
```

2: 1 Sample Loc [Tabs_1]

9: Average (P71)^4788 1: 2 Reps 2: 30 Loc [tref]

*Table 2 Program 02: 0.0000 Execution Interval (seconds)

*Table 3 Subroutines

End Program

-Input Locations-
1 Tabs_1 1 2 1
2 Tabs_2 9 2 1
3 Tabs_3 9 2 1
4 Tabs_4 9 2 1
5 Tabs_5 9 2 1
6 Tabs_6 25 2 1
7 tdiff_1 13 2 1
8 tdiff_2 9 2 1
9 tdiff_3 17 2 1
10 1 0 0
11 1 0 0
12 0 0 0
13 0 0 0
14 0 0 0
15000
16 0 0 0
17 0 0 0
18 0 0 0
19 0 0 0
20 0 0 0
21 0 0 0
22 0 0 0
23 0 0 0
24 0 0 0
25000
26 0 0 0
27 0 0 0
28 0 0 0
30 tref 121
31 battery 111
-Program Security-
0000
0000
0000
-Mode 4-
-Final Storage Area 2-
0
-CR10X ID-
0
-CR10X Power Up-
3

-CR10X Compile Setting-3 -CR10X RS-232 Setting--1 -DLD File Labels-0 -Final Storage Labels-0,Tabs_1_AVG~1,27583 0,Tabs_2_AVG~2 0,Tabs_3_AVG~3 0,Tabs_4_AVG~4 0,Tabs_5_AVG~5 0,Tabs_6_AVG~6 0,tdiff_1_AVG~7 0,tdiff_2_AVG~8 0,tdiff_3_AVG~9 1,tref_AVG~30,4788 1,battery_AVG~31 2,Tabs_1_STD~1,252 2,Tabs_2_STD~2 2,Tabs_3_STD~3 2,Tabs_4_STD~4 2,Tabs_5_STD~5 2,Tabs_6_STD~6 2,tdiff_1_STD~7 2,tdiff_2_STD~8 2,tdiff_3_STD~9 3,Year_RTM,16551 3,Day_RTM 3,Hour_Minute_RTM

Appendix B: Short manual LAI 2000

To make LAI2000 measurements or DIFN (fraction of sky visible to the sensor) in a forests it is almost necessary to use 2 sensors. One on top of the tower (Above=A) (or in a big open space) and the other one for measurements below or in the foliage (Below=B).

No measurements can be made in direct sunlight. To avoid this, follow the additional procedure: Use the 270° view cap (25% closed) on **both** sensors. Put the sensor for the A readings on top of the tower in the shade of e.g. a solar panel, rain gauge etc. in such a way the cap is blocking only this object but the sensor is in the shade. (e.g. the sensor is in the shade of the rain gauge but the sensor doesn't 'see' the rain gauge because it is in the part blocked by the cap). Make the B readings with your back to the sun, and the view cap blocking the sensors view of you and the sun. So make sure the sensor is shaded by you and all the obstacles you don't want in your measurements (yourself, tower parts etc.) are blocked by the view cap.

Switch on both consoles. Press [on] Press [break] Check if the day and time are equal for both consoles. If not press [FCT] [05] and synchronise.

Console for above readings:

Press [FCT] [11] This should give you 'rmt abv x'. Scroll with $[\uparrow \text{ or } \downarrow]$ to find the right mode. X means your sensor is connected to the left 15 pin D connector at the console. Press [enter] AVE = 0 LOG int 15 sec (logging every 15 seconds) Start time (insert the time you want the instrument to start measuring) Stop time (insert time to finish measuring) Put the sensor on top of the tower. Press [log] WHERE = cax (e.g.) WHEN = day number (we always use Julian day) Now it starts measuring and logging at the time you set. You can always stop the logging by pressing [break]. No data will be lost. Press [FCT] [09] to switch off

Console for below readings: Press [FCT] [11] This should give you 'rmt blw y'. Scroll with [\uparrow or \downarrow] to find the right mode. Y means your sensor is connected to the right 15 pin D connector at the console. AVE = 0 Press [log] WHERE = cax (e.g.) WHEN = day number (we always use Julian day) Start making your measurements by pushing the button on the sensor arm. Keep the sensor levelled straight. When finished press [break] Press [FCT] [09] to switch off

Use the Fv2000 software to download the data and to analyse your data and calculate the LAI and /or DIFN.

(DIFN = fraction of sky visible to the sensor (= diffuse non-interceptance); 0=no sky visible to the sensor, 1=no foliage visible to the sensor.)