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TITLE:

Field Measurement of Effective Leaf Area Index Using Optical Device in Vegetation Canopy

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SUMMARY:
Fast and precise leaf area index (LAI) estimation in terrestrial ecosystems is crucial for a wide range of ecological studies and calibrating remote sensing products. Presented here is the protocol for using the new LP 110 optical device for taking ground-based *in situ* LAI measurements.

ABSTRACT:
Leaf area index (LAI) is an essential canopy variable describing the amount of foliage in an ecosystem. The parameter serves as the interface between green components of plants and the atmosphere, and many physiological processes occur there, primarily photosynthetic uptake, respiration, and transpiration. LAI is also an input parameter for many models involving carbon, water, and the energy cycle. Moreover, ground-based *in situ* measurements serve as the calibration method for LAI obtained from remote sensing products. Therefore, straightforward indirect optical methods are necessary for making precise and rapid LAI estimates. The methodological approach, advantages, controversies, and future perspectives of the newly developed LP 110 optical device based on the relation between radiation transmitted through the vegetation canopy and canopy gaps were discussed in the protocol. Furthermore, the instrument was compared to the world standard LAI-2200 Plant Canopy Analyzer. The LP 110 enables more rapid and more straightforward processing of data acquired in the field, and it is more affordable than the Plant Canopy Analyzer. The new instrument is characterized by its ease of use for both above- and below-canopy readings due to its greater sensor sensitivity, in-built digital inclinometer, and automatic logging of readings at the correct position. Therefore, the

hand-held LP 110 device is a suitable gadget for performing LAI estimation in forestry, ecology, horticulture, and agriculture based on the representative results. Moreover, the same device also enables the user to take accurate measurements of incident photosynthetically active radiation (PAR) intensity.

INTRODUCTION:

Canopies are loci of numerous biological, physical, chemical, and ecological processes. Most of them are affected by canopy structures[1]. Therefore, accurate, rapid, non-destructive, and reliable *in situ* vegetation canopy quantification is crucial for a wide range of studies involving hydrology, carbon and nutrient cycling, and global climate change[2,3]. Since leaves or needles represent an active interface between the atmosphere and vegetation[4], one of the critical canopy structural characteristics is leaf area index (LAI)[5], defined as one-half of the total green leaf surface area per unit of horizontal ground surface area or crown projection for individuals, expressed in m^2 per m^2 as a dimensionless variable[6,7].

Various instruments and methodological approaches for estimating terrestrial LAI and their pros and cons in diverse ecosystems have already been presented[8–15]. There are two main categories of LAI estimation methods: direct and indirect (see comprehensive reviews[8–12] for more details). Mainly used in forest stands, ground-based LAI estimates are routinely obtained using indirect optical methods due to the lack of direct LAI determination, but they usually represented a time-consuming, labor-intensive, and destructive method[9,10,12,16]. Moreover, indirect optical methods derive LAI from more easily measure related parameters (from the viewpoint of its time-demanding and labor-intense nature)[17], such as the ratio between incident irradiation above and below the canopy and the quantification of canopy gaps[14]. It is evident that Plant Canopy Analyzers have also been widely used to validate satellite LAI retrievals[18]; therefore, it has been considered a standard for LP 110 comparison (see **Table of Materials** for more details about employed instruments).

The LP 110, as an updated version of initially self-made simple instrument ALAI-02D[19] and later LP 100[20], was developed as a close competitor for Plant Canopy Analyzers. As a representative of indirect optical methods, the device is hand-held, lightweight, battery-powered, without any need for a cable connection between the sensor and data-logger that uses a digital inclinometer instead of a bubble level and enables faster and more accurate positioning and value reading. In addition, the device was designed to note immediate readouts. Thus, the time estimate needed for collecting data in the field is shorter for the LP 110 than Plant Canopy Analyzer by approximately ⅓. After the export of readouts to a computer, the data are available for subsequent processing. The device records irradiance within the blue light wavelengths (i.e., 380–490 nm)[21,22] using an LAI sensor for making an LAI calculation. The LAI sensor is masked by an opaque restriction cap with 16° (Z-axis) and 112° (X-axis) fields of view (**Figure 1**). Thus, light transmittance can be noted using the device held either perpendicularly to the ground surface (i.e., zenith angle 0°), or at five different angles of 0°, 16°, 32°, 48°, and 64° to be able also to deduce canopy elements' inclination.

[Place **Figure 1** here]

Due to the higher sensitivity of the LAI sensor, its restricted field of view, in-built digital inclinometer, automatic logging of reading values at the correct position indicated by sound without a button press, the new instrument is also suitable for above-canopy readings at narrow valleys or even on broader forest roads to measure a wide range of sky conditions. Besides that, it enables quantification of mature stand canopies above the relatively high regeneration, and it attains higher accuracy of irradiance values than Plant Canopy Analyzer. Moreover, the price of LP 110 equals about ¼ of the Plant Canopy Analyzer. Contrariwise, the utilization of LP 110 in dense (i.e., LAIe at stand level over 7.88)[23] or very low canopies as grassland is limited.

The LP 110 can work within two operating modes: (i) a single sensor mode taking both below-canopy and reference readings (above the studied canopy or in a sufficiently widespread clearing located within the vicinity of the analyzed vegetation) performed before, after, or during below-canopy measurements taken with the same instrument and (ii) a dual sensor mode using the first instrument for taking below-canopy readings, whereas the second one is employed for automatically logging reference readings within a regular predefined time interval (from 10 up to 600 s). The LP 110 can be matched with a compatible GPS device (see **Table of Materials**) to record each below-canopy measurement point's coordinates for both the modes mentioned above.

The effective leaf area index (LAIe)[24] incorporates the clumping index effect and can be derived from measurements of solar beam irradiance taken above and below the studied vegetation canopy[25]. Thus, for the following LAIe calculation, transmittance (t) must be calculated from irradiation both transmitted below the canopy (I) and incident above the vegetation (I_o) measured by the LP 110 device.

$$t = I / I_0 \quad (1)$$

Since the irradiation intensity exponentially decreases as it passes through a vegetation canopy, LAIe can be calculated according to the Beer-Lambert extinction law modified by Monsi and Saeki[9,26]:

$$LAIe = - \ln (I / I_0) \times k^{-1} \quad (2),$$

Where, k is the extinction coefficient. The extinction coefficient reflects each element's shape, orientation, and position in the vegetation canopy with the known canopy element inclination and view direction[9,12]. The k coefficient (see equation 2) depends on the absorption of irradiance by foliage, and it differs among plant species based on the morphological parameters of canopy elements, their spatial arrangement, and optical properties. Since the extinction coefficient usually fluctuates around 0.5[9,27], equation 2 can be simplified as presented by Lang et al.[28] in a slightly different way for heterogeneous and homogenous canopies:

In a heterogeneous canopy

$$LAIe = 2 \times |\varnothing \ln t| \quad (3),$$

or

In a homogeneous canopy

$$LAIe = 2 \times |\ln T| \quad (4),$$

Where, t: is transmittance at each below-canopy measurement point, and T: is the average transmittance of all *t* values per measured transect or stand.

In forest stands, LAIe must be further corrected due to a clumping effect of the assimilation apparatus within the shoots[29–34] to obtain the actual LAI value.

The protocol is devoted to the practical utilization of the LP 110 optical device for estimating LAIe in a selected example of Central European conifer forest stands (see **Table 2** and **Table 3** for the site, structural, and dendrometric characteristics). LAIe estimation in a vegetation canopy using this device is based on a widely used optical method related to the transmittance of photosynthetically active radiation and canopy gap fraction. The paper aims to provide a comprehensive protocol for performing LAIe estimation using the new LP 110 optical device.

PROTOCOL:

NOTE: Before beginning to take planned field measurements, sufficiently charge the battery of the LP 110 device. Connect the instrument (USB connector, see **Figure 1**) and the computer through the attached cable. Battery status is shown in the left-upper corner of the device display.

1. Calibration before measurement

NOTE: For the LP 110, perform a dark calibration of the LAI sensor and in-built inclinometer calibrations before beginning each field measurement campaign.

1.1. LAI sensor's dark calibration

1.1.1. Turn on the instrument by pressing and holding the **Set** key for at least 1 s.

NOTE: The **Set** button serves as the Enter key.

1.1.2. Select **Settings** (the **Menu** key allows to shift up and down) and press the **Set** > **Lai Cal.**, press the **Set** key, and then check to see whether the LAI calibration constant is fixed to 1 (i.e., C = 1.0); if not, press the **Set** key repeatedly to adjust the constant to 1.0 and return back to the main menu (press **Menu | Return | Set**).

NOTE: When taking LAI measurements using the single sensor mode (see section 2), a constant value of 1.0 is recommended for all measurements.

1.1.3. Select **Settings** and press **Set | Lai zero | Set**. Completely cover the LAI sensor using, for instance, an opaque cloth or palm to avoid light interference during the whole calibration process. Afterward, press the **Set** key to maintain the zero value that appears on the display.

1.1.4. Press the **Menu** key repeatedly till **Return** is selected to return to the main menu, and then press the **Set** key.

1.2. Inclinometer calibrations

NOTE: Each LP 110 device is equipped with a built-in electronic inclinometer to ensure the correct inclination angle of readings. The internal inclinometer must be (re-)calibrated using a water level.

1.2.1. Vertical calibration

1.2.1.1. If the device is switched off, press and hold the **Set** key for at least 1 s to turn on the instrument.

1.2.1.2. Select **Settings** and press **Set | Vertical Cal**. **| Set** to activate the electronic inclinometer.

1.2.1.3. Hold the device vertically and place a water level on its lateral side along with the instrument.

1.2.1.4. Balance the device to the left or the right according to the water level bubble to achieve a zero or close-to-zero value for the X-axis. If not, press the **Set** key to adjust the readings until zero for the X-axis is read.

1.2.1.5. Place the water level along the device's rear side to complete the vertical calibration.

1.2.1.6. Tilt the device again to the left or the right and check whether the device display reads zero for the X-axis.

1.2.1.7. Hold the zero-angle position for the X-axis and simultaneously tilt the device forward or backward (the Z-axis) according to the water level bubble, making sure to keep the X-axis angle value at zero or close to zero.

1.2.1.8. Check to see whether the Z-axis reading equals zero or approaches zero. If not, hold the **Set** key and recalibrate the device to set zero readings for both X- and Z-axes.

1.2.1.9. Press the **Menu** key repetitively until **Return** is selected to return to the main menu, and then press the **Set** key.

1.2.2. Horizontal calibration

1.2.2.1. Select **Settings** and press the **Set | Horizontal Cal. | Set** to trigger the electronic inclinometer.

1.2.2.2. Hold the device horizontally. Then, place the water level along the device's rear side.

1.2.2.3. Level the device in the horizontal position according to the water level bubbles. Tilt the instrument to the left or the right and up or down along the X- and Y-axes, respectively.

1.2.2.4. After achieving the correct sensor position according to both water level bubbles, check to ensure that the reading for the Y-axis is zero or close to zero. If not, press the **Set** key to recalibrate the horizontal position of the instrument.

1.2.2.5. Press the **Menu** key repetitively until **Return** is selected to return to the main menu, and then press the **Set** key.

2. Single sensor mode for LAIe estimation

2.1. If the device is turned off, press the **Set** key for at least 1 s to switch on the instrument.

2.2. **Calibrate** the instrument before beginning each field measurement campaign according to steps 1.1 and 1.2.

NOTE: If calibration has already been performed, skip to step 2.3.

2.3. Afterward, set the current date and time (find **Settings** in the main menu by repeatedly pressing the **Menu** key. Then, press **Set | Time**; press the **Set** button again) and return to the main menu (select **Return** and hold the **Set** key).

NOTE: For an exact time setting, match the time with the computer as displayed in the relevant software (connect the LP 110 device to the computer through the attached cable. Open the software, press the **Setup | Device ID | Device**. Choose and press **Online Control | Time**. Then, tick the **Synchronize with Computer Time** option and press **Edit**).

2.4. Set the instrument to the single angle measurement mode using **Settings**. Press **Set | Angles | Set | Single** (confirm using the **Menu** key) and return to the main menu (select **Return** and hold the **Set** key).

2.4.1. If leaf angle inclination needs to be estimated, set the multi-angle measurement mode. **Settings | Angles | Multi** (press the **Menu** button) and return to the main menu (select **Return** and hold the **Set** key).

2.5. If a record concerning the positions of the measurements is needed, turn the relevant GPS device on (see the sections below for detailed instructions and the **Table of Materials**); if not, skip to step 2.6.

2.5.1. Check to be sure the device's time matches the computer.

NOTE: The time must be set correctly to reflect the time zone at the studied location.

2.5.2. Switch on the GPS device and wait a moment till the current position is found. Check the location on the display of the GPS device.

NOTE: Precision is contingent on the density of the canopy of the studied vegetation.

2.5.3. Carry both the LP 110 and the GPS device when taking all the field measurements.

2.5.4. After taking all the field measurements, connect both devices to the computer, download, and process the data in the relevant software (see **Table of Materials**) according to the LP 110 Manual and User Guide, Operation Instructions section[35].

2.6. Take a reference measurement in an open area or above the measured vegetation (i.e., an above-canopy reading). In sunny weather, prevent light from directly entering the view restriction cup (see **Figure 1**).

NOTE: For single sensor measurement mode, take both above- and below-canopy readings under constant light conditions during standard overcast, before sunrise, or after sunset (**Figure 2**) to avoid obtaining incorrect irradiance values.

[Place **Figure 2** here]

2.6.1. Select **Measurement** in the main menu (press the **Set** key), and then choose **Lai Ref**. After pressing the **Set** key, the reference measurement mode is activated.

NOTE: The current irradiance value appears on the display. This value is not yet stored in the device's internal memory (the measurement mode is triggered at this time).

2.6.2. Subsequently, press the **Set** key again to commence a search for the correct LAI sensor position (i.e., zenith angle 0°), and to activate both the built-in inclinometer and sound indicator.

NOTE: Simultaneously, the current position of the LAI sensor appears on the display for both X- and Z-axes.

2.6.3. Afterward, hold the device perpendicularly to the ground and make sure the LAI sensor is pointed up toward the zenith.

NOTE: The sound indicator increases in volume as it approaches the correct zenith angle.

2.6.4. Check the display, tilt the instrument both to the left and to the right, and forward and backward. The reference value is automatically acquired and stored immediately once the zenith angle defined by both the X- and Z-axes reach zero or less than 5 (the beeping tone stops).

NOTE: Considering the correct position must be attained in a very narrow range (i.e., mm), this step can be wearisome.

2.7. After taking reference measurement(s), return to the measurement menu by pressing the **Menu** key. Then, start to measure the level of transmitted irradiance below the canopy.

2.7.1. Define the positions for taking below-canopy readings and start taking light transmittance value measurements using the device's LAI sensor.

NOTE: The pattern of LAIe field measurements in different canopy structures is mentioned in detail by Černý et al.[36] and Fleck et al.[37].

2.7.2. Select **Lai** in the measurement menu. Press the **Set** key to activate the mode for taking transmitted irradiance measurements below the canopy.

NOTE: The current irradiance value appears on the display. This value is not yet stored in the device's internal memory (the measurement mode is triggered at this time).

2.7.3. Press the **Set** key again to record the below-canopy readings. The in-built inclinometer and sound indicator are triggered to obtain the correct LAI sensor position (i.e., zenith angle 0°).

NOTE: Simultaneously, the current position of the LAI sensor appears on the display for both X- and Z-axes.

2.7.4. Subsequently, hold the device perpendicularly to the ground and make sure that the LAI sensor is pointed up toward the zenith.

NOTE: The sound indicator increases in volume as it approaches the correct zenith angle.

2.7.5. Check the display, tilt the instrument both to the left and to the right, and forward and backward. All below-canopy readings are automatically acquired and stored immediately once the zenith angle defined by both the X- and Z-axes reach zero or less than 5 (the beeping tone stops).

NOTE: Considering the correct position must be attained in a very narrow range (mm), this step can be wearisome.

2.8. Proceed with taking further measurements of transmitted irradiance below the vegetation canopy, following steps 2.7.3–2.7.5.

NOTE: Reference readings can also be taken anytime between below-canopy measurements. For instance, after completing each transect, press the **Menu** button, select **Lai Ref** (hold the **Set** key) and continue according to steps 2.6.2–2.6.4. The more above-canopy readings taken during below-canopy measurements, the greater accuracy of reference calculations.

2.9. Immediately after finishing taking below-canopy measurements (press the **Menu** button, select **Lai Ref** and hold the **Set** key), take a measurement of the irradiance in an open area to obtain the last reference value, following steps 2.6.2. to 2.6.4.

2.10. Press the **Menu** key repetitively until **Return** is selected to return to the main menu, and then press the **Set** button.

2.11. After each measurement, the data is stored in the device's internal memory. Hold the **Menu** button for at least 1 s to switch off the device safely without erasing any data.

2.12. Connect the instrument to the computer; download and process the data. An example of field measurement and LAIe calculation is described in section 4.

3. Dual sensor mode for estimating LAIe

3.1. Turn on both instruments by holding the **Set** key for at least 1 s.

NOTE1: Instrument_1 and Instrument_2 are designated for above- (reference) and below-canopy readings, respectively. In dual sensor measurement mode, one device (Instrument_1) is mounted on a tripod in an open area (or at the top of a climatic mast above the canopy), while the second one (Instrument_2) serves for taking below-canopy measurements of transmitted irradiance. Instrument_1 automatically logs the reference signal in a predefined time interval (from 10 s up to 600 s). This approach collects a significant amount of reference data, thus increasing the accuracy when calculating reference values for individual below-canopy measurements.

3.2. Set the current date and time of both instruments (find **Settings** in the main menu by repeatedly pressing the **Menu** button. Then, press **Set | Time | Set**. Return to the main menu (choose **Return** and hold the **Set** key).

NOTE: For an exact time setting, match the time with the computer as displayed in the relevant software (connect the device to the computer through the attached cable. Open the software, and then press **Setup | Device ID | Device**. Next, choose and press **Online Control | Time**. Tick the **Synchronize with Computer Time** option and press **Edit**).

3.3. Afterward, set both the instruments to the single angle measurement mode. Select **Settings** (hold the **Set** key) **| Angles | Set | Single** (confirm with the **Menu** key). Return to the main menu (choose **Return** and hold the **Set** key).

3.3.1. If the leaf angle inclination within the studied vegetation canopy needs to be estimated, set Instrument_2 (below-canopy readings) to the multi-angle measurement mode. Select **Settings** (press the **Set** key) **| Angles** (press the **Set** button). Next, choose **Multi** (confirm with the **Menu** key), and then return to the main menu (choose **Return** and hold the **Set** key).

3.4. If a record concerning the positions of below-canopy measurements is required, turn the relevant GPS device on (see the sections below for detailed instructions and the **Table of Materials**); if not, skip to step 3.5.

3.4.1. Make sure the time displayed on the device used for taking below-canopy readings (Instrument_2) matches the computer.

NOTE: The time must be set correctly to reflect the time zone at the studied location.

3.4.2. Switch on the GPS device and wait for a moment till the current position is found. Check the location displayed on the GPS device.

NOTE: Precision is contingent on the density of the canopy of the studied vegetation.

3.4.3. Carry both the LP 110 used for taking below-canopy readings (Instrument_2) and the GPS device when taking all field measurements.

3.4.4. After taking all field measurements, connect both devices (Instrument_2 and the GPS device) to the computer. Download and process the data in the relevant software (see **Table of Materials**) according to the LP 110 Manual and User Guide, Operation instructions section[35].

3.5. Calibrate both the instruments before beginning each field measurement campaign according to sections 1.1 and 1.2.

NOTE: If calibration has already been performed, skip to step 3.5.1.

3.5.1. After calibrating both the LAI sensor and the in-built inclinometer, calibrate both LP 110 devices (Instrument_1 and Instrument_2) with each other.

3.5.1.1. For both devices, select **Settings** in the main menu (press the **Set** key) and choose **Lai Calibration** (press the **Set** button). Next, hold both the devices in a horizontal plane in the vertical position, and adjust the constant value (marked as C on the display) by repeatedly pressing the **Set** key on Instrument_1 (reference readings) to achieve the same values as depicted on the device's screen on Instrument_2. Then, press the **Menu** button and return to the main menu (choose **Return** and hold the **Set** key).

3.6. In sunny weather, prevent direct sunlight from entering the view restriction cup when taking all above-canopy readings (see **Figure 1**).

NOTE: For dual sensor measurement mode, take both above- and below-canopy readings under constant light conditions with standard overcast, before sunrise, or after sunset (**Figure 2**) to avoid obtaining incorrect irradiance values.

3.7. Attach Instrument_1 vertically either to a tripod placed in an open area or above the studied canopy (e.g., at the top of a climatic mast).

NOTE: This device will continuously record reference values (i.e., above-canopy readings).

3.7.1. First, select **Settings** in the main menu (press the **Set** key), and then choose **Auto interval** (again press the **Set** key). Next, repeatedly press the **Set** key, and then hold the **Menu** button to select the required interval for automatically logging reference values (from 10 up to 600 s).

NOTE: Set a shorter time interval to automatically log reference readings to increase the measurements' accuracy if light conditions change rapidly.

3.7.2. Press the **Menu** key, select **Return**, and hold the **Set** button to return to the main menu.

3.7.3. Subsequently, press the **Menu** button (hold the **Set** key) repeatedly to select **Measurement** in the main menu. Then, choose **Auto Lai Ref**. (press the **Set** key) to start searching for the correct LAI sensor position (i.e., zenith angle 0°).

NOTE: The current irradiance value appears on the display. This value is not yet stored in the device's internal memory (the measurement mode is triggered at this time).

3.7.4. Check the display, tilt the instrument both to the left and to the right, and forward and backward. After reaching the zenith angle defined by X- and Z-axes with zero or less than the value of 5 (i.e., both X- and Z-axes below the value of 5), fix the device firmly at the required position mentioned above, and then press the **Set** key.

NOTE: From this step, reference values (i.e., above-canopy readings) are automatically recorded and stored in the predefined time interval (each reading is accompanied by beeping). Avoid any deviation from the set position of Instrument_1; otherwise, the reference measurement will be interrupted. Considering the correct position must be attained in a very narrow range (mm), this step can be wearisome.

3.8. Afterward, start to measure transmitted irradiance below the vegetation canopy (below-canopy readings) using Instrument_2.

NOTE: During all below-canopy readings, keep the same orientation of the LAI sensor's field of view (Instrument_2) as the reference readings' LAI sensor (Instrument_1), for instance, perpendicularly to the north.

3.8.1. Define the positions for below-canopy readings and start the light transmittance value measurements using the device's LAI sensor.

NOTE: The pattern of LAIe field measurements in different canopy structures is comprehensively described in Černý et al.[36] and Fleck et al.[37].

3.8.2. In the main menu, choose **Measurement** (press the **Set** key) and select **Lai**. Press the **Set** key to activate the mode for transmitted irradiance measurement below the canopy.

NOTE: The current irradiance value appears on display. This value is not yet stored in the device's internal memory (just the measurement mode is triggered at this time).

3.8.3. Press the **Set** key again to obtain the value of transmitted irradiance below the canopy and trigger both the in-built inclinometer and sound indicator serving to find the correct LAI sensor position (i.e., zenith angle 0°).

NOTE: Simultaneously, the current position of the LAI sensor appears on display for both X- and Z-axes.

3.8.4. Then, keep the device perpendicularly to the ground surface to be the LAI sensor pointed up to the zenith.

NOTE: The sound indicator increases its tone by approaching the correct zenith angle.

3.8.5. Check the display, tilt the instrument both to the left and to the right and forward and backward. All below-canopy readings are automatically acquired and stored immediately once the zenith angle defined by both the X- and Z-axes reach zero or less than 5 (the beeping tone stops).

NOTE: Considering the correct position must be attained in a very narrow range (mm), this step can be wearisome.

3.9. Proceed with taking further measurements of transmitted irradiance (i.e., below-canopy readings), following steps 3.7.4–3.7.6.

3.10. After taking the below-canopy measurements (Instrument_2), press the **Menu** button and the **Menu** key repeatedly until **Return** is selected to return to the main menu, and then press the **Set** button.

NOTE: After completing all the reference readings (Instrument_1), use the same way as for

Instrument_2.

3.11. The data is saved in the instrument's memory after each reading. Hold the **Menu** button for at least 1 s to turn off the device safely without erasing any data.

3.12. Connect the instrument to the computer; download and process the data. An example of field measurement and LAIe calculation is described in section 4.

4. An example of field measurement and LAIe calculation

4.1. Define the measurement points for taking below-canopy measurements. Arrange the measurement layout in transect (or a regular grid) with equidistant measurement points to capture the vegetation canopy's heterogeneity caused by different sizes of gaps.

NOTE: A transect layout appropriate for vegetation planted in rows with a homogenous canopy is depicted in **Figure 3**. For more details about measurement layout, follow Černý et al.[36] and Fleck et al.[37].

[Place **Figure 3** here]

4.2. Take both above- and below-canopy measurements using either single or dual sensor mode according to section 2 or section 3, respectively.

4.3. After completing all the field measurements, download the data into the computer from the LP 110 device(s) used in either single or dual sensor mode to estimate LAIe.

NOTE: For dual sensor mode, follow the steps mentioned below for both instruments (i.e., Instrument_1 and Instrument_2).

4.3.1. Connect the instrument to the computer through the attached cable.

NOTE: For dual sensor mode, connect the device used for taking reference measurements (i.e., above-canopy readings) first.

4.3.2. Open the relevant software (see **Table of Materials**) and press the **Setup** key in the main bar. Then, select and press **Device ID**.

NOTE: Device: LaiPen appears in the bottom-left corner.

4.3.3. Press the **Device** button and subsequently click on **Download**.

NOTE: The software also enables the user to write down any remarks within the sheet entitled Notes displayed in the bottom-left corner. The software automatically matches the above-canopy readings with each below-canopy (transmittance) reading based on the measurement time.

4.3.4. Press the **File** icon in the main menu; choose and click on **Export**. Then, tick **ALAI** and press **OK** to export the data.

NOTE: In the exported file (txt., xls.), above- and below-canopy readings (transmitted irradiance) are marked, ReferenceIntensity and transmittance, respectively.

4.4. Calculate the transmittance (*t*) value for each measurement point within the transect (or grid) according to equation 1: t = I / Io (irradiance transmitted below the canopy divided by incident irradiance above the vegetation) resulting in $t_1, t_2, \ldots, t_n$, where n: is the number of below-canopy measurement points.

4.5. Calculate the average transmittance (*T*) of the studied vegetation canopy, for instance, in the first transect (T_1): $T_1 = (t_1 + t_2 ... + t_n) / n$, where n: is the number of below-canopy measurement points within the first transect.

NOTE: If measurements are taken in multiple transects, proceed with all transects (T_2, T_3, and T_4) in the same way.

4.6. Since irradiation intensity exponentially decreases as it passes through the studied canopy, calculate LAIe following the modified Beer-Lambert extinction law (see equation 2).

4.6.1. First, find the logarithm of the mean transmittance value (*T*) of the studied vegetation canopy, for instance, in the first transect (T_I): T_I = - ln T_1.

NOTE: If measurements are taken in several transects, proceed with all the transects in the same way (i.e., T_II = - ln T_2; T_III = - ln T_3; T_IV = - ln T_4).

4.6.1.1. Calculate the mean transmittance value (*T*) from all individual transects: T = [(- ln T_I) + (- ln T_II) + (- ln T_III) + (- ln T_IV)] / 4.

4.6.2. Afterward, calculate the final LAIe value using an extinction coefficient specified for each plant species according to equation 2.

NOTE: Extinction coefficients for the main tree species are listed in Bréda[9] in **Table 1**. In forest stands, LAIe must be corrected due to a clumping effect of the assimilation apparatus within the shoots[29–34] to obtain the actual LAI value.

REPRESENTATIVE RESULTS:

The spatial structure obtained from both tested devices obviously differed in all studied plots, i.e., thinned from above (A), thinned from below (B) and a control without any silvicultural intervention (C; see **Table 2** for more details). At the stand level, similar differences in LAI values obtained from the LP 110 and the Plant Canopy Analyzer were confirmed between thinned plots with various densities (A vs. B) using ANOVA and Tukey's test. For the Plant Canopy Analyzer, significantly higher LAI values were observed in the control plot with no silvicultural intervention than in the thinned ones (A, B). However, the values significantly exceeded LAI obtained from the LP 110 in the control plot. For the LP 110, LAI did not significantly differ in the C and B treatments. Contrariwise, a significant difference in LAI values between the C and A plots was found. Generally, LAI significantly decreased after applied thinning treatments in the studied stands. LAI estimated using the LP 110 (LaiPen LP110) decreased more evidently in plot A, whereas the LAI values obtained from the Analyzer (LAI-2200 PCA) decreased more in plot B. Nevertheless, these recorded differences were slight (**Figure 4).**

[Place **Figure 4** here]

The LAI values' spatial variability is illustrated in **Figure 5** for each thinning treatment in pure Norway spruce pole stands.

[Place **Figure 5** here]

The LP 110 underestimated LAI by 7.4% and 10.6% in plots A and C, respectively. Contrariwise, this device overestimated the LAI stand value obtained from the Plant Canopy Analyzer in plot B by 3.7%. If the total averages from all LAI values regardless of the thinning treatment applied were calculated and subsequently compared (LP 110 vs. Plant Canopy Analyzer), the LP 110 device underestimated LAI obtained by the Plant Canopy Analyzer by 5.8%. Subsequently, differences in specific LAI values measured above individual points arranged within the regular grid were calculated for both instruments, and these deviations were subsequently expressed as a percentage. Under these circumstances, the LAI values measured by the LP 110 and the Plant Canopy Analyzer differed profoundly (**Table 1**).

[Place **Table 1** here]

For all LAI data measured at a particular point level using the LP 110 and the Plant Canopy Analyzer, linear regression between both the employed devices was performed. The linear regression of $y = 0.8954x$ ($R^2 = 0.94$; RMSE = 2.11438) was found for all LAI data from both the tested instruments (**Figure 6**).

[Place **Figure 6** here]

FIGURE AND TABLE LEGENDS:

Figure 1: Physical features of the LP 110. The MENU key enables the user shift up and down throughout the display, and the SET button serves as the Enter key (**A**). The zenith view under different inclination angles (±8 due to the side view) and the horizontal view is fixed for LP 110 to 112° (**B**) similarly to the Plant Canopy Analyzer A (modified by restrictors).

Figure 2: Optimal weather conditions for taking LAIe measurements using the LP 110. The

optimal weather conditions when using the LP 110 are uniformly overcast skies with no direct solar radiation (**A**), or use either before sunrise or after sunset (**B**).

Figure 3: Transect's layout for estimating LAIe in homogenous vegetation cover. Transect I–IV: transect's number; ×: measurement point for taking the below-canopy reading. The first ten positions are labeled (1×–10×). Transects must be oriented perpendicularly to the rows of plants.

Figure 4: LAI values estimated using the LP 110 and the Plant Canopy Analyzer optical devices in Norway spruce pole stands under different silvicultural treatments. For estimating LAI, 81 below-canopy readings were taken in each studied stand. A: Thinning from above; B: Thinning from below; C: Control plot. The dots signify the mean LAI value. The whiskers display the standard deviations. Various letters indicate significant differences ($p < 0.05$) among the silvicultural treatments and different optical instruments using Tukey's Post-hoc test. This figure has been modified from Černý et al.[20].

Figure 5: Spatial heterogeneity of LAI estimated using the LP 110 and the Plant Canopy Analyzer at the level of individual measurement points under studied spruce canopy. A: Thinning from above; B: Thinning from below; C: Control plot. The numbers above arrows signify the lateral side length and spacing of measurement points within the regular grid. This figure has been modified from Černý et al.[20].

Figure 6: The linear regression among LAI values coming from the LP 110 and the Plant Canopy Analyzer at the level of individual measurement points in studied Norway spruce pole stands. This figure has been modified from Černý et al.[20].

Table 1: Mean LAI at the stand level and LAI differences expressed as a % between the LP 110 and the Plant Canopy Analyser at the level of individual measurement points. A: Thinning from above; B: Thinning from below; C: Control plot. This table has been modified from Černý et al.[20].

Table 2: Characteristics of the study site. This table has been modified from Černý et al.[20].

Table 3: Dendrometric and structural characteristics of the studied stands covering an area of 25 m x 25 m in 2014. In each studied stand, 81 below-canopy readings were taken within a regular grid (3 m x 3 m) under standard overcast skies (for more details, follow Černý et al.[20]). All LAI measurements were conducted in July and August when LAI values are most stable[9,38]. A: Thinning from above; B: Thinning from below; C: Control plot; DBH: stem diameter at breast height; $BA_{1.3}$: the basal area at breast height. For $BA_{1.3}$ at the stand level, the basal areas of each tree presented in the studied stand, calculated as: $BA_{1.3} = (\prod * DBH^2)/4$, was summed up. This table has been modified from Černý et al.[20].

DISCUSSION:

What are the differences between the LP 110 as a newly presented device for estimating LAI (or taking PAR intensity measurements) and the LAI-2200 PCA as an improved version of the previous standard LAI-2000 PCA for estimating LAI via an indirect method? Beyond the price being about fourfold higher for the Plant Canopy Analyzer compared to the LP 110, the number of output parameters, measurement conditions, methodological approaches, and possibilities of estimating LAI for different canopies, accuracy of results, etc., can be compared.

When comparing the hardware, the LP 110 seems to be more user-friendly. The LP 110 is a lighter device and does not require any cable connections between the sensors and the data-logger. Both sensors (i.e., for LAI and PAR measurements; see **Figure 1**) are integrated within the body of the device, allowing the operator to move easily throughout the studied ecosystem (e.g., in shrubs or dense forests). To ensure the reading value accuracy, a correct sensor position and value storage are essential. This position (either in the zenith or pre-set angles) is identified by a changing sound frequency if the sensor is close or far from the target position. Even under the most intensive sound (the volume can be corrected), the LP 110 held automatically saves the reading value. Contrariwise, finding the correct sensor position for the Plant Canopy Analyzer must be done with a manual bubble level on a hand-held stick. The operator must press the button to save the reading value simultaneously while checking the bubble level. However, the correct sensor position is routinely lost when pressing the button, resulting in decreased accuracy of the reading value. Since visually checking a bubble level is not necessary for taking LP 110 readings, there is also the possibility to hold the instrument on an extension rod, enabling the user to measure above canopies of natural or artificial regeneration, tall herbaceous or shrub layers. In this case, the correct sensor position can simply be found based on the changing sound frequency.

There are differences between the LP 110 and the Plant Canopy Analyzer in respect of LAI sensor construction, especially with regard to sensor sensitivity and the sensors' fields of view (FOV). If the LAI sensor of the Plant Canopy Analyzer is exposed to open-air, it can fog up under high air humidity conditions, which commonly occur in the early morning in open areas. Contrariwise, the LAI sensor of the LP 110 is fog-free as it is located inside the restrictor view cup (**Figure 1**). Although the restrictor of the LP 110's LAI sensor is removable, it has a fixed FOV; however, the FOV of the LAI sensor of the Plant Canopy Analyzer can be modified both in the azimuthal and zenith directions using different restrictors (opaque view caps) and by using a masking procedure during data post-processing, respectively. Even though the FOV of the LP 110's LAI sensor (**Figure 1**) is relatively narrow and cannot be manipulated compared to the Plant Canopy Analyzer, the sensitivity of this sensor is about tenfold higher. This higher LAI sensor sensitivity enables the user to take measurements using the LP 110 under conditions of low irradiance and also to take above-canopy (reference) readings on extremely narrow open plots, for instance, on narrow forest roads or lines. Furthermore, the above to below-canopy readings' ratio is higher, leading to increased accuracy of the measured transmittance and thus better LAIe estimation. On the other hand, it is necessary to increase the number of below-canopy readings per transect owing to the narrow FOV of the LP 110's LAI sensor.

There are some similarities between the LP 110 and the Plant Canopy Analyzer, for instance, in measuring conditions and in modifications of the LAI sensor zenith angle view (in directions of 0°, 16°, 32°, 48°, and 64° for the LP 110; and 7°, 23°, 38°, 53°, and 68° for the Plant Canopy Analyzer) to quantify the inclination angle of canopy elements. Similar to the Plant Canopy Analyzer, the LP 110 diminishes the effect of light reflectance and measures a real light absorption part of the light by foliage due to specific sensor wavelength characteristics. Other optical-based instruments such as SunScan, AccuPAR, TRAC[39], or DEMON[9,40] (for more details, see **Table of Materials**) measure under relatively wider light intervals regardless of the light reflectance. In dual sensor mode, it is possible to take automatic measurements with one sensor ordinarily placed in an open area to take above-canopy (reference) readings in time intervals ranging from 10–360 s and 5–3600 s for the LP 110 and the Plant Canopy Analyzer, respectively, and there is the possibility to add GPS positions to individual measurements. For both the instruments, it is impossible to measure LAIe: i) during and immediately after rain conditions, as wet canopy elements, including stems enhance both light reflectance and transmittance values below the canopy; thus, actual LAIe is underestimated under such conditions; ii) during windy conditions when canopy elements are moving, and transmittance values vary greatly even though the sensor position is stable, and iii) during unstable synoptic situations when light conditions change rapidly. The last condition is not so limiting for the LP 110 due to the sensor's narrow FOV. Also, a distance of obstacles need to be considered. However, a suitable sensor orientation lessens the problem. For both devices, it is likewise possible to estimate LAIe during a sunny day, mainly close to sunrise or sunset. Except for midday when direct sun rays can enter the LAI sensor through the restrictor cap slot, taking LAIe measurements is feasible throughout the whole day; even if the LAI sensor is perpendicularly oriented toward the sun (relevant for the LP 110) or the back of the operator (relevant for the Plant Canopy Analyzer). However, some correction procedures presented by Leblanc and Chen[41] must be applied. If the above-canopy readings vary by more than ±20% during a short time span (approximately 1–2 min), continuing to take LAIe measurements is useless due to the expected extremely high LAIe estimation error. That problem could be avoided with a precise synchronous estimation of above- and below-canopy readings in dual sensor mode employing two units with the same accurate time setup and calibration. The next critical step for estimating LAIe using the LP 110 is a selection of a suitable open area for above-canopy readings, especially for single sensor mode (the maximal time lag between above and below-canopy readings, i.e., forest stand and open plot, must be 15–20 min), where the size of the open area must respect the sensor FOV. Besides that, the LP 110 is similar to the Plant Canopy Analyzer, not suitable for accurately estimating LAIe in too dense (i.e., LAIe at stand level over 7.88)[23], very low canopies grassland, or the transmittance below 1%.

All the obtained values of incident light and light transmittance below the canopy with a time entry are post-processed using specific software, providing many output parameters, especially with the Plant Canopy Analyzer. Contrariwise, the software for processing the data obtained from LP 110 needs to be improved to be more automatic and user-friendly, such as the software relevant to Plant Canopy Analyzer. Moreover, it is advisable to modify the restriction cup for the LP 110 by the producer to change or adjust the sensor FOV.

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The authors have nothing to disclose. The representative results were used from the article Černý, J., Krejza, J., Pokorný, R., Bednář, P. LaiPen LP 100 – a new device for estimating forest ecosystem leaf area index compared to the etalon: A methodologic case study. *Journal of Forest Science.* **64** (11), 455-468 (2018). DOI: 10.17221/112/2018-JFS based on the Journal of Forest Science editorial board's kind permission.

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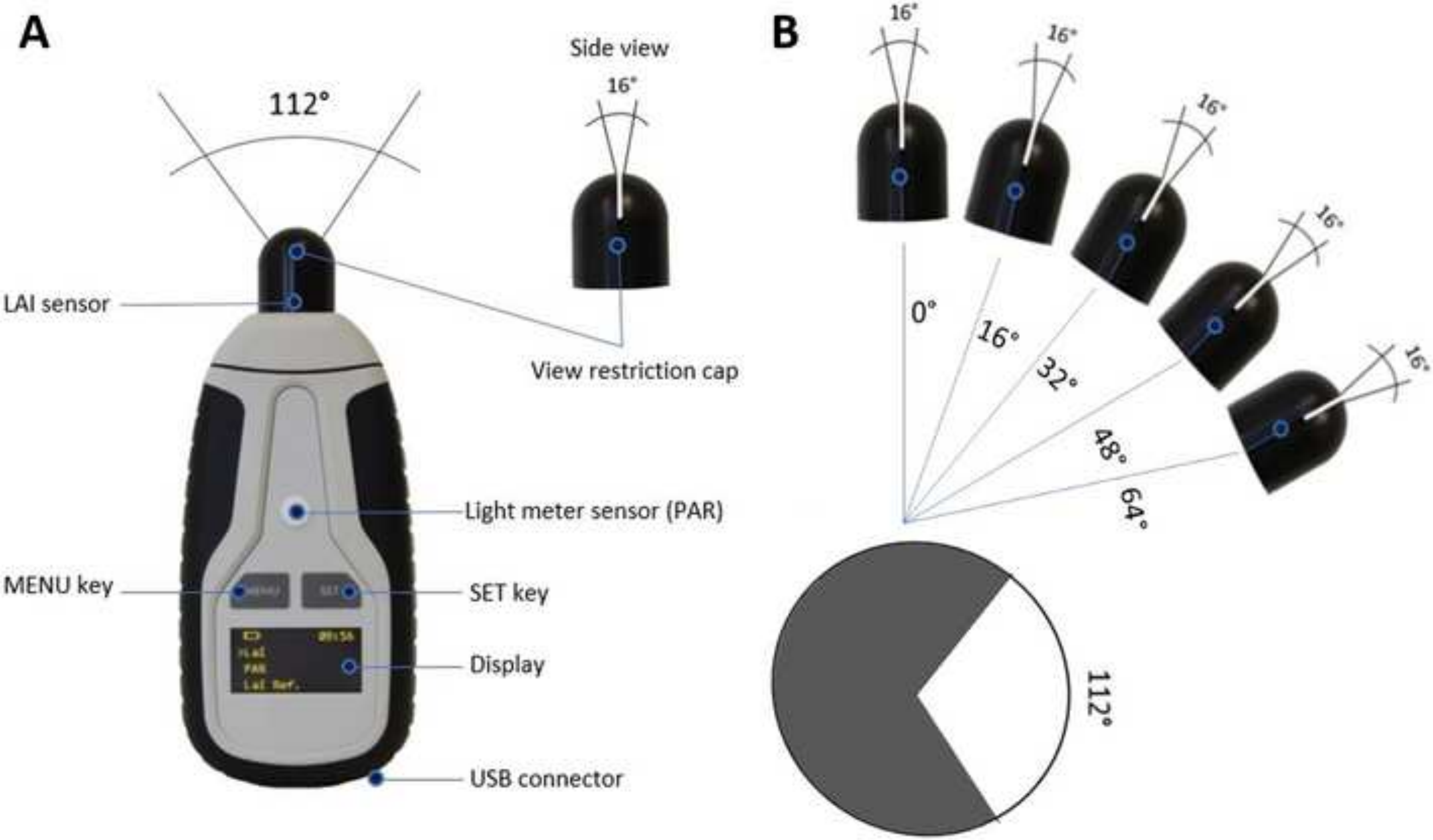
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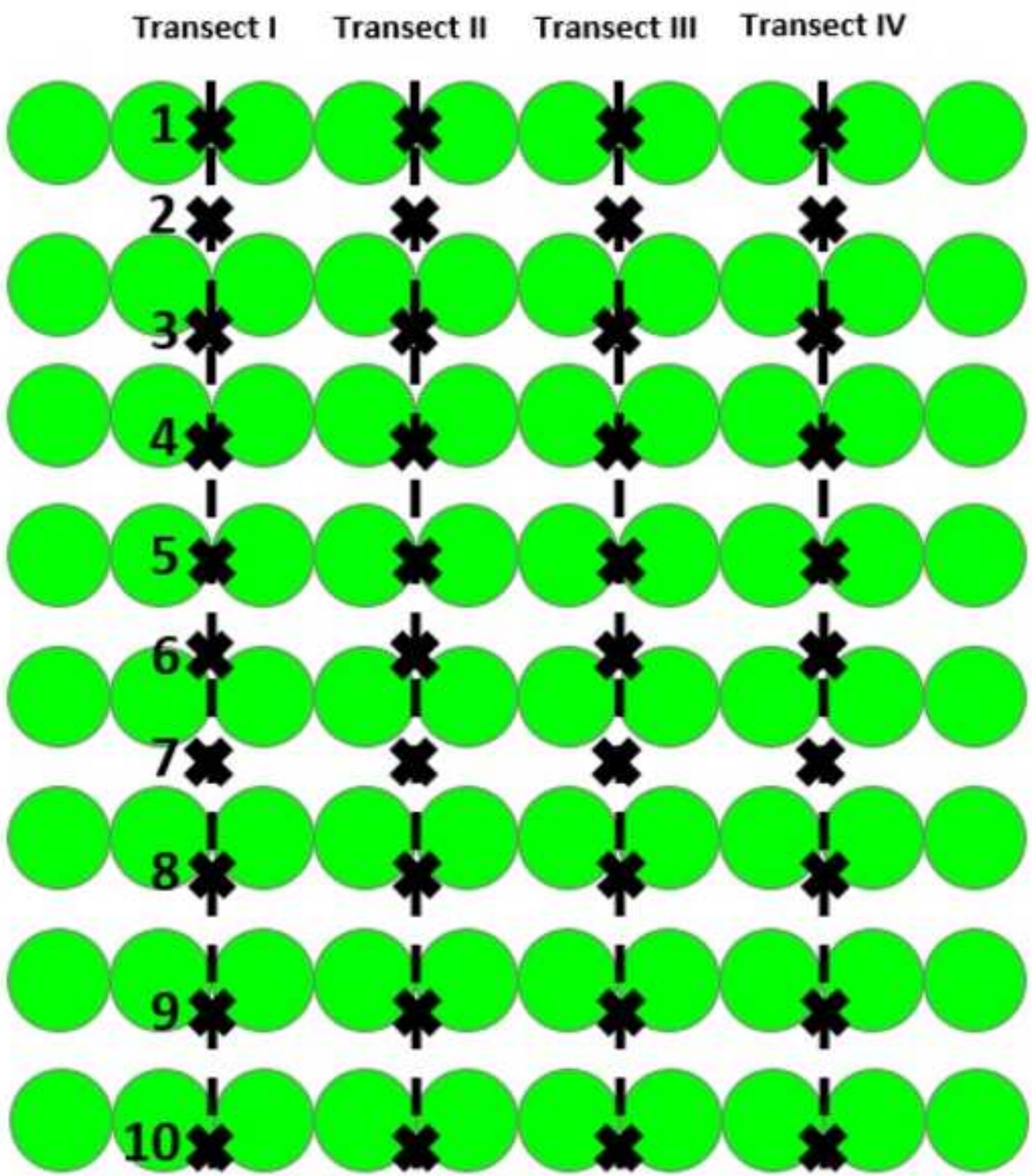
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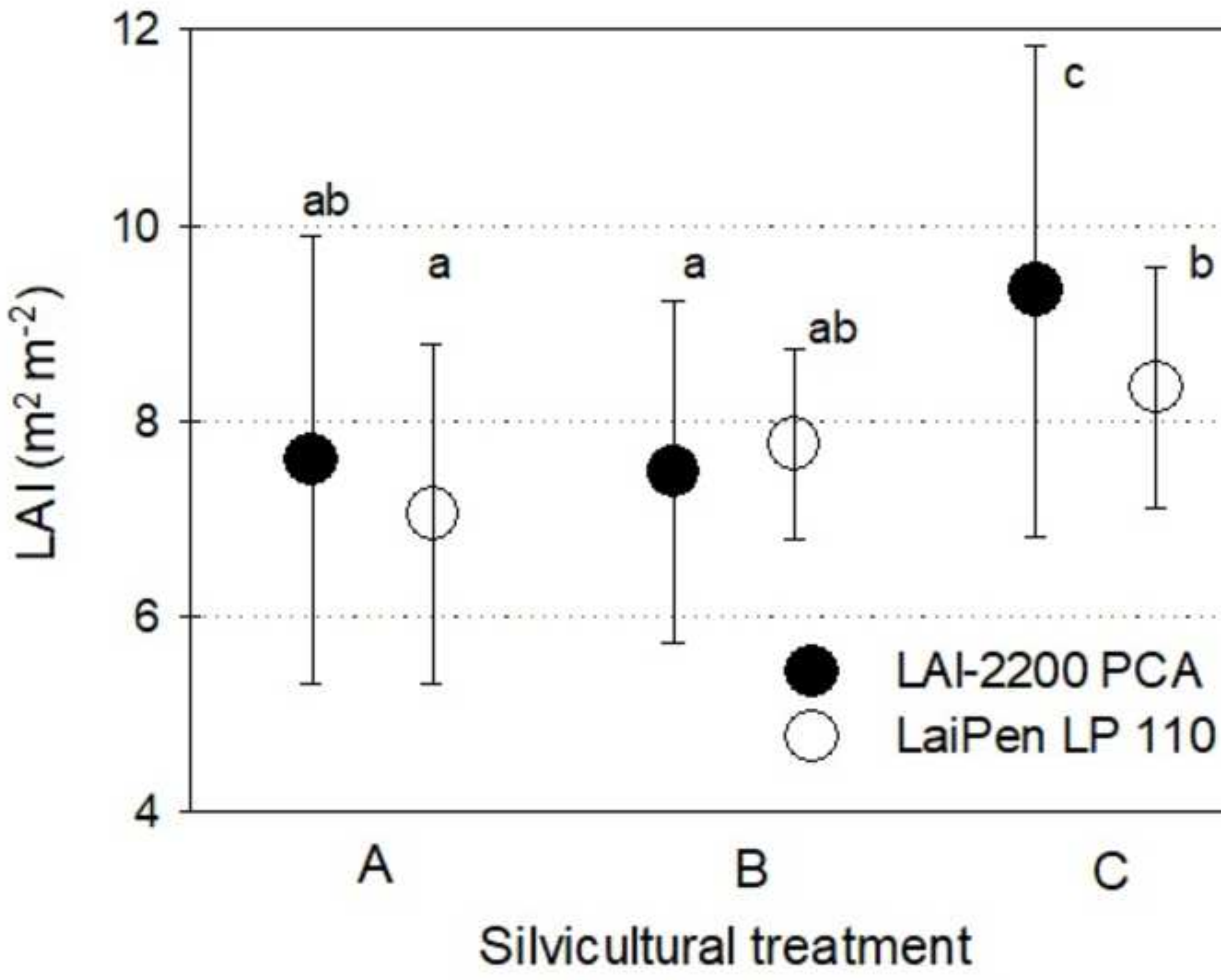

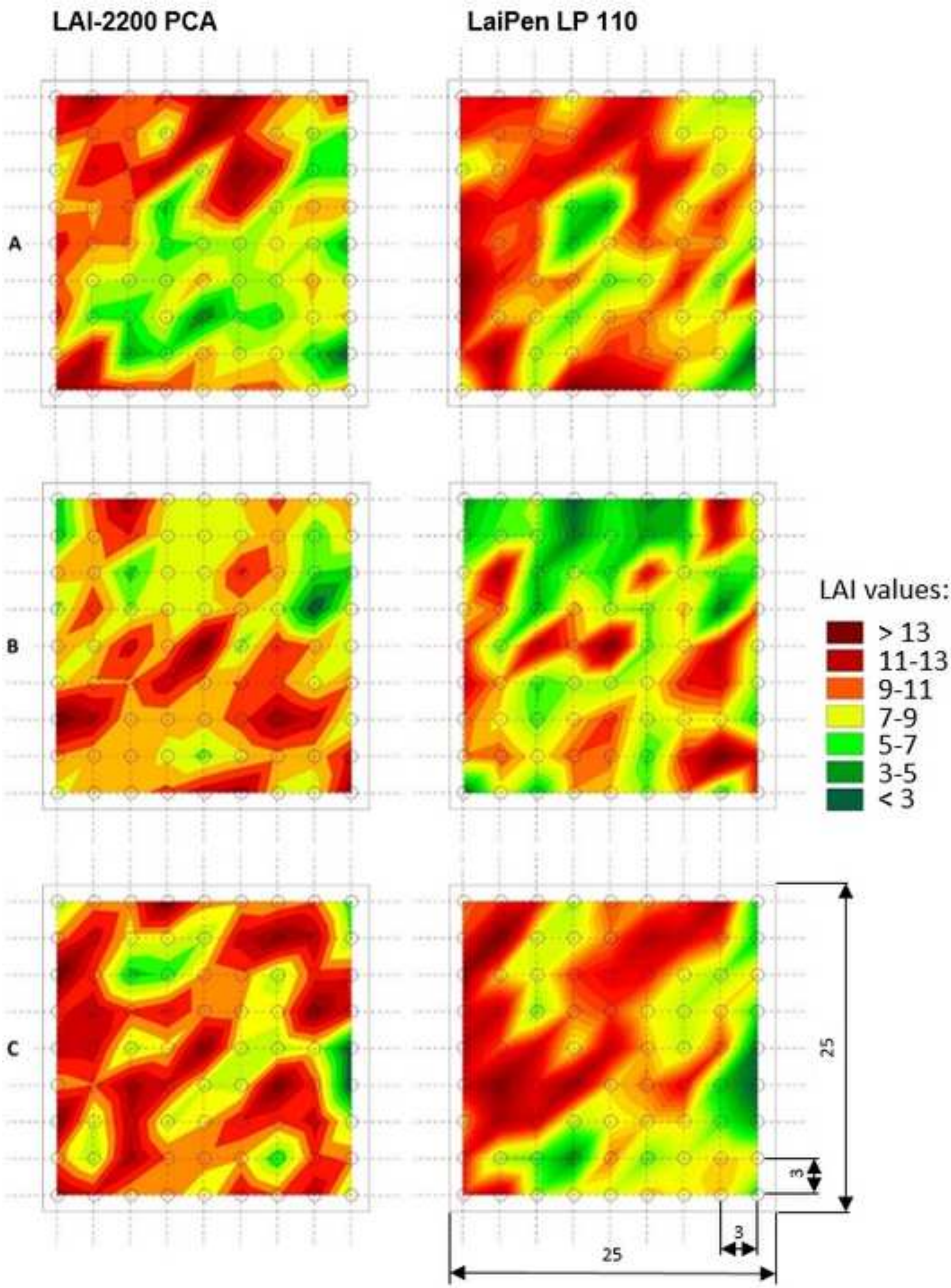
LAI-2200 PCA
LaiPen LP 110
A
B
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LAI values:
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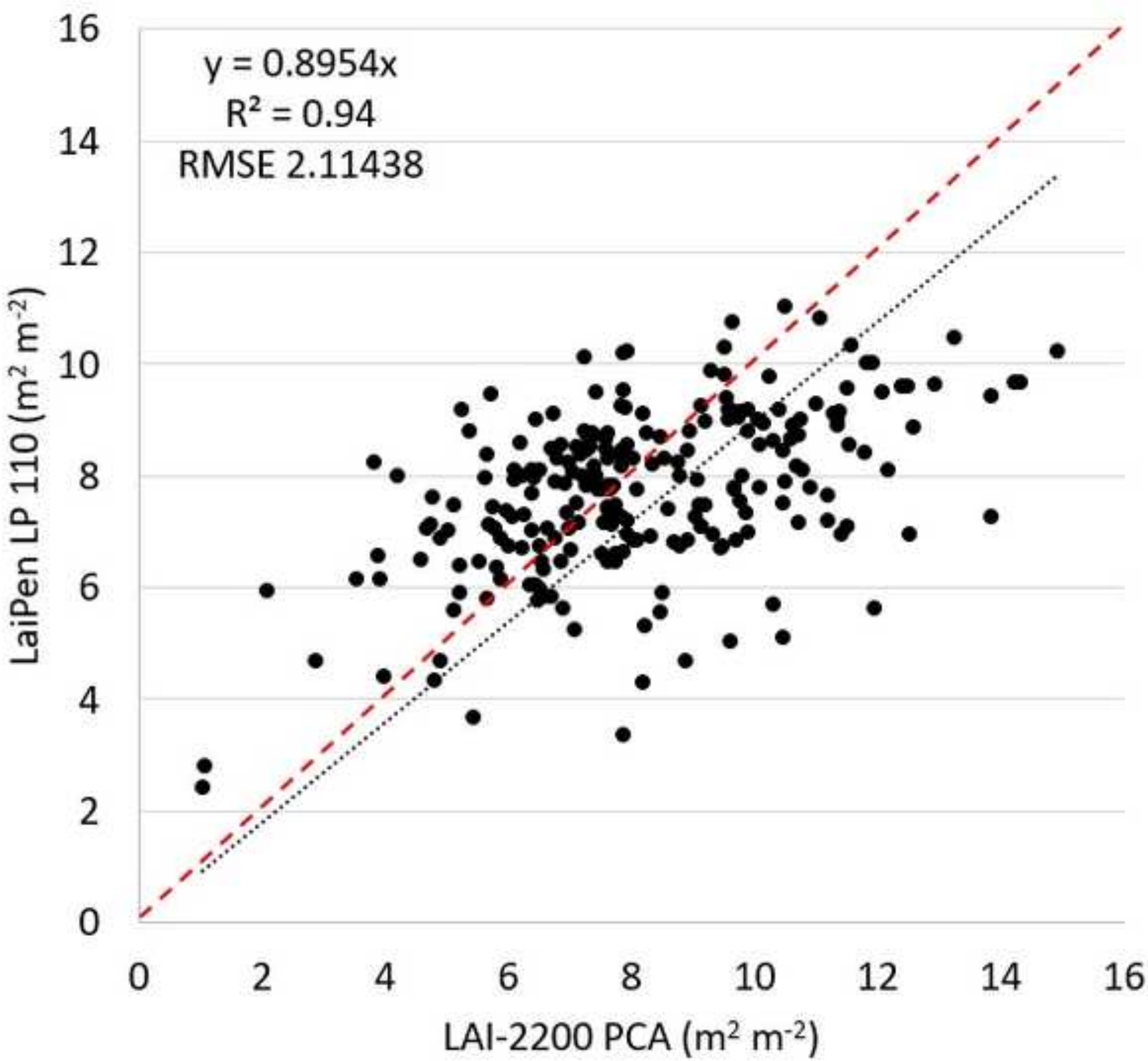

Silvicultural treatment	**Forest stand LAI**	
	LaiPen LP 110 (m^2 m^{-2})	LAI-2200 PCA (m^2 m^{-2})
A	7.05 ± 1.73	7.61 ± 2.29
B	7.76 ± 1.36	7.48 ± 1.75
C	8.35 ± 1.23	9.34 ± 2.51

Relative differences (%) among LAI from LaiPen LP 110 compared to LAI-2200 PCA at the level of individual measurement points
1 ± 37 (-58; 156)
8 ± 30 (-33; 183)
-5 ± 26 (-48; 115)

Geographic coordinates	49°29'31" N, 16°43'30" E
Altitude	610-625 m a. s. l.
Mean annual air temperature	6.5 °C
Mean annual precipitation	717 mm

Table 3

Plot	Age of stand (years)	Stand density (trees ha^{-1})	Height (m)	DBH (cm)
A	36	1.930	14.14 ± 3.73	14.84 ± 6.13
B	36	1.915	16.33 ± 2.37	15.81 ± 4.47
C	36	4.100	12.72 ± 2.68	10.97 ± 4.81

$BA_{1.3}$ ($m^2 \cdot ha^{-1}$)	Growing stock ($m^3 \cdot ha^{-1}$)
36.60 ± 0.25	250.02 ± 2.00
43.41 ± 0.17	290.07 ± 1.32
36.96 ± 0.19	287.12 ± 1.39

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Table of Materials
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Original comments from the editor and our response (yellowed)

Editorial comments:

1. Please revise the following lines to avoid previously published work:477-478, 617-618. Please refer the iThenticate report attached.
All lines mentioned above were rewritten to avoid overlapping with previously published papers.

2. Please reduce the usage of the term "LaiPen LP110" in the manuscript text. JoVE cannot publish manuscripts containing commercial language.
We avoided all terms "LaiPen LP110" in the whole manuscript text.

3. Comments to be addressed are included in the attached manuscript file.
All editorial comments were taken into account and implemented in the text of the manuscript (Ln. 608-610; 656; 673-674).

4. The references must be numbered in the order of citation. The reference order breaks in line 61 (ref 36 appears after ref 14). Please revise.
All references were ordered upwardly in the reference list based on their citation order in the text of the manuscript.

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Authors: Jakub Černý, Jan Krejza, Radek Pokorný, Pavel Bednář

Doi: https://doi.org/10.17221/112/2018-JFS

originally published in the Journal of Forest Science can be used (the research results) in the article of Jakub Černý that will published in the Journal of Visualized Experiments, with the proper citation of the original source.

Thank you for your cooperation.
Best regards,

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Title of Article:	FIELD MEASUREMENT OF EFFECTIVE LEAF AREA INDEX USING OPTICAL DEVICE LaiPen LP 110 IN VEGETATION CANOPY
Author(s):	JAKUB ČERNÝ, RADEK POKORNÝ

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