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# TreeTalker: a new device to monitor tree functional traits, from calibration to forest monitoring

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*“In Natural science the principles of truth ought to be confirmed by observation.”*

Carl Linnaeus





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## **Abstract**

Climate change is an increasing concern and understanding the role of forest ecosystems in the mitigation process is key. Continuous monitoring of forests can help in defining the environmental factors influencing tree responses to external changes. Nowadays technology is developed for continuous and large-scale forest monitoring by advancing of the Wireless Sensor Network to connect several sensors without the usage of any wire or cable. Thanks to this advanced technology, a new tool for extensive forest monitoring has been built, the TreeTalker.

The TreeTalker (TT) is a device capable to measure simultaneously different plant and environmental factors. It is equipped with Internet of Things (IoT) and the radio LoRa protocol for the real-time data transmission in order to early and remotely check trees' responses to environmental conditions. Another advantage is the low cost and the energy efficiency, in fact, the battery works continuously for one month and TT records hourly data. This new device has been tested along the Italian peninsula, where different trees' species have been investigated. TreeTalker has been used extensively for the first time in forest ecosystems in this study and for this reason calibrations and automatization of data processing were initially needed.

The role of my PhD inside this framework was to manage the 92 TTs installed in Tuscany (Central Italy) where the devices were installed on two of the most important species of the region: *Fagus sylvatica* L. and *Abies alba* M. The first activity was to automatize the TTs

data processing, due to the large number of data recorded each hour, to check the functionality of each TT. In fact, an automatic daily routine using R has been developed to early detect technological issues related to battery consumption and internet signal for the communication between server and TT-Cloud, such as sensors damages. The development of R package early identified low battery levels of TTs and TT-Clouds, low GPRS signal and pending records still to be sent to the server due to one of the two conditions described earlier. Moreover, the output, as a pdf report, collects daily graphs with sap flow, radial growth, temperature and battery trends for each site and each TT installed (Paper I). Before proceeding for forest monitoring by using the TT, calibrations were carried out through the comparison of the TT with another reliable and already commonly used sensor, the TreeWatch (TW). Therefore, experiments of validation and calibration of TT sensors were performed in cooperation with researchers at the University of Ghent. Two different trees have been equipped with both devices and then monitored for an entire month to check the functionality of the sap flow sensor of TT. As a result, the sap flow measured with the two sensors was comparable even if TT underestimated maximum daily fluxes and it did not accurately estimate low or close to zero water fluxes. Another experiment has been carried out by installing the TT on a stem segment in a climate chamber by using the Mariotte system. This method, based on Mariotte's bottle principle, is used to test the accuracy of sap flow measurements on cut trees' stem

permitting to have constant water flow and pressure during the experiment. Moreover, two stem segments were left in a climate chamber under zero flow conditions to test the capability of TT sap flow sensors to detect it. The results demonstrated low accuracy of the sap flow sensor of TT when the flow rate is low (Paper II).

These preliminary studies were important to accomplish ecophysiological research. A monitoring of tree functionality was performed along a hillslope in the forest by using TT. As a results, we assessed differences in sap flow and growth rates in trees in different hillslope locations (*bottom, mid* and *top*). The main force driving sap fluxes and tree radial growth was soil moisture, with the result of maximum radial growth at the *bottom* location, where soil moisture content was constant during the entire vegetative season. Lower and more stable transpiration fluxes were recorded at *bottom* compared with the other two locations by highlighting the assumption of high soil moisture content (Paper III). These activities were needed to validate the TreeTalker, by considering no studies have been published yet using this device and by supporting future activities in trees' functional traits monitoring in response of environment.





## **List of papers**

### **Paper I**

Zorzi I., Francini S., Chirici G., Coccozza C. (2021). The TreeTalkersCheck R package: An automatic daily routine to check physiological traits of trees in the forest. *Ecological Informatics*, 66, 101433. <https://doi.org/10.1016/j.ecoinf.2021.101433>

### **Paper II**

Asgharina S., Renzi F., Zorzi I., Leberecht M., Gebhardt T., Coccozza C., Niccoli F., Opgenoorth L., Kompanizare M., Steppe K., Valentini R. Principles and Applicability of TreeTalker (TT+): A Two-Needle Transient Thermal Dissipation Enabling Real Time Sap Flux Density Measurement

### **Paper III**

Zorzi I., Fabiani G., Verdone M., Giovannelli A., Dani A., Penna D., Coccozza C. Does tree location along a hillslope affect sap flow rates and tree growth? A case study in a beech forest stand in Central Italy.

## **Abbreviations**

DSDV	Daily Stem Diameter Variation
HPV	Heat Pulse Velocity
IoT	Internet of Things
ITT-Net	Italian TreeTalker Network
LoRa	Long Range
NTG	Natural Temperature gradient
SDV	Stem Diameter Variation
SFD	Sap Flux Density
TDP	Thermal Dissipation Method
TT	TreeTalker
TTD	Transient Thermal Dissipation
TW	TreeWatch
WSN	Wireless Sensor Network

# 1. Introduction

Climate change is an increasing environmental process influencing all the terrestrial ecosystems (Anderson-Teixeira et al., 2015). Global mean temperature in 2021 was already 1.1°C above pre-industrial average and there is the 50% chance that it will reach 1.5°C in the time between 2022 and 2026, discarding IPCC (2019) prediction of temperature increase of 1.5°C starting by 2030. (WMO, 2021). Studying the terrestrial ecosystems' role in mitigating these changes is key. Forests are one of the most important ecosystems in the planet, covering approximately 31% of terrestrial surface (FAO and UNEP, 2020) and they are crucial for their mitigation role (Bonan, 2008). There are limited studies on the dynamics climate change can arise, since it is difficult to have a large-scale forest's monitoring system, for the high costs and the difficulties in managing a large number of sensors.

Climate influences species composition and distribution (Lindner et al., 2014) and it is urgent to know the responses of different species to these changes to better support trees resilience and adaptation (Bosela et al., 2016; Ciccarese et al., 2012; Trambly et al., 2020), and to promote sustainable forest management (Magh et al., 2019). There are significant factors to consider, such as water availability and forests productivity, to detect the potential of environmental changes on forests (Hatfield & Dold, 2019). To determine water use efficiency and tree growth in response to environment, trees physiological variations may be assessed using sap flow sensors and

dendrometers (Mencuccini et al., 2017), requiring high-frequency data acquisition (Torresan et al., 2021; van der Maaten et al., 2018). Sap flow sensors can be employed in this framework to monitor sap flow rates, which can be used to analyse tree transpiration with high resolution information, using different methods. The first method, Heat Pulse Velocity (HPV) was described by Marshall (1958). This method can measure different parameters, such as zero flow, reverse flow and positive flow rates (Marchionni et al., 2019). With this method the fluctuation of sap flow under changing environmental conditions can be rapidly detected (Jones et al., 1988), but it seems to be noisy when under low flux densities (Steppe et al., 2010). Low cost and ease of usage (Reyes-Acosta et al., 2012) make the thermal dissipation (TDP) the most used method to measure sap flow (Lu et al., 2004; Vergeynst et al., 2014). Developed by Granier (1985), it consists of two probes, a heated and a reference one, inserted radially into the tree trunk. The flow is measured as temperature difference between the two probes. As per the technology that requires continuous heating, the TDP results as a high energy demanding method (Čermák & Kučera, 1981; Lu et al., 2004; Vandegehuchte & Steppe, 2013). Moreover, natural temperature gradient (NTG) influences negatively sap flow measurements by causing errors of over 100% (Do & Rocheteau, 2002a, 2002b; Lubczynski et al., 2012; Reyes-Acosta et al., 2012). To overcome this issue, a new method has been assessed by Do & Rocheteau (2002a, 2002b). They proposed the transient thermal dissipation method (TTD), where the

functioning consists of heating and cooling cycles (minimum 10 minutes per phase) (Do et al., 2011). The TTD method was initially proposed using the two probes to measure SFD, but the cycling system permits the usage of just one single probe as proposed by Mahjoub et al. (2009), permitting to measure sap flux as temperature gradient between the needles during and after the heating phase, with the advantage of a unique probe (Mahjoub et al., 2009). Even if the above-mentioned methods are appropriate to assess sap fluxes, currently ecophysiological measurements are still done using traditional sap flow sensors, which require manually recording and/or downloading of the data (Zweifel et al., 2021), requesting large manpower.

While assessing tree water use can be done using different method with the usage of sap flow sensors, tree growth can be assessed using other instruments called dendrometers. The usage of this sensors is important as tree growth defines their capability to adapt to the environment (Aryal et al., 2020).

Dendrometers are used to measure short- and long-term changes in stem radius. High resolution dendrometers can be classified as: point dendrometer, measuring radial or diametral variations, and band dendrometer, measuring circumferential variations (Breitsprecher & Hughes, 1975). Point dendrometers are measuring stem changes in one single point of tree stem and it can be placed in different locations around the trunk (Young, 1952). Band dendrometer, instead, measures the variations of the entire circumference resulting

in a better estimate of the mean of the radial increments (Keeland & Sharitz, 1993). Already relevant improvements have been done in high resolution dendrometers, as the first versions, developed 90 years ago (Daubenmire, 1945; Reineke, 1932), had the disadvantage of the manually reading of the data, reducing temporal and spatial resolution (Drew & Downes, 2009; Dyer & Fritts, 1976). Nowadays, data recorded by the dendrometers are collected and stored in electrical data loggers (Mencuccini et al., 2017; Urban et al., 2015; Zhang et al., 2016; Zweifel et al., 2021). However, due to the high cost of the dendrometers and data collection, most studies consider just few repetitions (Pesonen et al., 2004).

Although advancements in terms of automatic and high-resolution data acquisition were obtained, progresses in term of automatization of data collection, processing and real time transmission can be reached (Matasov et al., 2020; Siqueira et al., 2020; Stankovic et al., 2005; Vandegehuchte & Steppe, 2013; Zweifel et al., 2021).

Combining sap flow and dendrometer sensors to forest monitoring is nowadays still rarely done and, usually, the studies consider few trees (Giovannelli et al., 2019; Neuwirth et al., 2021). Studies on the synchronism between plant functions and environmental factors are still lacking (Cocozza et al., 2018), as well as environmental conditions are mostly assessed with weather stations usually far from the monitored forests (Ackerman & Goldblum, 2021; González de Andrés et al., 2018; James et al., 2013; Lüttschwager & Remus, 2007; Rita et al., 2014).

To automatise data collection, avoiding the continuous need for human interaction, sensors networks can be implemented (Vilenski et al., 2019). Technology helps in building large scale monitoring system taking advantage of the wireless sensor network (WSN) (Bayne et al., 2017). Such networks enable, not only data collection, but also real time data transmission (e.g., Steppe et al., 2016) thanks to the IoT (Internet of Things) and LoRa (Long Range) technologies. Recently, new opportunities are given by Industry 4.0 (Roblek et al., 2016) for its wide range applications and benefits, such as low-cost solutions (Matasov et al., 2020). Although networks that combine data around the world exist (e.g. SAPFLUXNET, (Poyatos et al., 2016), EUROFLUX (Tenhunen et al., 1998), TreeNet (Zweifel et al., 2021)), a system to collect simultaneously environmental and trees data by using the same device was missing. Specifically, these networks combine data taken from different projects spread around the world which requires data harmonization. Moreover, frequently, such systems, take into account just few tree traits (e.g. SAPFLUXNET works with sap flow measurements). Thus, to implement a system with a continuous tree monitoring combined with high-frequency environmental measurements a new tool has been developed, the TreeTalker device (Valentini et al., 2019).

## **1.1 TreeTalker device**

The TreeTalker (Nature 4.0 SB Srl, Italy) (TT) is a device built to monitor continuously trees with the advantage of real-time data

transmission. The TT is equipped with several sensors, recording hourly and simultaneously, tree traits, such as tree radial growth, sap flow and stem moisture content, and environmental parameters, namely light penetration through the canopies, air temperature and humidity.

The TT is constituted by a battery package equipped with a small solar panel that requires to be changed once per month. Although, the TT is a new device, already new developments have been released, such as new batteries lasting 4 months, allowing the TT system to work independently without a continuous control of battery level.

The need for low energy consumption has brought the producers to choose the sensors based on their energy efficiency, adopting to measure sap flow, the TTD, proposed by Do & Rocheteau (2002a, 2002b), with default cycling phases of 10 minutes heating and 50 cooling. The TT permits to use the single probe approach (Mahjoub et al., 2009) and to easily change the timing of the phases. Moreover, the TTD sensors are constituted by probes 20mm x 3mm with a low impact on stem.

The tree radial growth is measured by the TT through a growth sensor, an infrared distance sensor has been chosen, consisting in two fibre sticks to be inserted into the tree trunk. These sensors measure the seasonal radial growth, with the work principle of a point dendrometer.



The monitoring system using the TTs can be considered a WSN. As such technology, generate large volume of data, there is the need of an automatised and real-time data transmission. For these reasons, the TT uses the LoRa protocol to communicate to a data logger (TT-Cloud). The TT Cloud is equipped with a SIM card and thanks to GPRS connection communicates with a computer server where all the data recorded by the TTs are stored. In this way, no interaction is needed for data recording, downloading and storage, as the process is completely automatized. The TT-Cloud works in cluster, in fact a single TT-Cloud can host up to 48 TTs (Matasov et al., 2020), permitting the contemporary acquisition of many data, of many trees. The TT-Cloud works continuously due to the battery connected to a big solar panel.

Tree monitoring using the TreeTalker device facilitates data harmonization, as the output is equal wherever the devices are installed, overcoming the common problem to compare different studies where different sensors are used (Poyatos et al., 2016). Moreover, the simultaneous data acquisition is innovative by considering the sensors arrangement in the device, furthermore real-time data transmission permits the instant checking of trees functionalities. The challenge to adopt this technology is to have a large amount of data acquired at the same time in different part of the world, in different forests, on different species, assessing trees adaptation to the changing environment. Currently, monitoring projects using the TT are carried out in Italy and Russia and the

network is expanding. At present, not many studies have been done using this technology due to the innovation of the product as required calibrations are still ongoing.

To maintain the production cost and the high frequency data acquisition the accuracy of the sensors is not yet optimal (Asgharina et al., 2022), but the low maintenance needed to manage the entire system makes the TT a convenient tool to work with.

## **1.2 The first TreeTalker monitoring network**

The first monitoring system built to test the TT device has been put in place in Italy, representing the first worldwide example of large-scale forest monitoring, starting from hourly measurements, then scaled to daily, monthly, and seasonal, allowing to assess the variability and the dependency of trees' responses to environmental disturbances. Thanks to a PRIN project, the Italian TreeTalker network (ITT-Net), 500 TTs have been installed along the entire Italian Peninsula, comprehending forests from Trentino-South Tyrol to Sicily, with the advantage of using the same sensors, devices and technologies. The aim of the ITT-Net is the assessment of the variability of forest responses to environmental changes at a regional scale and comparing trees' behaviours. Moreover, data collected across a geographical gradient will help forest owners in managing forests regarding climate change adaptation and mitigation. Massive single trees' data availability is crucial for the upscaling strategy used in forest modelling (Fabrika et al., 2019).

*Fagus sylvatica* L. has been chosen as a common species to monitor, for its significance throughout all Europe, as a result of its extensive distribution (von Wühlisch, 2008) in order to start responding to the societal challenge of assessing climate change impacts on forests. Moreover, European beech is present in all the Italian regions considered in the network. Additionally, each research unit involved in the project had to choose the most prevalent species of their area to assess how those species are adapting to the changing environment.

## **2. Background motivation and aims**

Inside the ITT-Net, my PhD serves to start the TT monitoring in Tuscany (Central Italy), helping in implementing automatization of data processing and sensor calibrations, beyond assessing the impact of environmental changes on forest ecosystems. To accomplish this goal, in Tuscany, 40 TTs were installed in a European beech pure stand, in the Sant'Antonio Forest. Moreover, to assess the adaptation of different species to climate change, another specie has been selected. In the case of Tuscany, one of the most common forest species is *Abies alba* M. and this has been chosen to be monitored, equipping 40 trees with the TTs in the Vallombrosa forest. Another study site was then set up in the Rincine forest, where 12 TTs were installed in a *F. sylvatica* pure stand in a watershed. In total, we had 92 trees monitored spread around Tuscany.

The objectives of this project were multiple, starting by the fact the TreeTalker is a new developed device, made for massive data collection and processing. It registers simultaneously parameters of trees, such as tree radial growth and sap flux density, and environmental factors, as spectral bands of light below canopy, air temperature and humidity. Each TT, records hourly 43 different attributes resulting in a daily database of 1032 attributes, multiplied for the many TTs installed, it produces a high dimension daily database. Since it was hard to assess the operation of the many TTs installed, the initial goal was to automate sensor checking and create a clean database for data processing. As a new developed tool, some calibrations still were needed before the usage to detect tree functionalities, especially about wood moisture and sap flux density measurements. To calibrate the capacitive sensor, comparison with other already well known and commonly used sensors needed to be done. As part of my PhD, during the abroad period, spent at the Ghent University, I compared TreeTalker devices with the already known and used TreeWatch, installed on three *F. sylvatica* in the Gontrode forest (Belgium). During this stay I, moreover, calibrated the sap flow sensors using the Mariotte system and the collaboration with other researchers brought to the TT sap flow sensors calibration procedure. The final aim, thanks to the automatization of data processing and the calibration of the system, was to use the TT technology to implement the first case study, done in the Rincine forest (Tuscany, Central Italy) to assess tree response to

environment. The aim of the work was to define difference in tree radial growth and sap flow rates along a hillslope in a watershed assessing different plants behaviours, driven by soil available water and temperature. The initial aims of my PhD were partially addressed as more physiological studies were initially planned, but some complexities were faced during this period. Firstly, the initial installation planned for March 2020 was delayed by the pandemic and brought us to the loss of one growing season, having been able to install the first devices just at mid-July 2020 and the first entire season data were collected at the end of September 2021. This made us lose the first growing season, which was important for implementing case studies about responses of trees under different management systems. In fact, the cuttings have not been done yet, waiting for the acquisition of at least two complete growing seasons. For the same reason, studies on different species behaviour under the same environmental conditions have not been completed. Moreover, the TT being a new device, needed still some calibration before the complete usage of it. For these reasons some case studies in tree ecophysiology were postponed.



### 3. Papers

#### 3.1 Paper I: Checking TreeTalker functionalities

**The TreeTalkersCheck R package: an automatic daily routine to check physiological traits of trees in the forest**

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## **Abstract**

Monitoring of tree traits and tree response to the environment can be integrated with sensor technology. High-resolution monitoring requires a frequent data acquisition, that generally results in hourly measurements for the definition of daily processes, then scaled at monthly, seasonal and annual levels, by producing very large databases. The TreeTalker (TT) technology is an example of a tool for forest monitoring that measures hourly and simultaneously tree parameters and environmental variables, by collecting 1032 attributes per tree per day (43 variables, such as sap flow and stem diameter variation measured every hour). Therefore, we present the TreeTalkersCheck R package built to facilitate the remote checking of the functioning of the TreeTalkers devices. This package contains functions to download, process raw data obtained with TreeTalker devices, and produces a report with all the alerts detected and a complete database with preliminary processing of the data. The package was tested on a database obtained by 60 TTs installed on 60 trees in three sites in Tuscany (Central Italy).

**Keywords:** TreeTalker, sensor decoding, tree monitoring, R coding



## **1. Introduction**

Monitoring tree functional traits and tree response to the environment is a tool by knowing the responses of forests to environmental changes, for supporting forest management and the improvement of their resilience (Magh et al., 2019). Climate variations drive forest growth (Oberhuber et al., 2014), becoming disturbing elements of forest dynamics when extreme events occur. In environments characterized by frequent drought seasons, with consequent mortality and anomalies of species distribution (Lindner et al., 2014), data on water use efficiency and tree growth allow forecasting the impact of environmental constraints on tree functionality (Cocozza et al., 2018).

Nowadays monitoring systems, constituted by sensors, are developed and widely used to accomplish measurements of tree functionality (Dietrich et al., 2018; Steppe et al., 2016; Su et al., 2019; Peters et al. 2020). These monitoring systems benefit from technologies, like Industry 4.0, to achieve high efficiency in controlling data and productivity in data acquisition (Roblek et al., 2016). The research interest by studying the tree functionality in response to the environment, namely ecophysiology, requires a high-frequency data acquisition. High-frequency data acquisition generally results from hourly measurements for the definition of daily, monthly or seasonal behaviour processes (van der Maaten et al., 2018). Research questions addressed to quantify plant water use and health in terms of growth can be answered by using dendrometers and sap flow

sensors (Mencuccini et al., 2017) but several issues related to data acquisition exist. Usually, when monitoring ecophysiological variables, several studies consider few trees (Giovannelli et al., 2007; Nourtier et al., 2014; Oberhuber et al., 2014), due to the difficulties of having a lot of repetitions, maintaining the monitoring systems, and the high cost of the probes. Moreover, when comparing different studies, where different types of sensors are used for data collection, harmonization of data is often required. To overcome these issues a new device, named TreeTalker (TT) (Valentini et al., 2019), has been developed.

In this study, we show a monitoring system realized with the TreeTalker (TT) technology that collects hourly and simultaneously tree parameters, such as tree trunk radial growth (through a dendrometer sensor), water transport (through a sap flux sensor), light transmitted through the canopies (through a spectrometer) and its spectral components, tree stability (through an accelerometer), as well as air temperature and humidity (Valentini et al., 2019). Furthermore, the system is equipped with a data logger, TT-Cloud, that directly communicates in real time with the TT and with a computer server. This enables to obtain complete databases, presenting environmental and tree variables, collected simultaneously, in a unique server. Along with its real time data transmission, this facilitates the instant control of the TT functionalities and trees responses to environmental conditions, with the possibility of having the TTs installed in several sites, on several

trees, due to their low cost (Valentini et al., 2019). Another important step forward can be the standardisation of the measurements made by all the sensors installed, creating a really large database, with the same data format, as done by the SAPFLUXNET (Poyatos et al., 2020), with the value of having them collected directly in the same server and without needing a preliminary dataset preparation.

Due to the high number of sensors installed and the considerable number of attributes recorded every hour, it is difficult to have a quick overview of the functionality of all the TTs placed in the forest and to act if issues occur. Moreover, the raw data, collected by the device and stored directly in the server, are complex to read, due to the fact there are no variable names in the server and the data of different devices, TT and TT-Cloud, are stored all together. For all these reasons, this study was aimed to present the new `TreeTalkersCheck R` package, specifically designed to check the functionality of TT devices. The package automatically generates the report of the day before (customizable) - where any malfunction of each TreeTalker and each TT-Cloud is shown - and a complete database for further data processing. Moreover, the report graphically shows the daily trend of battery level, air temperature, dendrometer, and sap flow sensors.

`TreeTalkersCheck R` package can be used for TT devices requiring no programming skills and, if needed, it can be easily adapted to work with similar devices too.

## 2. Methods

### 2.1 TreeTalkers sensors and installation

TTs were installed in three different sites located in Tuscany (Central Italy) in July 2020. Two sites were located in the Vallombrosa forest and are characterized by a pure silver fir (*Abies alba*) stand. In the third site, located in the Sant'Antonio Forest, the devices were installed in a pure European beech (*Fagus sylvatica*) stand. Tree age ranged from 55 years of European beeches and 100 years of silver firs and the average diameter at breast height was 34 cm and 37 cm for the two species respectively. In each study site, 20 TTs and one TT-Cloud were installed, to monitor a total of 60 trees (40 silver firs and 20 European beeches).

The installation of the TT consists of the insertion of the probes measuring sap flux and the dendrometer into the trunk. The sap flux is measured as flux density, through a thermal dissipation method, with a cyclic heating system (10 minutes heating and 50 minutes cooling), by using two probes, a reference one and a heated one. The two probes are vertically and horizontally placed with a separation of 10 and 2 cm, respectively. The dendrometer measures stem diameter variations through an infrared pulsed distance sensor. The infrared sensor is positioned a couple of centimeters away from the trunk and it is anchored in the xylem by two carbon fiber sticks. In the TT device's box air temperature and humidity sensors are placed. They are covered by a Goretex membrane to avoid water fill and to

be permeable to water vapor. Moreover, an accelerometer records oscillation of tree due to gravity, using Spherical Coordinate System, to be used for monitoring responses against wind impacts.

The TT-Cloud uses GPRS signal to communicate with a computer server via internet connection (Valentini et al., 2019). The TT device uses the LoRa (“Long Range” is a low-power wide-area network modulation technique) protocol for communication with the TT-Cloud. The Internet of Things (IoT) system for the communication between the TT-Cloud and the server allows the remote control and the real time data transmission of various tree and environmental variables. The monitoring system is entirely carried out by the wireless sensor network technology, which permits a group of sensors to be connected and communicate with each other without using wires or cables (Bayne et al., 2017). However, low GPRS connection, such as low battery voltage of the device, can limit a punctual data transmission, which results in a semi real time communication.

## **2.2 TreeTalker database**

Every day, each TT collects 43 attributes by returning 1032 attributes per day, 30960 attributes data per month, that multiplied per several TT produces a database of large dimensions (afterward DB). The development of an automated check of TT functionality is necessary

to maintain efficient battery levels, not lose any data, and directly control the functioning of the different sensors.

The 60 TTs installed send more than 60k attributes every single day, about two million per month. The data are stored in different web pages on [altervista.org](http://altervista.org), one for each TT-Cloud installed. In our study, we have three TT-Clouds (three forest sites), likewise three web pages. Each webpage contains a `ttcloud.txt` file with all the data registered by all the TTs associated with the relevant TT-Cloud. Each TT (model TT+ 3.2, Nature 4.0 SB Srl, Rovereto, Italy) sends two different records per hour: (1) TT sensors recorded data (device type 4D), (2) TT spectral bands recorded data (device type 49) (Table 1 and 2). The two records generated each hour by the TT-Cloud contain (1) information about the status of the TT-Cloud (4B), (2) information about the communication between TT-Cloud and all the TTs (4C) (Table 1 and Table 3). Examples of four possible types of data records, representing the two devices (TT and TT-Cloud) used in our monitoring system, are shown (Table 1, Table 2, and Table 3). Recorded data are sent to the server and stored in the database. The TT database is defined by records displayed as digital numbers to be converted. These records are the input file run with the `TreeTalkersCheck R` package and there is no need of preparing the dataset before running it.

---

12.10.20 12:08:20, 52050499; 18BF9; 4D; 1602486000  
 12.10.20 12:08:37, C0200103; 18BF4; 4B; 1602486000  
 12.10.20 12:08:53, C0200103; 18BFB; 4C; 1602486000  
 12.10.20 12:08:13, 52050467; 18BFC; 49; 1602486001

---

<b>Date and time when the record is sent to the server</b>	<b>Device number</b>	<b>serial number</b>	<b>Progressive record number</b>	<b>Device Type</b>	<b>UNIX timestamp</b>
12.10.20 12:08:20	52050499		18BF9	4D	1602486000
12.10.20 12:08:37	C0200103		18BFA	4B	1602496800
12.10.20 12:08:53	C0200103		18BFB	4C	1602496800
12.10.20 12:12:13	52050467		18BFC	49	1602486001

---

Table 1. Example of data registered from the diverse device type part of the TreeTalker technology system. Top, are shown the raw records registered by TTs.

<b>Device type 4D</b>		<b>Device type 49</b>	
43696;44291;49388;43323 ;17;84;59;-3790;0;- 169;0;- 1476;0;43839;40898;1751 7; 70157		1685;1424;1755;1479;151 5; 1651;2368;3261;3633;263 2;2663;2035;50;3	
<b>Tref_0</b>	43696	<b>AS7263_610</b>	1685
<b>Theat_0</b>	44291	<b>AS7263_680</b>	1424
<b>growth sensor</b>	49388	<b>AS7263_730</b>	1755
<b>adc_bandgap</b>	43323	<b>AS7263_760</b>	1479
<b>number of bits</b>	17	<b>AS7263_810</b>	1515
<b>air relative humidity %</b>	84	<b>AS7263_860</b>	1651
<b>air temperature*10°C</b>	59	<b>AS7262_450</b>	2368
<b>g_z(mean)</b>	-3790	<b>AS7262_500</b>	3261
<b>g_z(std.dev)</b>	0	<b>AS7262_550</b>	3633
<b>g_y (mean)</b>	-169	<b>AS7262_570</b>	2632
<b>g_y (std.dev)</b>	0	<b>AS7262_600</b>	2663
<b>g_x (mean)</b>	-1476	<b>AS7262_650</b>	2035
<b>g_x (std.dev)</b>	0	<b>integration time</b>	50
<b>Tref_1</b>	43839	<b>gain</b>	3
<b>Theat_1</b>	40898		
<b>StWC [freq (Hz)]</b>	17517		
<b>adc_Vbat</b>	70157		



Table 2. Data registered from the TreeTalker device (type 4D and 49). Tref\_0, Tref\_1, Theat\_0, Theat\_1 are used to calculate the sap flux density, being respectively the reference probe temperature before and after heating, and the heated probe temperature before and after heating. Adc\_bagap, adc\_Vbat are used to calculate the battery level. G\_z, g\_y, g\_x are used to calculate the tree stability.



<b>GSM field level (from 0 to 32)</b>	17		
<b>Battery level (mV)</b>	3678		
<b>Firmware Version</b>	rel.5.0d		

Table 3. Data registered from the TT-Cloud (type 4B and 4C)

### 2.3 The TreeTalkersCheck R package

TreeTalkersCheck was built to daily monitor the status of TT devices and TT-Clouds. The package consists of an automatic alert system to quickly identify potential malfunctions. Failures of TT functions might be no signal or too many pending records of TT-Cloud, a too low battery level of devices, or irregular data collection. The key point of TreeTalkersCheck is user-friendliness, as there is no need of having programming skills to use it, and no data preprocessing operations are needed.

The general workflow to obtain daily reports using the package is to: (1) download and install R (<https://cran.r-project.org/>), (2) download TreeTalkersCheck, (3) define input parameters, and (4) schedule a daily execution using, for example, the Windows Task Scheduler (<https://docs.microsoft.com/en-us/windows/win32/taskschd/task-scheduler-start-page>) or the Macscheduler (<https://www.macscheduler.net>) or, depending on the operating system, any scheduler designed to enable a user to automatically perform routine tasks on a chosen computer. A simple but exhaustive guide for package installation and usage is available on Github (<https://github.com/saveriofrancini/TreeTalkersCheck>).

The main function of TreeTalkersCheck, `TreeTalkersCheck()`, executes all functions, (1) `checkRequirements()`, (2) `downloadNatureTalkers()`, (3)

`readServerData()`, (4) `checkCloud()`, (5) `checkTT()`, (6) `Plot()` and (7) `Render()` (Fig. 1).

The `checkRequirements()` function is executed to prepare data and to handle any exception that can occur each time a new user executes the `TreeTalkersCheck` package for the first time. `checkRequirements()` includes functions to (1) handle path of files and R installations, (2) download required R and R Markdown packages, and (3) check for the required input files presence. If any error is encountered a specific message is printed, otherwise, the `downloadNatureTalkers()` function is executed which, as stated before, downloads the DBs associated with the servers declared in the input file.

The data downloading step takes place by appending all DBs in a single file ("`TT_CloudDB.csv`"), which is updated with new records each time `TreeTalkersCheck` is executed. The "`TT_CloudDB.csv`" file contains the records as stored in the server, as shown in the previous chapter and how described in Tables 1, 2 and 3. The complexity of reading the data directly into the server makes this DB hard to work with and understand. It is also clearly unsuitable for analysis purposes, so, reorganizing data is needed. `readServerData()` serves this need, merging the records of the TT-Cloud status and the records of communication between TT-Cloud and TTs (4B-4C) and TT sensors and spectral bands recorded data (4D-49) in DBs by producing two files, one for TTs

("ttDB.csv") and one for TT-Clouds ("cloudDB.csv"), both stand by for further analysis.

The "ttDB.csv" and the "cloudDB.csv" are the inputs of the `checkTT()` and `checkCloud()` functions, respectively. Those functions are designed to check the status of the devices and the quality of the data sent by the devices. The checking occurs over a specific day that can be defined by the user. The default day is set to `Sys.Date()-1`, producing the report of "yesterday". This is because data sending requires from seconds to hours, depending on GPRS signal, to complete daily data collection and to produce a report for "yesterday". On the other hand, if required, any day can be selected by the user using a string (e.g. "2021-01-01"). Both `checkCloud()` and `checkTT()` output two files: (1) the filtered DBs (e.g., by selecting data in "2021-01-01", the outputs are `2021-01-01_TT.csv` and `2021-01-01_cloud.csv`) and (2) the alert DBs (e.g., by showing anomalies in `2021-01-01_TTAlert.csv` and `2021-01-01_cloudAlert.csv`). The `checkTT()` function, in addition to filter data over a specific day, calculates additional parameters, using formulas included in the user manual, (1) battery level, (2) sap flux density and (3) stem diameter variation, then registered in the output file (e.g. `2021-01-01_TT.csv`). Regarding the alert files, several checks are performed, and the results are presented in the final reports. In particular, `checkCloud()` checks for (i) the minimum TT-Clouds signal (9 GSM field level), (ii) the maximum number of pending records (10 records), (iii) the minimum TT-Clouds battery

level (4000 mV) and (iv) the minimum signal between TT-Clouds and TTs (-100 RSSI radio signal strength), as indicated in the TT manual, or experienced by our research group, to guarantee the functionality of the system. The check of the TTs battery level, carried out by `checkTT()`, is established at the threshold not lower than 3.5 V.

The `Plot()` function calculates and graphs daily trends of (1) air temperature, (2) stem diameter variation, (3) sap flux density, and (4) battery levels of each TT in each site. Finally, a graph of the stem diameter variation (SDV) daily trend is produced for each TT. The first attempt at the data analysis of dendrometer was made through the use of rough data. However, the pronounced noise on the signal due to the different stem diameter sizes of each tree did not allow a direct comparison in the graphs, then it was necessary to normalize stem diameter to clearly visualize daily trends. A Daily normalized Stem Diameter Variation (DSDV) was calculated for each TT in the range 0-1 (eq. 1), where 0 corresponds to the daily minimum and 1 to the daily maximum of each tree:

$$DSDV = \frac{SDV - \min(SDV)}{\max(SDV) - \min(SDV)} \quad (eq. 1)$$

where  $\min(SDV)$  and  $\max(SDV)$  refer respectively to a daily minimum and maximum SDV values.

The sap flux density is calculated, firstly converting the digital numbers present in the server to temperatures (°C), then using the method proposed by Do & Rocheteau, 2002, applying the following formula (eq. 2):

$$\begin{aligned} \text{sap flux density} \\ &= 118.99 \left[ \frac{\Delta T_{max}}{\Delta T_{on} - \Delta T_{off}} \right. \\ &\quad \left. - 1 \right]^{1.231} \quad [l \text{ dm}^{-2} \text{ h}^{-1}] \quad (eq. 2) \end{aligned}$$

Where:  $\Delta T_{max}$  is the maximum temperature gradient between the reference and the heated probe after heating;  $\Delta T_{on}$  is the temperature difference between the reference and the heated probe after the heating period;  $\Delta T_{off}$  is the temperature difference between reference and heated probe before heating.

The last function to be executed is the `Render()` function. It consists in an R Markdown document (<https://rmarkdown.rstudio.com/>) outputting the report in docx and html formats (e.g. 2021-01-01\_TT.html and 2021-01-01\_TT.docx). Daily reports include graphs produced by the `Plot()` function, a table with issues detected by the `checkCloud()` and `checkTT()` functions and sentences stating the lower battery level and the device that did not send any data in the selected day (again, by default “yesterday”). To see an example of a daily report produced by our package see the appendix.



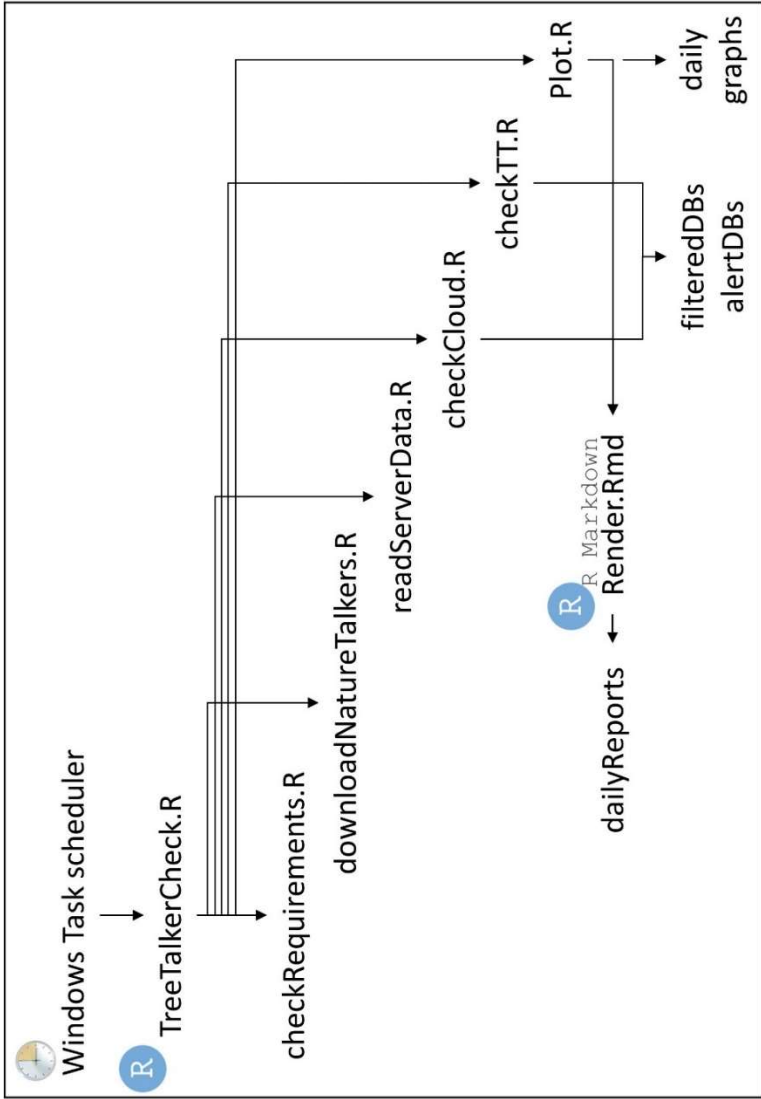


Figure 1. TreeTalkersCheck R package workflow and outputs generated

## **2.4 TreeTalkersCheck testing**

We tested the TreeTalkersCheck R package in three study sites taking into account the data recorded by 60 TTs and three TT-Clouds during 15 days in August 2020.

To simplify the checking of the report, we named the three different sites, as site1, site2 and site3, and each TT with a number from 1 to 20, followed by the site name (e.g., 1site1, 2site1, 3site1, etc.).

The execution of the daily routine produces, as stated before, a daily report with, in the case of the database used, 72 graphs (60 graphs of stem diameter variation of 60 TTs); four graphs of air temperature, three sites and a summary one, likewise four for battery level, three graphs for sap flux density and one graph of battery levels of TT-Clouds. An example of a daily report can be found in the appendix and it shows the report generated using the data collected on 27/08/2020.

The data used for the testing are provided along with the TreeTalkersCheck R package and can be found in GitHub (<https://github.com/saveriofrancini/TreeTalkersCheck>).

## **3. Results**

The TTs permitted us to efficiently monitor in continuous, with hour detail, 60 trees located in three different sites in Tuscany. The R package represented a crucial tool to check the devices' functionality and to preliminary process raw data. Specifically, the output report allowed to quickly visualize the daily trend of measured variables

and to take action when needed, without manually checking all the records sent by the devices.

The graphs generated show the trends of different variables in a day of monitoring (Fig. 2): the battery voltage (V) use for the TT functionality (Fig. 2a); the air temperature ( $^{\circ}\text{C}$ ) variation (Fig. 2b); the sap flux density ( $\text{l dm}^{-2} \text{h}^{-1}$ ) (Fig. 2c); the stem diameter variation, normalised in a 0-1 scale (eq. 1) (Fig. 2d).

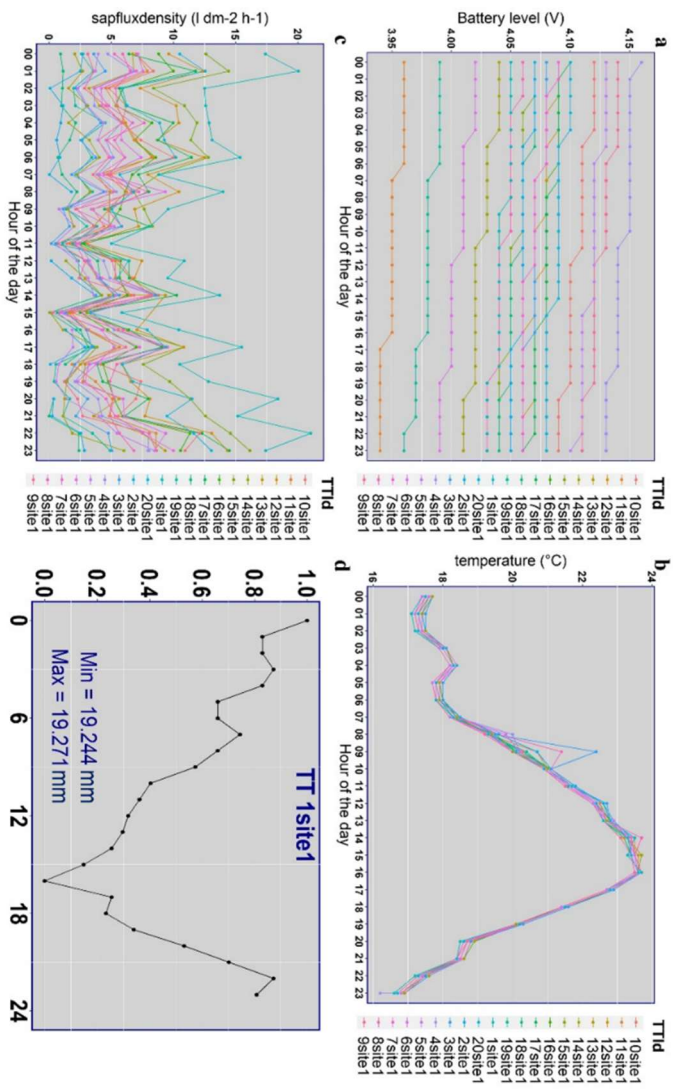


Figure 2. Example of daily graphs produced by the TreeTalkersCheck R package in a site with 20 TTs: (a) battery level; (b) air temperature; (c) sap flux density; (d) stem diameter variation of TT number 1, while in the report there is one graph per each TT.

## 4. Discussion

The TT is a new device and, so far, not much research has been done using this monitoring system. For this reason, this study represents an important step towards an automatization of this technology that brought to the first R package development to simplify the TT management reaching two important goals: (1) the fast and easy check of the functionality of the devices installed in remote forests and (2) quick data visualisation starting from a large complete database. Finally, the `TreeTalkersCheck` R package is a starting point in the direction of the full automatization of data collection and processing, bringing us to have a package performing analysis automatically. The data workflow will implement a set of advanced time-series and machine-learning tools for enhancing the interpretability and enable scientific exploitation, for 1) extracting relevant sub-signals on different time scales (i.e. diurnal, synoptic, and seasonal scales as well as trends), 2) identifying anomalous and extreme ecophysiological events, 3) quantifying (event) synchronization with ancillary time-series data (i.e. climate data).

At this stage, we focused our attention on some sensors assembled in the TreeTalker, but the script might be implemented for other variables registered by TTs, such as the tree position and the stem moisture, both very interesting for the day-to-day monitoring. The R coding permits to customize the package to specific requirements, implement functions and scripts to make it more suitable depending

on user needs. Moreover, although the package has been developed and tested on TT devices and databases, it can be adapted to work with different devices with high-frequency data acquisition. Indeed, the TreeTalkersCheck R package facilitates the management of the large number of sensors that can be installed in the forest. The TT is a powerful device and the simultaneous recording of the data, the hourly and real time acquisition, and the possibility to check remotely the functioning are valid features from a scientific perspective. The package allowed to enhance these potentialities, supporting the user to easily check the monitoring system performance. Despite this, there are still some limits due to the occasional long lack of internet connection or not optimal battery voltage, sometimes data were not in real time. This compromised the functionality of TTs, resulting in daily reports with missing data. Being the TT an electronic device, the data acquisition could be interrupted by bad weather conditions, low battery supply, or wild animals damaging wires. The aim of the package was also to reduce long data gaps by promptly identifying the issues and correcting them.

In the future, the package will be further improved to obtain the automatization of the full data processing and algorithms for segmentation of time series (Keogh et al., 2001), to identify the relevant breakpoints, and to remove noise from time series of data registered by TT. The development of the package is expected to greatly expand the network of TT devices, by establishing a largest

database, to implement ecological research infrastructures exploiting data acquired with high-frequency.

## **5. Conclusions**

A large database obtained by the hourly and simultaneous collection of tree parameters and environmental variables reaches high value if timely checked to guarantee complete and correct data storage. The `TreeTalkersCheck` package has been developed to automatically and daily check a `TreeTalker` database, and it was tested with 60 devices (61920 attributes per day considering 60 trees) spread through three sites in Tuscany. The package outputs a daily report that graphically shows the trend of the TT in the different sites instead of daily checking 61920 records in the server, to detect the issues and if needed correct them. This package represents the first step towards the full automatization of the functionality checking of the devices, to arrive at the full mechanisation of all data processing.

### **Package Availability Statement**

The `TreeTalkersCheck` R package is available in GitHub (<https://github.com/saveriofrancini/TreeTalkersCheck>).

This package is a preliminary step towards the complete automatization of the TT system, and it can be improved by those using those and similar devices. A brief TT database can be freely downloaded and used to test `TreeTalkersCheck` R package. Any

feedback and suggestion for possible implementation of the package or any possible bug to solve can be submitted through email or directly on GitHub.



## 6. Appendix

Example of a daily report generated by TreeTalkersCheck R package.

2020-08-27

This report was automatically produced by the **TreeTalkerCheck** package

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Saverio Francini  
Gherardo Chirici  
Claudia Cocozza

### 1. Detecting issues

No issues related with the TT where automatically identified

The number of cloud issues are 212

Issues related with clouds

tipology	issue	cloudId	timeStamp	Location.x
cloudSignal	0	C0200100	2020-08-27 07:00:00	site3
cloudSignal	0	C0200100	2020-08-27 15:00:00	site3
cloudSignal	0	C0200103	2020-08-27 01:00:00	site2
cloudSignal	0	C0200103	2020-08-27 02:00:00	site2
pendingRecords	11	C0200100	2020-08-27 05:00:00	site3
pendingRecords	13	C0200100	2020-08-27 12:00:00	site3
pendingRecords	17	C0200100	2020-08-27 15:00:00	site3
pendingRecords	69	C0200100	2020-08-27 16:00:00	site3
pendingRecords	54	C0200100	2020-08-27 17:00:00	site3
pendingRecords	17	C0200100	2020-08-27 18:00:02	site3
pendingRecords	11	C0200100	2020-08-27 19:00:00	site3
pendingRecords	49	C0200100	2020-08-27 23:00:00	site3
pendingRecords	13664	C0200102	2020-08-27 00:00:02	site1
pendingRecords	13664	C0200102	2020-08-27 00:00:02	site1
pendingRecords	13581	C0200102	2020-08-27 01:00:00	site1
pendingRecords	13581	C0200102	2020-08-27 01:00:00	site1
pendingRecords	13519	C0200102	2020-08-27 02:00:00	site1
pendingRecords	13519	C0200102	2020-08-27 02:00:00	site1
pendingRecords	13467	C0200102	2020-08-27 03:00:00	site1
pendingRecords	13467	C0200102	2020-08-27 03:00:00	site1
pendingRecords	13371	C0200102	2020-08-27 04:00:00	site1
pendingRecords	13371	C0200102	2020-08-27 04:00:00	site1
pendingRecords	13260	C0200102	2020-08-27 05:00:00	site1
pendingRecords	13260	C0200102	2020-08-27 05:00:00	site1
pendingRecords	13187	C0200102	2020-08-27 06:00:00	site1
pendingRecords	13187	C0200102	2020-08-27 06:00:00	site1
pendingRecords	13074	C0200102	2020-08-27 07:00:00	site1

tipology	issue	cloudid	timeStamp	Location.x
battery	3970	C0200102	2020-08-27 21:00:00	site1
battery	3964	C0200102	2020-08-27 22:00:02	site1
battery	3964	C0200102	2020-08-27 22:00:02	site1
battery	3962	C0200102	2020-08-27 23:00:02	site1
battery	3962	C0200102	2020-08-27 23:00:02	site1
battery	4000	C0200103	2020-08-27 04:00:00	site2
battery	4000	C0200103	2020-08-27 05:00:00	site2
battery	3990	C0200103	2020-08-27 06:00:00	site2
battery	3984	C0200103	2020-08-27 07:00:00	site2
battery	3982	C0200103	2020-08-27 08:00:00	site2
battery	3974	C0200103	2020-08-27 09:00:00	site2
battery	3966	C0200103	2020-08-27 10:00:01	site2
battery	3968	C0200103	2020-08-27 11:00:00	site2
battery	3964	C0200103	2020-08-27 12:00:00	site2
battery	3962	C0200103	2020-08-27 13:00:00	site2
battery	3964	C0200103	2020-08-27 14:00:01	site2
battery	3954	C0200103	2020-08-27 15:00:00	site2
battery	3954	C0200103	2020-08-27 16:00:00	site2
battery	3946	C0200103	2020-08-27 17:00:00	site2
battery	3938	C0200103	2020-08-27 18:00:00	site2
battery	3938	C0200103	2020-08-27 19:00:00	site2
battery	3936	C0200103	2020-08-27 20:00:00	site2
battery	3928	C0200103	2020-08-27 21:00:00	site2
battery	3924	C0200103	2020-08-27 22:00:00	site2
battery	3924	C0200103	2020-08-27 23:00:00	site2
sensorSignal	18,21	C0200100	2020-08-27 00:00:00	site3
sensorSignal	1,18,21;25	C0200100	2020-08-27 01:00:00	site3
sensorSignal	1,2;3,4;21;25	C0200100	2020-08-27 02:00:00	site3
sensorSignal	1,2;3,4;5,6;7;8;9;10;11;12;13;14;15;16;17;18;21;25	C0200100	2020-08-27 03:00:00	site3
sensorSignal	18,21	C0200100	2020-08-27 04:00:00	site3
sensorSignal	1,2;3,4;5,6;7;8;9;10;11;12;13;21;25	C0200100	2020-08-27 05:00:00	site3
sensorSignal	18,21	C0200100	2020-08-27 06:00:00	site3
sensorSignal	21	C0200100	2020-08-27 07:00:00	site3
sensorSignal	1,2;3;18;21;25	C0200100	2020-08-27 08:00:00	site3
sensorSignal	1,2;3,4;5,6;7;8;9;10;11;12;13;14;15;16;17;21;25	C0200100	2020-08-27 09:00:00	site3
sensorSignal	18,21	C0200100	2020-08-27 10:00:00	site3
sensorSignal	17,21	C0200100	2020-08-27 11:00:00	site3
sensorSignal	1,2;3,4;5,6;7;8;9;18;21;25	C0200100	2020-08-27 12:00:00	site3
sensorSignal	12,21	C0200100	2020-08-27 13:00:00	site3
sensorSignal	15,18;21	C0200100	2020-08-27 14:00:00	site3

typology	issue	cloudId	timeStamp	Location.x
sensorSignal	1,2,3;4;5;6;7;8;9;10;11;12;13;14;15;16;21;22;23;24;25	C0200102	2020-08-27 16:00:00	site1
sensorSignal	1,2,3;4;5;25	C0200102	2020-08-27 16:00:00	site1
sensorSignal	22;23;24	C0200102	2020-08-27 17:00:00	site1
sensorSignal	1,2,3;4;5;25	C0200102	2020-08-27 17:00:00	site1
sensorSignal	1,2,3;4;5;6;7;8;9;10;11;12;13;14;15;16;17;18;19;21;22;23;24;25	C0200102	2020-08-27 18:00:00	site1
sensorSignal	1,2,3;4;5;25	C0200102	2020-08-27 18:00:00	site1
sensorSignal	1,2,3;4;5;6;7;8;9;10;11;12;13;14;15;16;17;18;19;21;22;23;24;25	C0200102	2020-08-27 19:00:00	site1
sensorSignal	1,2,3;4;5;25	C0200102	2020-08-27 19:00:00	site1
sensorSignal	22;23;24	C0200102	2020-08-27 20:00:00	site1
sensorSignal	1,2,3;4;5;25	C0200102	2020-08-27 20:00:00	site1
sensorSignal	1,2,3;4;5;6;7;8;9;10;11;12;13;14;15;16;17;18;19;21;22;23;24;25	C0200102	2020-08-27 21:00:00	site1
sensorSignal	1,2,3;4;5;25	C0200102	2020-08-27 21:00:00	site1
sensorSignal	1,2,3;4;5;6;7;8;9;10;11;12;13;14;15;16;17;18;19;21;22;23;24;25	C0200102	2020-08-27 22:00:02	site1
sensorSignal	1,2,3;4;5;25	C0200102	2020-08-27 22:00:02	site1
sensorSignal	22;23;24	C0200102	2020-08-27 23:00:02	site1
sensorSignal	1,2,3;4;5;25	C0200102	2020-08-27 23:00:02	site1
sensorSignal	1,25	C0200103	2020-08-27 00:00:00	site2
sensorSignal	1,2,3;4;25	C0200103	2020-08-27 02:00:00	site2
sensorSignal	1,2,3;4;5;6;7;8;9;10;11;12;13;25	C0200103	2020-08-27 04:00:00	site2
sensorSignal	1,2,3;4;5;6;7;8;9;10;11;12;13;14;15;16;17;25	C0200103	2020-08-27 11:00:00	site2
sensorSignal	1,2,3;4;5;6;7;8;9;10;11;12;13;14;15;16;17;18;19;20;25	C0200103	2020-08-27 13:00:00	site2
sensorSignal	1,2,3;4;5;6;7;8;9;25	C0200103	2020-08-27 16:00:00	site2
sensorSignal	1,2,3;4;25	C0200103	2020-08-27 23:00:00	site2

The lower level of battery on 2020-08-27 is 3.87

On the 2020-08-27 the total number of devices that sent the data are 60

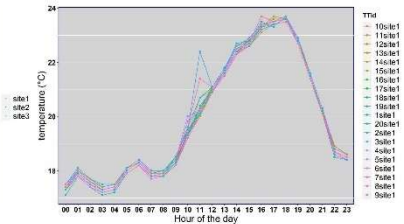
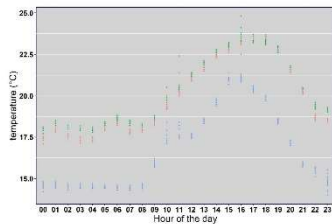
On the 2020-08-27 the total number of devices that didn't send the data are 0

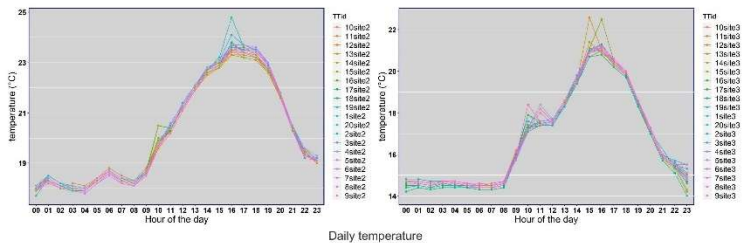
On the 2020-08-27 The devices that didn't send the data are

## 2. TT Daily graphs

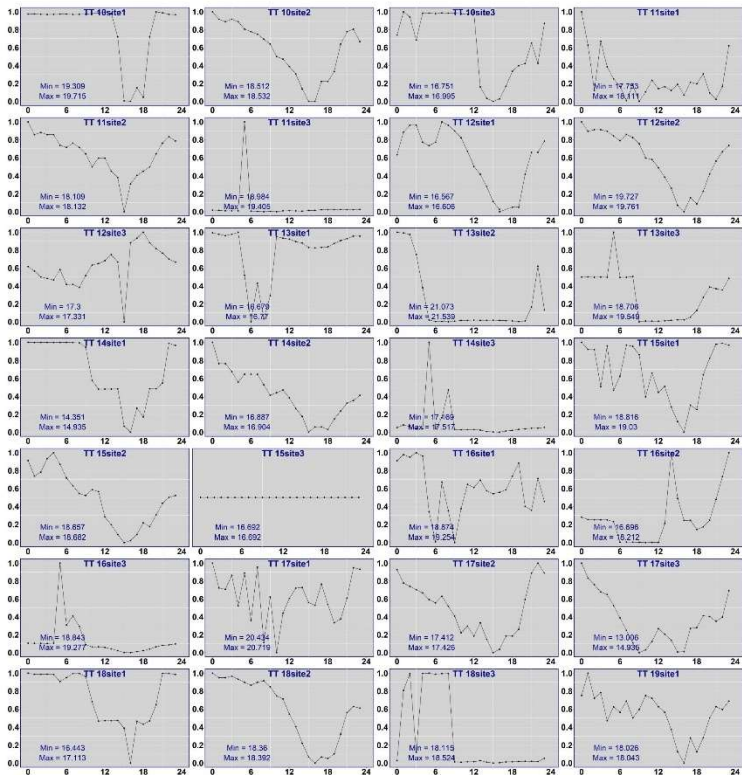
The daily graphs are important to understand if the sensors are working properly and to detect the daily trend of temperature, sapflux density and stem diameter variation per each site.

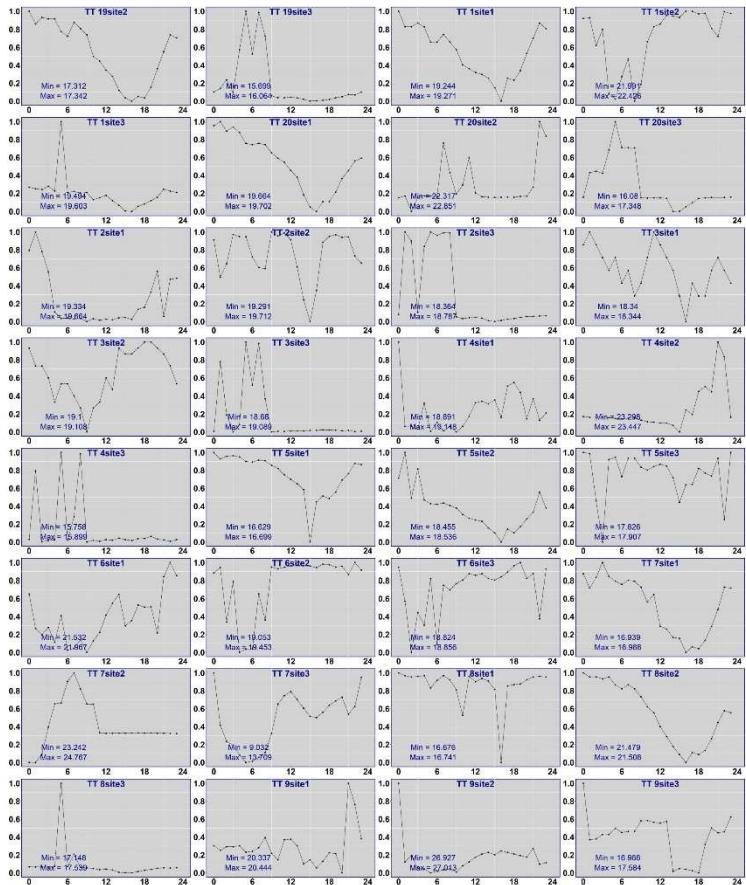
### 2.1. Temperature





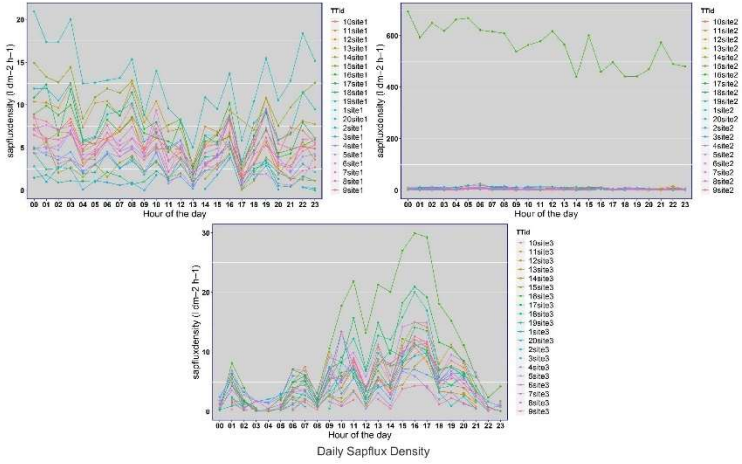
## 2.2. Stem diameter variation





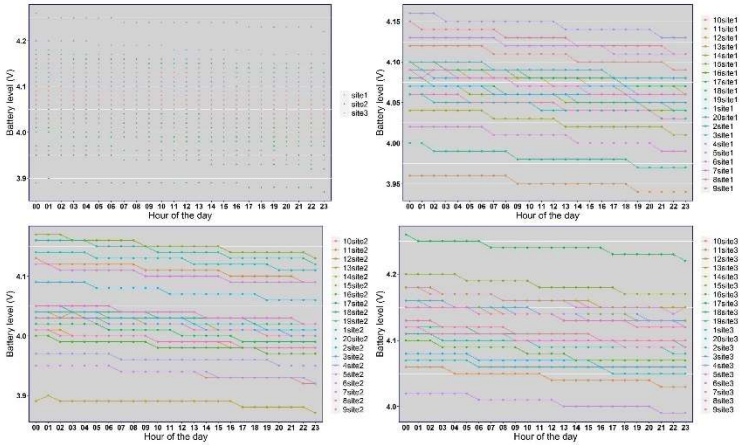
Daily stem diameter variation

### 2.3. Sapflux Density



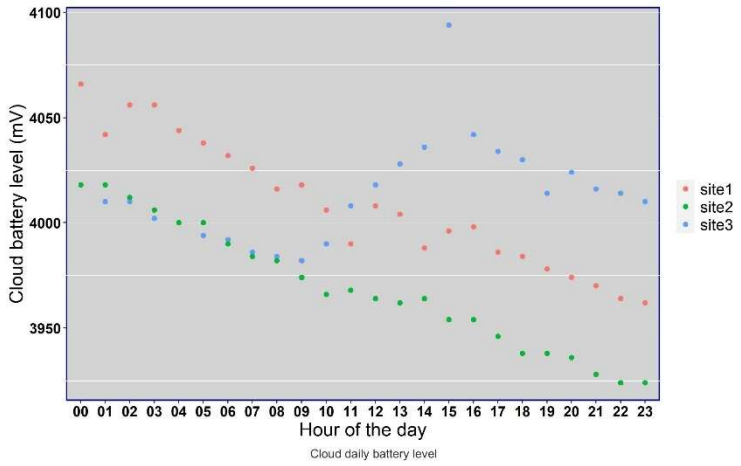
Daily Sapflux Density

### 2.4. Battery Level



Daily Battery Level

### 3.Cloud battery level



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## 3.2 Paper II: Sap Flow sensor calibration

### **Principles And Applicability of TreeTalker (TT+): A Two-Needle Transient Thermal Dissipation Enabling Real Time Sap Flux Density Measurement**

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## Abstract

Because of the low-cost, non-destructive, multifunctional nature of the IoT-based TreeTalker (TT+), both the transient thermal dissipation (TTD) system and constant thermal dissipation method (CTD) methods have become more accessible and interchangeable. Using this advance in technology, its application, we aim to assess the applicability and thus merit of the TreeTalker toward sap flux density measurement and computation. Capability analysis of TT+ is verified both under a lab scenario using an artificial hydraulic column of sawdust and a stem segment of *Fagus sylvatica* L. in the field via mounted TT+ devices and with comparison of a commercial sap flow sensors on different species. and in different cyclic TTD systems on *Populus nigra* L. and *Pinus pinea*. Installing a TT+ on the artificial flow system, temperature evolution data from heating and reference probes are recorded both in heating and cooling phases to compute values of different flow indices ( $K_{\text{Classic}}$ : (Granier, 1985), K1 & K2: (Doet al., 2018; Do & Rocheteau, 2002b), K3: (Mahjoub et al., 2009) under different flux densities. Applied continuous heating mode and a transient regime with four different combinations of heating and cooling times (in minutes) 10/10, 5/10, 15/45, and 10/50 are tested by TT+ and calibration of flux density vs flow indices conducted by applying optimal fitting curve on the source data up to  $8 \text{ L dm}^{-2}\text{h}^{-1}$ . Results Employing TT+ and revisiting different thermal approaches, we confirmed that the best possible empirical approach is the transient regime using cooling phase data to value flow index “K3”,

applying the temperature of probes after the heating current is switched off. The relationship between sap flux and K3 for different cyclic TTD systems was found to be linear with better coefficients of determination in the cooling phase ( $R^2 = 0.79 - 0.94$ ) with respect to the heating phase by valuing K1 and K2 ( $R^2 = 0.71 - 0.90$ ). Obtained calibration equations for sap flux density vs K1 were applied to data from different species across different locations and compared with thermal dissipation and heat pulse velocity methods to validate TT+ ability under field circumstances.

## **1. Introduction**

Sap flow as a proxy for transpiration and as an indicator of plant water status has become increasingly important in plant science (Vandegehuchte & Steppe, 2013). Numerous studies have reported a range of different sap flow measurement methods predominantly based on the application of heat to the sapwood area with the subsequent literature in this field being both broad and robust (Do et al., 2011; Do et al., 2018a; Flo et al., 2019; Granier, 1985; Huber & Schmidt, 1937; Isarangkool Na Ayutthaya et al., 2010; Marshall, 1958; Masmoudi, et al., 2012; Nadezhdina et al., 2012; Ren et al., 2020a; Vandegehuchte et al., 2012). Transporting heat as a tracer by the ascent of sap within xylem tissue to derive sap flow based on the principles of heat conduction–convection was, in fact, reported 90 years ago by Huber (1932). The heat conduction–convection equation in sapwood has been developed by Marshall (1958a) and is

used worldwide as an analytical solution of the heat partial differential equation in a specified isotropic medium. For an instantaneous heat pulse, Marshall (1958) proposed the following analytical solution:

$$\text{Equation 1. } \Delta T = \frac{Q}{4\pi kt} \exp \left[ -\frac{(x-Vt)^2 + y^2}{4Dt} \right]$$

with  $\Delta T$  (K) the temperature differences between two sap flow needles at position (x, y), t is the time in seconds, Q (K m<sup>2</sup>) is defined as the temperature to which the amount of heat liberated per unit length of the line would raise a unit volume of the substance, D the thermal diffusivity (m<sup>2</sup> s<sup>-1</sup>), and V the heat pulse velocity (m/s) (Vandegheuchte et al., 2012). This heat pulse velocity (HPV) is directly proportional to sap flux density. HPV method as a theoretical approach with the capability of capturing reverse, zero, and positive flow rates, is limited by measuring the flow rate greater than 0.54 m/hr and it is dependent on the thermal characteristics of the wood (D, thermal diffusivity).

Among the available thermal methods to measure sap flux density, the thermal dissipation method (TDP) is the most used in application and research to estimate plant transpiration (Vergeynst et al., 2014). The continuous heating technique was developed by Granier (Granier, 1985, 1987) which comprises two needle-sized sensors, a heater, and reference probes, inserted radially into the sapwood area. The flow rate is estimated from a dimensionless flow index,  $K_{\text{classic}}$ ,

which is related to the differential measurement of temperature between heated and reference probes in the heating phase.

$$\text{Equation 2. } K_{classic} = (\Delta T_{max} - \Delta T_i) / \Delta T_i$$

Where  $\Delta T_i$  is the actual temperature gradient between two probes and  $\Delta T_{max}$  is the maximum temperature gradient between the probes measured during a period of zero flow condition.

TDP method depends on the zero-flux condition and supports low, average, and high sap flux density estimations (Lu et al., 2002, 2004). Granier performed an empirical and species-independent calibration for the TDP system which is valid for various tree species (Cabibel, 1991; Granier, 1985).

$$\text{Equation 3. } SFD = 119 * K_{classic}^{1.231} * \frac{3600}{100000}$$

$$\text{in linear form } SFD = 4.28 * K_{classic}$$

Where SFD is the sap flux density ( $l \text{ dm}^{-2} \text{ h}^{-1}$ ).

However, the TDP method is not capable of measuring reverse flow, often underestimates the sap flux density by increasing wound impact due to continuous heating resulting, has a dependency on zero-flux, and is limited by high power consumption (Čermák et al., 1973; Čermák & Kučera, 1981; Granier, 1985, 1987; Lu et al., 2002, 2004; Sakuratani, 1984; Siqueira et al., 2020; Vandegheuchte & Steppe, 2013). Moreover, natural temperature gradients (NTG) in



stems of trees growing in open stands give rise to errors when measuring sap flow by the continuous thermal dissipation probe method (Do & Rocheteau, 2002b; Lubczynski et al., 2012). The influence of NTG on the TDP method has been extensively studied and has been shown to lead to errors of over 100% if not corrected (Do & Rocheteau, 2002a, 2002b; Lu et al., 2004; Lubczynski et al., 2012; Vandegehuchte & Steppe, 2013). Therefore, a novel cyclic heating system with a new flow index was developed by Do and Rocheteau (2002a) to reduce significantly the influence of NTG. Cyclic heating system works in transient thermal dissipation (TTD) conditions by introducing a relatively short cycle of heating and cooling (a minimum of 10 minutes heating and 10 minutes cooling) (Do et al., 2011). The transient signal ( $dT$ ) is the relative change in temperature over the heating period, between the differential cooled temperature ( $\Delta T_c$ , after cooling) and the maximum differential temperature reached after the period of heating ( $\Delta T_h$ ):

$$\text{Equation 4. } dT = \Delta T_h - \Delta T_c; \text{ given } K_1 = (dT_{max} - dT_i)/dT_i$$

Where  $dT_{max}$  is the maximum transient signal obtained under zero-flux conditions and  $dT_i$  is the measured signal at a given sap flux density.

The transient signal is normalized by its value at zero-flux and a non-species-specific calibration method is used to derive the sap flux

density equation in ( $l \text{ dm}^{-2} \text{ h}^{-1}$ ) (Do & Rocheteau, 2002a, 2002b; Isarangkool Na Ayutthaya et al., 2010).

$$\text{Equation 5. } SFD = \left(11.3 * \frac{K_1}{1-K_1}\right)^{0.707},$$

*in linear form: SFD = 12.95 \* K<sub>1</sub>*

To improve the TTD system, (Nhean et al., 2019) proposed using the incremental rise of temperature from 30 to 300 s time window after commencement of heating to derive  $K_2$  while normalizing as  $K_2 = (dT_{max} - dT_i)/dT_{max}$ . The assessed linear calibration for  $K_2$  yielded:

$$\text{Equation 6. } SFD = 6.42 * K_2$$

The possibility of measuring sap flow density (SFD) by a single heated probe using the transient regime (TTD) just after the heating current is switched off was proposed by (Mahjoub et al., 2009; Masmoudi et al., 2012). A new flow index ( $K_3$ ) is presented, which involves the transient signal (dT) at the beginning and intermediate times of the cooling kinetics. The recorded temperature at the end of the cooling phase where the equilibrium point is considered as the stem temperature.

$$\text{Equation 7. } K_3 = \frac{1}{t_i} \ln \frac{dT_{t_0}}{dT_{t_i}}$$

Where,  $t_0$  when the heating current is switched off,  $t_i$  is *intermediate time after kinetics*  $\approx 20$  seconds,  $dT_{t_0}$  is temperature decrease at the initial time of kinetics and  $dT_{t_i}$  is the temperature decrease at *intermediate time*. Using an olive stem segment, a non-species-specific calibration method is used to derive the sap flux density equation in ( $l\ dm^{-2}\ h^{-1}$ ) having  $K_3$  from cooling phase data.

$$\text{Equation 8. } SFD = a K_3 + b$$

when  $t_i = 20\ sec$   $a = 180$  and  $b = -8.7$

The above equation applies the classic 10/10 cyclic regime as introduced by (Do & Rocheteau, 2002b) and it is only valid for the positive flow when  $K_3 > -b/a$ .

Transient thermal dissipation (TTD) systems provide a simple way to measure xylem sap flux with a dual or a single Granier-type probe (Do et al., 2011; Mahjoub et al., 2009; Masmoudi et al., 2012; Ren et al., 2020), but the possibility of reducing the heating duration (minimum 5 minutes) while keeping enough sensitivity and accuracy in the response to flux density as well as lower energy consumption as suggested by (Nhean et al., 2019; Ren et al., 2020). However, TTD system has weak performance under low flux rates ( $< 1\ l\ dm^{-2}\ h^{-1}$ ) since with the minimum heat input, the system 95% is sufficient to reach  $dT_{max}$  (maximum temperature gradient under zero-flux) due to the nocturnal hydraulic activity of trees regarding the stem water

content (Do & Rocheteau, 2002a, 2002b). Stem water content may decline during the day to contribute to transpiration rate and as such  $dT_{\max}$  increases when WC decreases (0.1 to 2°C) (Vergeynst et al., 2014). Consequently, the sap flow measurement methods based on  $dT_{\max}$  under zero-flux (TDP and TTD) might introduce errors from 16 to 68.2% in daily sap flow measurement (Vergeynst et al., 2014). Although the above-mentioned sap flow measurement methods, the heat pulse method (HPV) (Marshall, 1958), continuous heating (TDP) (Granier, 1985), and cyclic regime (TTD) (Do & Rocheteau, 2002a) are well-established, exploiting an innovative in-situ IoT monitoring network for a fast assessment of forest transpiration rate with real-time data, low power consumption at very low cost is still under discussion (Ren et al., 2020; Siqueira et al., 2020; Vandegheuchte & Steppe, 2013) Thus, a new tool (called TreeTalker) developed with UNITUS, offers a unique feature of tailored firmware to measure plant water transport (Matasov et al., 2020; Tomelleri et al., 2022; Valentini et al., 2019; Zorzi et al., 2021). TreeTalker (TT+) is the automated setting of both described methods TDP and TTD, respectively. In TTD method, TreeTalker can accept the manipulation of any preferred heating/cooling durations to suit climatic variability and species-independent preference. Furthermore, the TT+ platform permits the recording of high-frequency data of heat dissipation in the sapwood area. This demonstrates a novel approach to the application of both methods cost-effectively and conveniently furthering scientific precision and

accurate based measurements of sap flux density. By default, TreeTalker is designed on a non-steady-state regime utilizing the transient thermal dissipation (TTD) method (Do et al., 2011; Do et al., 2018; Do & Rocheteau, 2002b, 2002a; Isarangkool Na Ayutthaya et al., 2010) which by theory is very close to thermal dissipation probe (TDP) method (Granier, 1985). However, the TTD method has been chosen for the TreeTalker development for the lower energy consumption which is a critical issue for the long-term operation of large sensor networks in remote areas.

The main purpose of this study is the validation of TreeTalker performance toward sap flux density measurements using hydraulic bench experiments and a beech stem segment in the lab, comparing with TDP and HPV methods as established throughout topical literature. Additionally, the proposition of a numerical solution to estimate sap flux density based on *heat flow equation capturing full heat dissipation curve* by TT+. Fig. 1 shows a flowchart with step-by-step processing of this study.

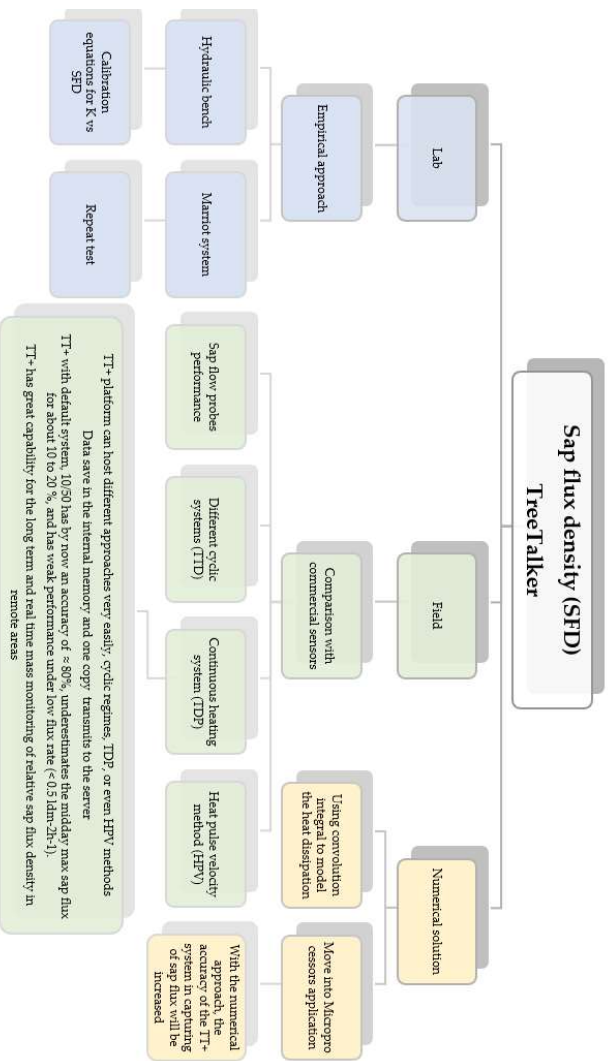


Figure 1. A flowchart of the study process for TreeTalker principles and applicability toward sap flow measurement

## **2. Materials and Methods**

### **2.1. Sensor design**

The TT+ sap flow measurement technique is based on the temperature difference between a pair of probes, the reference probe and the heater probe, which are inserted radially in the tree trunk with a vertical separation of 10 cm, facing north and covered by a reflective shield to avoid direct solar heating which may impact registered temperatures (Granier, 1985). One of the probes is heated with a power input of 0.2 watts while the other is used to measure the stem temperature, and stem water content using a thermistor and capacitance sensor (Asgharinia et al., 2022). TT+ sap flow measurement by default is set on the transient thermal dissipation (TTD) technique with a cyclic heating system (10 minutes of heating/50 minutes of cooling) to measure sap flux density (Do et al., 2011; Doet al., 2018; Isarangkool Na Ayutthaya et al., 2010; Masmoudi et al., 2012; Nhean et al., 2019). The length and diameter of sap flow probes are 20 and 3 mm, respectively.

### **2.2. Lab experiment**

Sap flux density measurements are a key feature and focus of the TreeTalker technology and thus require correct calibration of the system. Therefore, three explicit yet separate laboratory experiments were designed to obtain a calibration equation to convert flow index outputs from TreeTalker to sap flux. The three experiments included:

a) A hydraulic bench filled with sawdust and b) Using a stem segment of a felled beech tree. Each experiment utilizes the gravimetric water method as reference data where digital balance is used to collect water mass data, continuously.

### **2.2.1. Experiment using an artificial sapwood column**

TreeTalker sensor testing to quantify water transport in woody species was conducted under controlled laboratory conditions applying the calibration experiment method (Lubczynski et al., 2012; Wiedemann et al., 2016) in the laboratories of Tuscia University, Viterbo. For this purpose, two sap flow probe pairs of TreeTalkers are inserted into a plastic pipe with a horizontal separation of 10 cm (Fig. 2). The plastic pipe with a diameter of 3.2 cm and length of 65 cm, was filled with pine sawdust (Fig. 2). The pipe is supported by a 5 liters water tank in the head to pass the water through the plastic tubing (Fig. 2), and a pressure gauge, water container, and digital balance in the tail (Fig. 2). To provide constant water pressure, the main tank feeds water continuously from the bottom overflow tank by way of a mini pump which pumps water into the head tank (Fig. 2). A pressure gauge and 2 valves at either end of the poly plastic tubing are utilized to set the hydraulic bench on different levels of flux rates, essentially controlling the flow rate. In this experiment, under different flux rates, TT+ is programmed to provide high-frequency temperature records (every second) from Ref and Heater probes to capture the heating and cooling phases before and after the current switches off in each cycle. Capturing this high-frequency



data in this investigation aims to a.) Using different time windows from the temperature evolution records to provide calibration equations between different flow indices in the lab using hydraulic bench b.) To see the performance of the Ref probe and heater probes under different duration and amounts of heat input. In this experiment, the flux density rate was measured and collected via a digital balance. The digital balance specifically is utilized to facilitate the gravitational lab experiment where the calculation of stem water transport from the change in liquid mass on the balance to conduct non-species-specific calibration equations to convert the flow index of the TT+. This experiment covers sap flux density range between 0 to 8 ( $l\ dm^{-2}\ h^{-1}$ ).

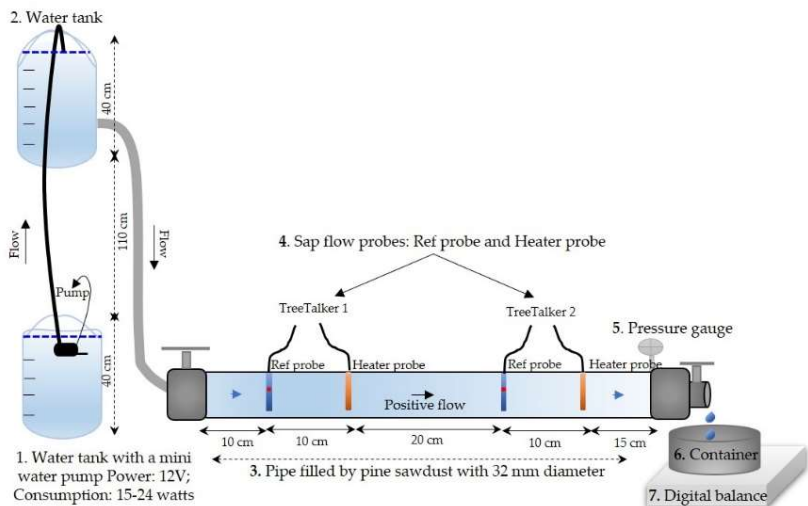


Figure 2. Hydraulic bench filled with pine sawdust for empirical calibration of TT+ regarding the sap flow measurement.

### 2.1.2. Experiment using a stem segment

Three European beech trees (*Fagus sylvatica* L.), beech 1, beech 2 and beech 3 were harvested at the experimental forest of Ghent University (Gontrode, Belgium) to retrieve cut stem segments with a length and diameter of approximately 22 and 14 cm, respectively. Each segment was stored in the fridge while wrapped with plastic film to retain its physiological integrity (avoid physiological deterioration) in preparation for hydraulic experiments. The first segment, beech 1 was installed as per the Mariotte-based verification system (Steppe et al., 2010). Firstly, a blade is used to remove the first layer of damaged vessels from the sample caused by destructively harvesting the stem. Secondly, a 2 cm collar of bark on one end of the segment is removed to attach and seal the plastic tubing column filled with water to avoid leakage. To improve the seal, a water-proof silicone glue is applied to fix the segment inside the tube where the bark was removed (Fig. 3.a). TT + sap flow sensors (Ref and Heater probes) were installed on the cut stem segment with a vertical separation of 10 cm, considering upstream and downstream directions in the segment (Fig. 3.b). In this experiment, the cycling mode of the TT+ was set to 10 minutes of heating and 50 minutes of cooling. Once sealed, probes inserted and TT+ ready to log the data, the segment and Mariotte system are placed in a climate chamber with a fixed temperature and humidity of 20° C and 70 %, respectively (Fig. 3.b). The Mariotte system with a principle of constant water pressure (McCarthy, 1934), can provide

different levels of water flow in the segment by changing the height of the Erlenmeyer flask (Steppe et al, 2010). Parallel measurements of the temperature difference between the TT+ probes are taken on an hourly scale, while water throughflow via the stem segment is recorded by a digital balance, every 2 minutes. Given our 5-hour time window, 5 data points were collected for this experiment. To control and check TT+ probe depth is correctly aligned with sapwood depth, dye in the form of food coloring is applied (Fig. 3.c). In this particular case, the sapwood depth of 3.5 cm is recorded and was sufficient to obtain both the correct installation of the probes and their functioning.

Beech 2 and beech 3 segments are used for detecting the capability of TT+ for measuring zero-flux. In this experiment, mounting two TT+ including the pair of sap flow probes (Ref and Heater probes), the hourly transient signal was measured in fresh-cut stem segments for approximately 2 days.

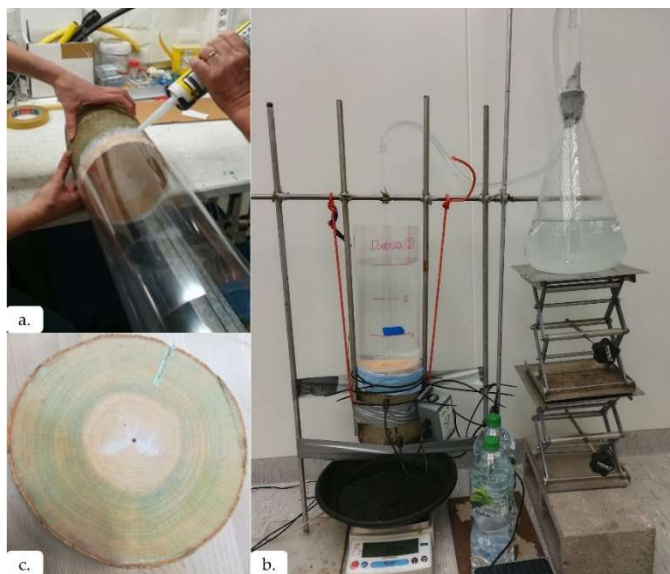


Figure 3. a. Preparation of the harvested stem cut for Mariotte system, b. Mariotte system with fresh cut beech stem segment for TT sap flow sensor calibration and c. detecting the sapwood depth with coloring technic.

## 2.2. Sap flux density measurements: field experiment

### 2.2.1. Time curves of temperature evolution for different cyclic regimes

To analyze the Ref and Heater probes responses under different flux rates with different heating duration, four TreeTalkers (TT+) were installed on two selected *P.nigra* trees at the experimental site of Tuscia University (Fig. 4). TreeTalkers are installed vertically toward the north at DBH height. On each tree, two pairs of Ref and heater probes were placed on the vertical axis with 10 cm of spacing.

In this experiment, TT+ was programmed to collect high-frequency records (every 2 sec) of heat dissipation data under different heating/cooling cycles in the living trees.



Figure 4. Installation of two pairs of TT+'s sap flow probes on *P. nigra*, recording high frequency data of heat dissipation, Unitus, Italy.

### **2.2.2. Effect of heating duration on sap flux measurement in a living tree**

A *Pinus pinea*, native to the Mediterranean region, was chosen to apply 3 TreeTalkers with 3 different cyclic TTD systems in Campania, Italy. Heating/cooling cycles selected for each TreeTalker were 50/10, 10/50- and 10/10-minutes, respectively.

### **2.2.3. Comparison of TT+ with HPV and TDP system across different species in different locations**

TreeTalker were installed on different species, at different locations across Europe spanning several yet not identical growing seasons. Investigations focus on the accuracy of sap flux density measurement methods in comparison with other commercially available sensors.

- a. To evaluate the TT+ capability in terms of measuring the absolute value of sap flux rate, two TT+ were installed on European beech trees (*Fagus sylvatica* L.) at the experimental forest of Ghent University (Gontrode, Belgium) (Fig. 5). On the same trees, TreeWatch sensors were installed which are performed based on the heat pulse velocity (HPV) system. The DBH of the selected trees, beech 1 and beech 2 were 26 and 63 cm, respectively. Moreover, the sapwood areas for the above-mentioned trees are equal to 130.5 and 710.6 cm<sup>2</sup>.



Figure 5. TT+ and TreeWatch installation on a beech tree, UGent, Belgium.

- b. *Picea abies* as a native large pyramidal evergreen conifer in the alpine region of Switzerland was selected to apply commercial the UP Sap Flow-System (TDP based) and TreeTalker. The UPS sensor operates on a continuous heating technique and applies 0.2 watts of power consumption. The sensor includes the Granier-type probe with a total length of 33 mm and a heating zone of 20 mm. TT+ functions with the same characteristics, approximately. The heater probe of TreeTalker has a length of about 20 mm with heat across its entire surface area. In this study, TreeTalker was set on a non-steady heating regime with 10 minutes of heating and 20 minutes of cooling cycle. Sap flux density (SFD,  $l\ dm^{-2}\ h^{-1}$ ) was measured and compared in 5

randomly selected *P. abies* using TT+ and UPS sensors mounted at diameter at breast height and both facing south.

- c. *Fagus sylvatica* L. and *Quercus rubra* L. were chosen to continue the investigation of TT+ sap flow sensor capability in capturing sap flux density in comparison with the TDP system in different locations with the collaboration of Technische Universität München (TUM) and Philipps-Universität Marburg. Mounting TT+ and TDP system, beech and oak with DBH of 30.4 and 20.7 cm are monitored from mid-June to the end of July 2021 and oak in Technische Universität München. In addition, for two consecutive years (2020 and 2021), TT+ and TDP systems are installed on a beech tree with DBH of 52 cm at MOF study area with the collaboration of Philipps-Universität Marburg.

### **3. Results and discussion**

#### **3.1. Temperature gradient under different heating/cooling durations in hydraulic bench**

One of the benefits obtained via the TT+ platform is its flexibility and user-specified firmware capabilities allowing the capture of a high frequency of heat flow data (Fig. 6). Benefiting from these capabilities and using tailored firmware, we demonstrate the capture of temperature evolution during the cyclic heating and cooling



phases of the heater and reference probes under different flux rates and with several possible cyclic regimes (Fig. 6) in the lab with a hydraulic bench. The aim of recording the full curve of heat dissipation was to extract temperature gradient data of different time windows and thus giving rise to different flow index evaluations. For instance, in the heating phase, using initial  $\Delta T$ ,  $\Delta t_{\min}$  and  $\Delta t_{\max}$  can fulfil evaluating K1, while using  $\Delta T$  at 30 and 300 seconds provided a different flow index, K2 introduced by (Nhean et al., 2019). In addition, in the cooling phase, getting  $\Delta T$  at the initial point after turning off the heater current and 20 seconds after, we can apply K3 flow index.

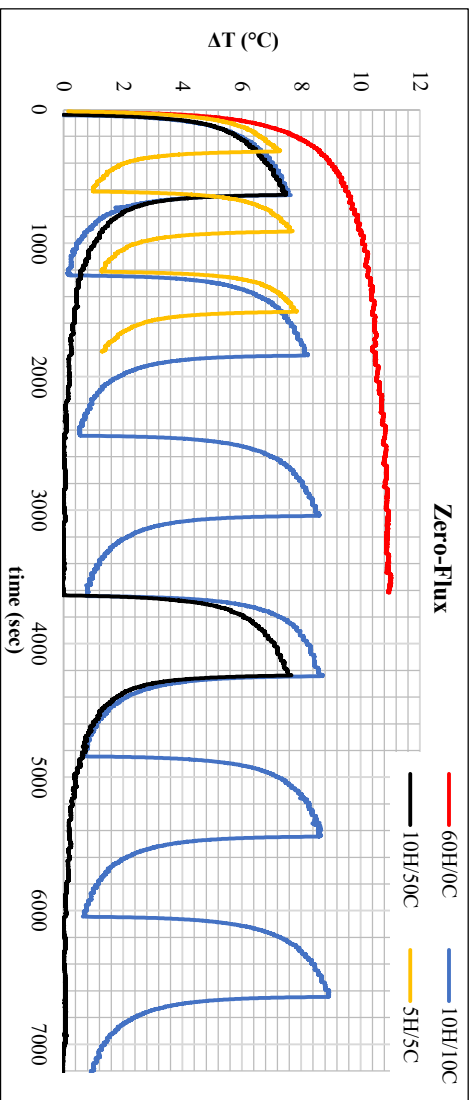


Figure 1. TT+ performance in recording heat dissipation data using different heating/cooling durations, e.g., 60/0, 5/5, 10/10, and 10/50.

Comparing the classic cyclic system 10/10 proposed by (Do & Rocheteau, 2002a, 2002b) to the 10/50 cycle which is the default of TT+ for the sake of low power consumption, shows that under low and fast-flux rate, irrespective of cooling duration, both of the cycles, 10/10 and 10/50 are reaching the steady-state as well as converging to each other within 600 sec, whereas, under the zero-flux condition, neither of the two stated conditions can be satisfied (Fig. 7). Moreover, cycle 10/50 in comparison with cycle 10/10 is underestimating the  $\Delta T_{max}$  by about 1 °C (Fig. 7).

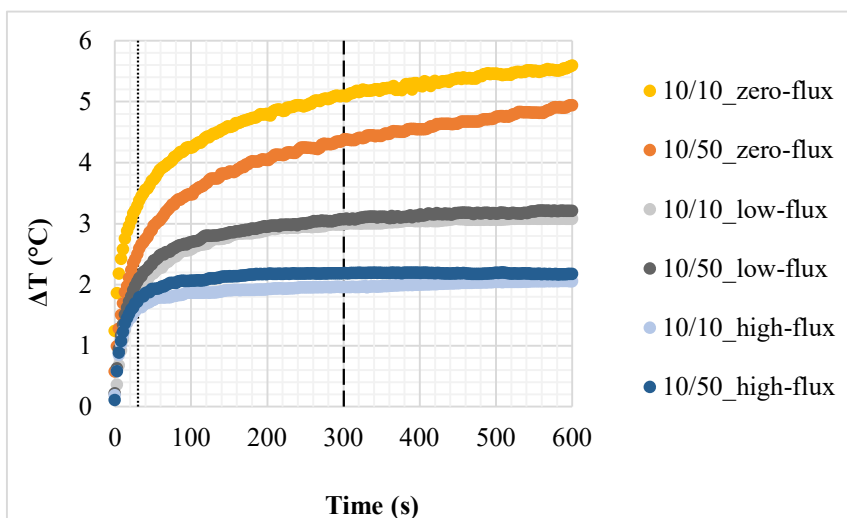


Figure 7. Comparison of the classic cyclic regime, 10/10 to TT+ default with 10/50 system under zero, medium ( $1.7 \text{ l dm}^{-2} \text{ h}^{-1}$ ), and fast-flux ( $5.6 \text{ l dm}^{-2} \text{ h}^{-1}$ ) rate.

Figures 8a and 8b captures changes in  $\Delta T$  according to different flux rates from hydraulic bench experiments of heating and cooling phases using TT+ set on a 10/50 cycle with tailored firmware. In Fig.8, time windows are highlighted by the red vertical lines to separate time windows as a reference to curves, in the heating phase 30, 300, and 600 seconds and in the cooling phase 20 seconds.

Focusing on time to reach a steady-state in the heating phase, where the plateau of the curve is, we see that under higher flux rates (SFD=1.7, 4.1, 5.6), a steady-state is achieved within the 600 seconds heating regime. On the contrary, however, for low and near null flux (SFD =0 and 0.36) the 600 seconds heating regime is not enough to reach a steady state implying modification of heat duration input. As such, problems may arise when using this heating regime with 600 seconds to estimate  $\Delta t_{max}$  for empirical approaches. Regarding the cooling phase, the heat dissipation curves for all SFD vary in exponential form, and the slope of each curve and respective  $\Delta t_{max}$  change according to each SFD. Considering the empirical method of (Mahjoub et al., 2009), which is independent of  $\Delta t_{max}$  under zero-flux conditions as well as heating duration, using the cooling phase data can lead to a more accurate estimation of the flow rate. Importantly for both heating and cooling phases, the magnitude of  $\Delta T$  is distinguishable at the end of the heating phase and the start of the cooling phase for different flux rates.

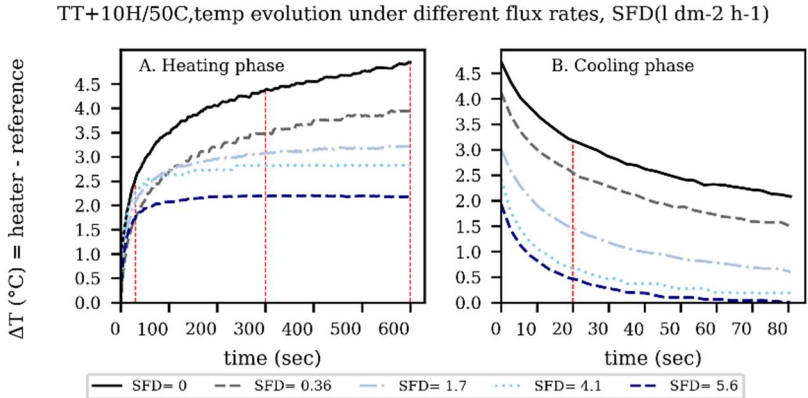


Figure 8. The temperature gradient in heating and cooling phases under different flux rates, data captured by TT+ set on 10/50 cyclic regime

### 3.2. Sap flux density vs flow indexes

Captured data for heat dissipation in the heating phase and cooling by using two sets of the Heater and Ref probes allowed the estimation of different flow indices K1 (K\_Heating phase), K2 for the time window of 30 to 300 sec (K: 30 to 300 sec\_Heating phase) and K3 (K: 0 to 20 sec\_Cooling phase) to find calibration equations between flow indices and gravimetric SFD. Calibration involved the y variable as SFD captured by digital balance and the x-axis the different flow indices (Fig. 9). The different heating and cooling phases and durations are presented for each scenario. The results from this set of graphs demonstrate respectable correlations between the K1 and SFD under all scenarios with variations in intercepts and  $R^2$  values (table 1). Fig. 9.a shows the relationship between K1 and

SFD, where  $K1$  is derived as the initial  $\Delta T$  at 0 seconds and  $\Delta t_{max}$  from the heat dissipation curves in the heating phase and R-value ranging 0.88-0.95 and slope ranging from 2.93 and 7.13 respectively. Here heat input consequently has a significant influence according to different flux. For example, the blue line or continuous heating approach, the TDP method, has a slope of 2.93, whereas the 15/45 TTD approach is 7.13. In Fig. 9.b,  $K2$  and SFD, where  $K2$ , is still in the heating phase, applies the subsequent derivatives of  $\Delta T$  from a range of 30-300 seconds as introduced for the transient regime (Nhean et al., 2019). Again, we report similar linear forms, however, R-values differ in magnitude between 0.81 and 0.94 and intercepts between 7.33 and 10.07. In Fig. 9.c,  $K3$  and SFD, where  $\Delta T$  derivatives are taken from the cooling phase rather than the heating phase and not surprisingly similar regarding slope given the natural behavior of heat loss over time and increased SFD. The poorest correlation between 5/10 may be explained by the lack of sufficient heat input of substrate volume not receiving a large enough heat pulse to be influential. Both Fig. 9a and 9b show that the derivatives of flow indexes are highly varied under different heating/cooling durations whereas the cooling phase, 9c is irrespective of heating duration. In general, the linear regression model is not the best form to represent the relation between flow indexes and SFD, polynomial models or sigmoid functions are recommended to capture the K behavior vs SFD (Do & Rocheteau, 2002a, 2002b). However, the table 1 was the preliminary analysis to evaluate and compare the results in terms of

different  $K$ , and different heating/cooling durations under different flux rates.

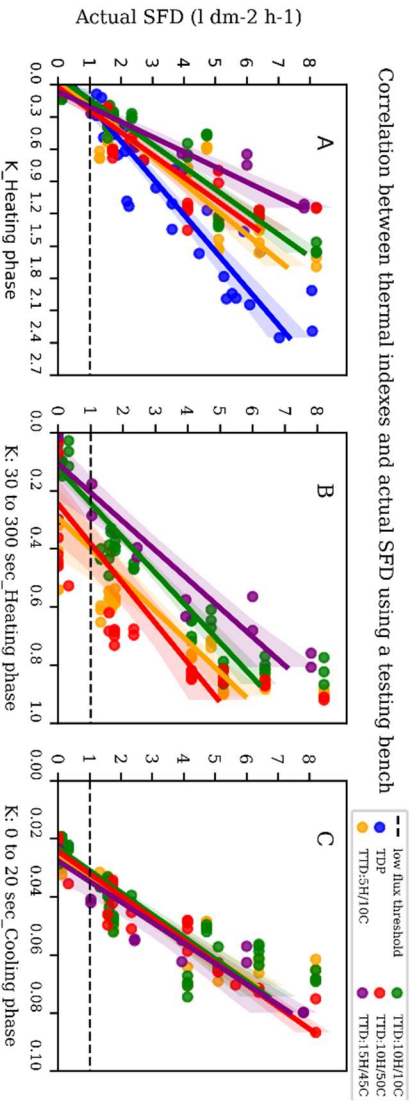


Figure 1. Linear correlation between flow indexes driven from TreeTalker vs gravimetric sap flux density a. K\_Heating phase (K1), b. K: 30 to 300 sec\_Heating phase (K2), and c. K: 0 to 20 sec\_Cooling phase (K3), and Y-axis: actual SFD measured by digital balance.



Table 1. regression analysis between flow indices and gravimetric SFD for different scenarios in linear standard form (SFD=aK+b)

Var.	K_Heating phase					K_30 to 300 sec_Heating phase					K3_Cooling phase				
	TDP	5H/10C	10H/10C	10H/50C	15H/45C	5H/10C	10H/10C	10H/50C	15H/45C	15H/45C	5H/10C	10H/10C	10H/50C	15H/45C	15H/45C
slope	2.93	4.18	4.79	4.79	7.13	9.40	8.34	7.33	10.07	10.07	143.36	131.08	130.91	141.44	
intercept	0.45	0.16	0.33	-0.13	-0.47	-2.76	-1.03	-1.81	-1.09	-1.09	-3.81	-3.06	-3.20	-3.91	
rvalue	0.93	0.92	0.95	0.88	0.95	0.84	0.94	0.81	0.93	0.93	0.89	0.91	0.97	0.94	
pvalue	7.1E-14	2.0E-17	1.4E-26	1.2E-10	7.38E-07	1.77E-12	4.42E-25	1.53E-08	4.63E-06	4.63E-06	9.72E-19	1.5E-26	1.3E-27	2.5E-06	
stderr	0.22	0.29	0.23	0.49	0.71	0.95	0.43	0.97	1.21	1.21	10.45	7.47	5.01	15.98	
n	30	41	53	31	13	43	53	33	13	13	53	68	44	13.000	

For the default mode of TT+, 10/50, the regression analysis for the hydraulic bench data, K\_Heating phase, K: 30 to 300 sec\_Heating phase, and K: 0 to 20 sec\_Cooling phase were represented in the following section applying the polynomial model, as well (Fig. 10, table 2). A second-order polynomial function in the standard form of  $SFD = a + bK + cK^2$  was applied to the data. In comparison to the offered linear regression forms (table 1), for K1, R-squared slightly degraded from 0.77 to 0.73, while K2 and K3 improved from 0.66 to 0.91, and 0.94 to 0.96, respectively (table 2). Applying polynomial function, with respect to RMSE and R-squared, in the heating phase K2, and in the cooling phase K3, offers the best empirical estimation of SFD (table 2). However, using the preferred flow index in the heating phase (K2) is limited for the low flux rate ( $< 1 \text{ l dm}^{-2} \text{ h}^{-1}$ ) and the flow index driven from the cooling phase is only applicable for the positive flow.

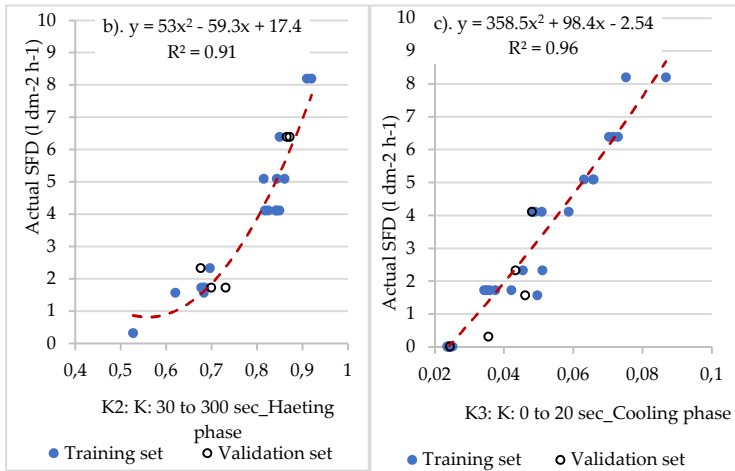
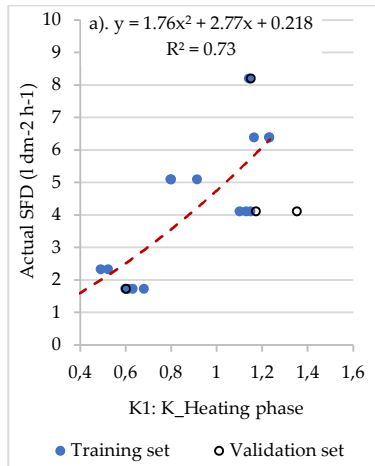


Figure 10. Quadratic polynomial model between flow indexes driven from TreeTalker (10/50) vs gravimetric sap flux density, a. K\_Heating phase, b. K: 30 to 300 sec\_Haeting phase, and c. K: 0 to 20 sec\_Cooling phase.

Table 1. Regression analysis between flow indices and gravimetric SFD for different scenarios in second-order polynomial form ( $SFD = a + bK + cK^2$ )

non-linear regression for 10/50 regime	Parameters	Value	Standard error	Lower bound (95%)	Upper bound (95%)	MSE	RMSE	R-squared	Observations	restriction
SFD vs K_Heating phase	a	0.218	1.180	-2.298	2.734	1.508	1.228	0.73	25	-
	b	2.772	3.442	-4.564	10.109					
	c	1.766	2.308	-3.154	6.685					
SFD vs K:30 to 300 sec_Heating phase	a	17.414	7.967	0.433	34.395	0.503	0.709	0.91	25	K>0.55
	b	-59.301	21.842	-105.857	-12.746					
	c	52.971	14.728	21.579	84.362					
SFD vs K: 0 to 20 sec_Cooling phase	a	-2.544	0.653	-3.876	-1.212	0.319	0.565	0.96	40	K>0.025
	b	98.380	30.805	35.552	161.207					
	c	358.486	314.050	-282.023	998.995					

### 3.3. Stem segment results

Fig. 11.a is representative of calibration equations for TT+ with 10/50 regime mounted on a stem segment, beech 1 placed in the climatic chamber using the Mariotte system. The results of the Mariotte system experiment provide a calibration equation between flow index, K1 (derived from TT output) and SFD (balance data). The resulting linear equation gives a slope of 3.5 which is quasi-close to the value achieved using sawdust in a hydraulic bench (4.79) for the mentioned cyclic system. The differences between the two may be explained by the lack of sufficient data from the Mariotte system. Therefore, the Mariotte system experiment should be repeated to obtain more data points to cover a greater range of SFD.

Fig. 11.b instead, is a comparison of TT+ performance using the 10/50 regime aiming to detect zero flux. Here, two freshly cut stem segments, beech 2 and beech 3 were placed in a climatic chamber with independent TT+ mounted to record changes in SFD. As the results demonstrate, the TT+ is detecting a very low SFD up to  $0.2 \text{ l dm}^{-2} \text{ h}^{-1}$  for the first 18 hours. The expected zero-flux was achieved after this period. Indeed, this is considered as the relative error in SFD measurement under zero flux conditions and may be attributed to fluctuations in the stem water content of freshly cut stems. Furthermore, these results confirm the overestimation of the SFD near null flux by applying empirical thermal approaches which is

reported by several studies, as well (Čermák et al., 2007; Vandegehuchte & Steppe, 2013; Vergeynst et al., 2014).

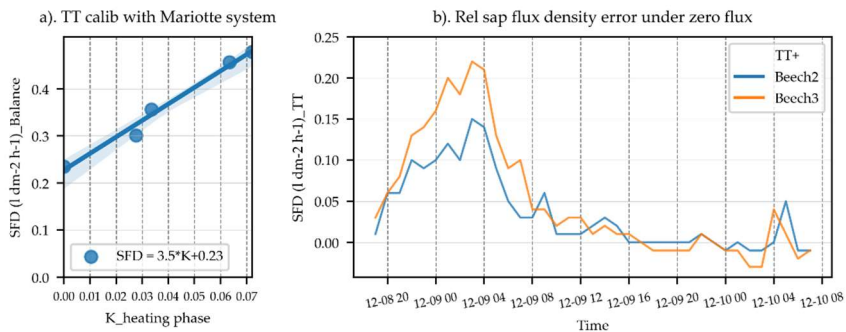


Figure 11. a. Correlation between flow index (K1: K<sub>Heating phase</sub>) and gravimetric SFD using stem segment of fresh-cut beech mounted in Mariotte system, b. SFD data are recorded with two TT+ installed on two stem segments of fresh-cut beech to analyze the capability of the sensor in zero-flux measurement.

### 3.4. Reference and heater Probes' performance in living trees

Fig. 12 displays the results of temperature fluctuation derived from the heater and reference probes with different cycling regimes (10H/50C and 10H/10C) applied to a living tree, *P. nigra* from 07:00 am 25th of May to 07:00 am 26th of May. The highlighted sections denoted as a and b were randomly chosen from the data set to reflect day and night-time to discuss in detail such observations in the following section. The reference probe provides stem temperature data which is shown by the grey line in Fig. 12 and displays a max

stem temperature at 18:00. The temperature oscillation of both heat probes (Black dashed and Blue solid lines) is closely aligned since both methods have 10 minutes of heat input, however, the cyclic 10/10 approach better responds to the maximum transpiration rate in this tree (occurring at 6 pm) This is also reflected in Fig. 12 by the respective  $\Delta T$ .

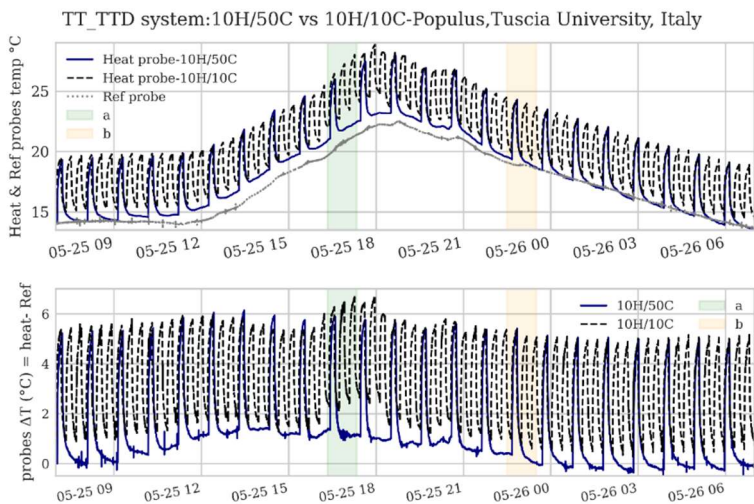


Figure 12. temperature evolution data for 10/10 and 10/50 regimes recorded by TT+ on *P. nigra*, Unitus, Italy.

Using the highlighted a and b sections from the above experiment, Figs. 13.a and 13.b provide a snapshot to see the impact of differing cycling regimes on the performance of the heater and reference probes during the day and night. Of note, Fig. 13.a demonstrates that recorded daily  $\Delta T$  with the 10/10 approach (4.2 °C) is smaller than the same data provided by 10/50 approach (4.4 °C). This is further

highlighted by the respective SFD estimations for both cycle regimes which show midday underestimation of about 20% and nighttime over estimation of flux rates with 10/50 system (Fig. 13.b). To estimate SFD, derivatives of  $\Delta T$  contributed to the flow index equation (K2\_Heating phase) and eventually provided SFD results based on the calibration equations for each cyclic system (Fig. 13.c). Overestimation of SFD may occur under very low flux rates (here  $\approx < 10 \text{ g m}^{-2} \text{ s}^{-1} / 0.36 \text{ l dm}^{-2} \text{ h}^{-1}$ ) and higher fluctuation of stem water storage (Vergeynst et al., 2014).



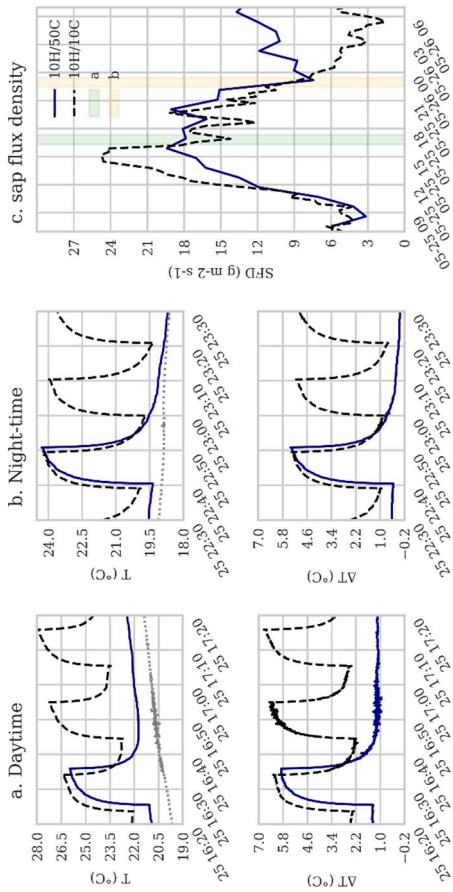


Figure 1. a. heat dissipation curve by 10/10 and 10/50 regimes in the daytime, b. heat dissipation curve by 10/10 and 10/50 regimes in the nighttime, c. estimated sap flux based on flow index of heating phase for both cyclic systems,

*P.nigra*, Unitus, Italy

### **3.5. Heating/cooling duration effect on SFD data in *P. pinea***

In this section, a comparison of different heating/cooling durations and their impact or effect on flow index and SFD are presented. To examine the variation of the measured sap flux by different cyclic systems, 3 TreeTalkers with heating/cooling regimes of 10/50, 50/10 and 10/10 minutes were mounted at diameter at breast height DBH on a *P. pinea* in Campania, Italy. As the 10/10 regime represents the original formulation established by (Do & Rocheteau, 2002a), in Fig. 14 we have considered it as a reference and thus the independent variable on the x-axis. The result of flow indexes of 10/50 and 50/10 modes are sat on the y-axis. The flow indices for all cyclic regimes are estimated based on eq.4, K1 and then applied in the SFD calibration equations provided in table 1. As we don't have calibrations for 50/10 we assume the TDP method to derive SFD for this particular cyclic regime.

Having data for 7 days, from 12<sup>th</sup> to 18<sup>th</sup> April 2020, it is apparent that TreeTalker set on 50H/10C mode overestimates the flow index and subsequently SFD by  $\approx 2.3$  and 1.5 times, respectively. In contrast, the average results associated with 10/50 suggest a plausible underestimation of the flow index and subsequently also SFD by  $\approx 0.65$  and 0.8 times compared with 10/10 method. In addition to capturing SFD using various heating/cooling regimes, we confirm that the max SFD occurs around midday irrespective of the regime applied to see Fig. 15. In Fig. 15, the Gustafsson noise filtering

method (Gustafsson, 1996) slightly is applied to sap flux density results to smooth the curves while keeping the shape and magnitude of the SFD rate unchanged.

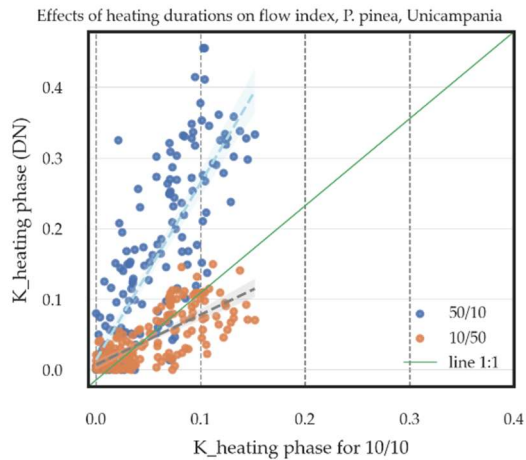


Figure 14. Comparison of flow indexes (K1) of TreeTalkers in 10/10 mode with 50H/10C and 10H/50C modes.

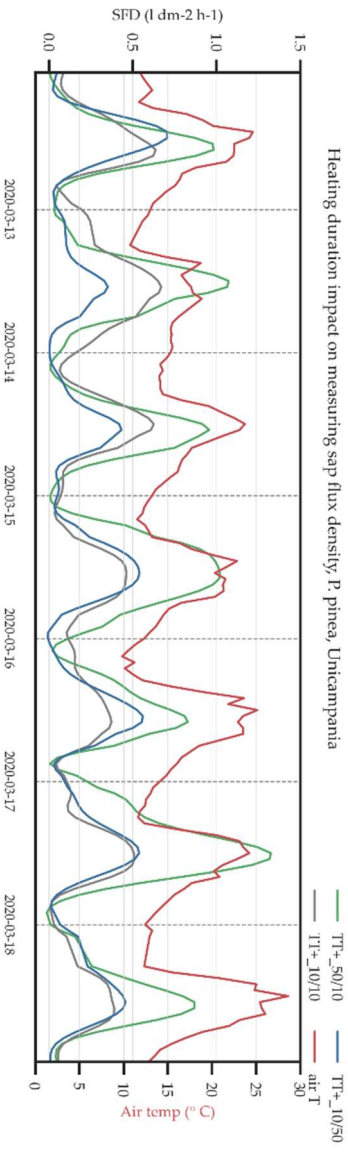


Figure 1. Results of hourly sap flux density and air temperature in a *P. pinea* measured by TreeTalker with 3 different sets of heating/cooling durations (50/10, 10/50, and 10/10)

### **3.6. Comparison of TT+ with Heat pulse velocity method**

As the heat pulse velocity method (HPV) is a non-empirical method where SFD is based on thermal dissipation from theoretical ideas of conduction (material substrate wood) and convection (water velocity sap) offering an absolute measurement of SFD in living trees. The result of this investigation between TT+ with 10/50 regime (grey solid line) and the HPV method (orange solid line) yields similar patterns regarding SFD measurements (Fig. 16). Interestingly, toward the end of the growing season when beech transpiration undergoes a reduction as they enter the dormancy phase for winter, the TT+ demonstrates a good capacity ( $\approx 80\%$ ) for capturing the SFD in comparison with the HPV method (Fig. 16). However, TT+ again underestimates max daily SFD by approximately 20%. Furthermore, near null fluxes are difficult to detect accurate flux patterns utilizing TT+ as well as HPV method (Fig. 16). It should be considered that HPV method is overestimating the SFD rate under low flux conditions (Fig. 16) (Ren et al., 2020). In Fig. 16, applying the Gustafsson noise filtering method, the smoothed curve for TT+ is presented as a blue solid line with the label of TT+/ Gustafsson, as well.

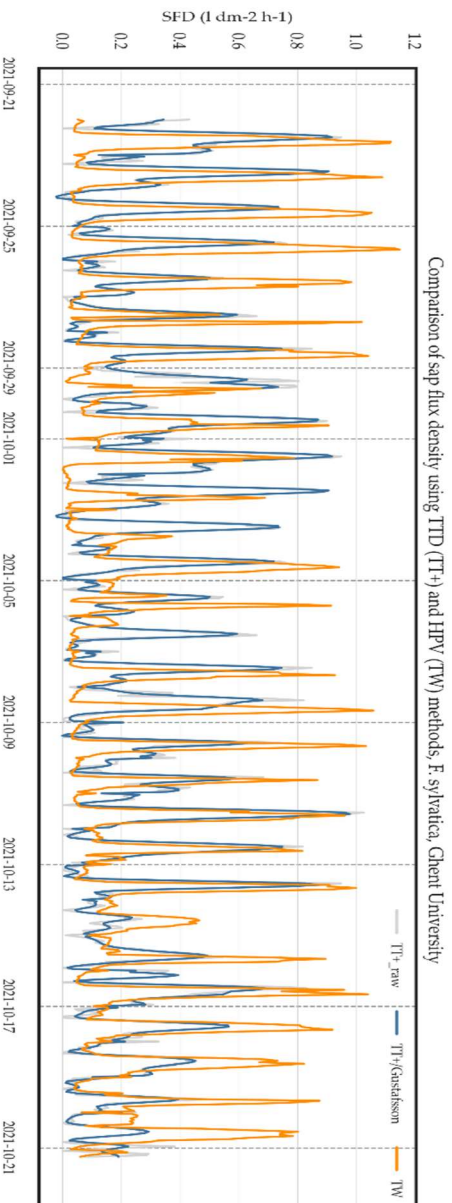


Figure 1. SFD measurement with Transient regime (TT+ with 10/50 mode) vs HPV method using Tree Watch (TW) in beech, UGent, Belgium.

### **3.7. Comparison of TT+ with 10/50 mode to the continuous heating system (TDP)**

In the comparison of TT+ and HPV systems, the next phase involved a comparison of another well-established method, the continuous heating technique (TDP).

The total measurements of TT+ and TDP on a living beech tree for two consecutive growing seasons, 2020 and 2021 from the MOF study area, Philipps-Universität Marburg are displayed in Fig. 17.a and 17.b. The result of this experiment between hourly SFD data of TT+ (10/50) (muted blue solid line) vs TDP (muted green solid line) as well as daily max SFD of TT+ (10/50) (orange solid line) vs TDP (red solid line) are presented in Fig. 17.a and 17.b for two growing seasons, from July to mid-October, 2020 and 2021. In addition, Fig. 17.a and 17.b show the variations in average daily air temperature (black solid line) at the sampling site. When the annual average of SFD measured by TDP system is  $< 1 \text{ l dm}^{-2} \text{ h}^{-1}$ , the relative error of SFD measurement with TT+ utilizing the 10/50 system is higher (Fig. 17.a), whereas, for the annual average flux rate  $> 1 \text{ l dm}^{-2} \text{ h}^{-1}$ , TT+ (10/50) shows better performance in capturing SFD (Fig. 17.b). However, for the cyclic system with short heat input (10 min heating), a relative error of SFD measurements, about 41% is anticipated under a low flux rate ( $< \approx 0.5 \text{ l dm}^{-2} \text{ h}^{-1}$ ) (Isarangkool Na Ayutthaya et al., 2010).

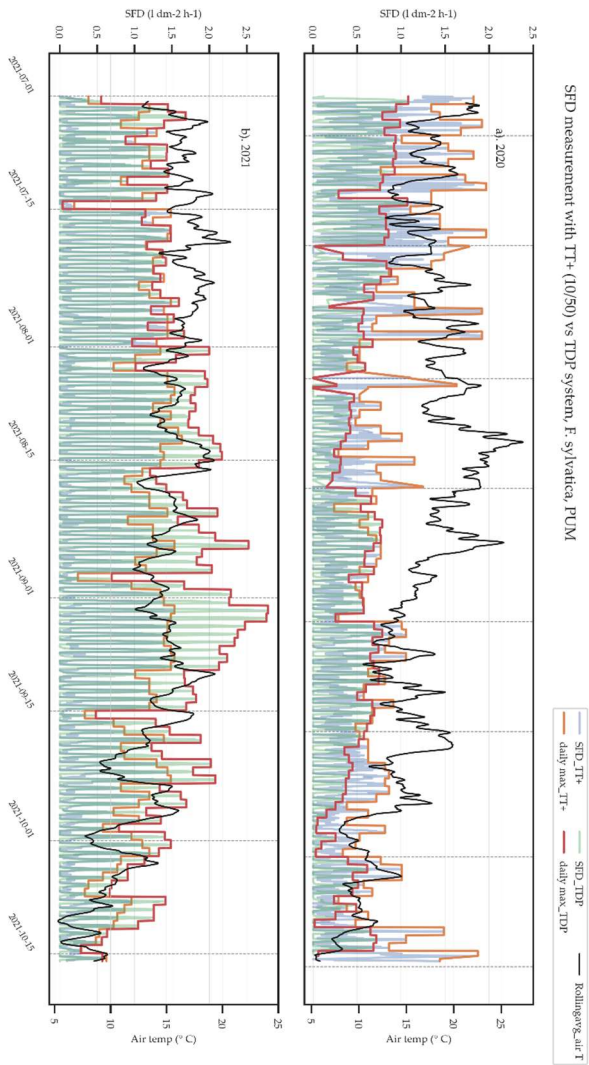


Figure 1. Comparison of TTP+ with the transient regime (10/50) with TDP method on beech, MOF study area, Philipps-Universität Marburg.



To compare the results of the two methods, the error of SFD is estimated using the following equation (Nourtier et al., 2011).

$$\text{Equation 9 } SFD\_Error = (SFD_{max-24h} - SFD_i)_{TT (10/50)} - (SFD_{max-2} - SFD_i)_{TDP}$$

When capturing data for the growing period with a heat wave in 2020 in comparison with 2021 records, TT+ and TDP systems, both are similarly aligned in terms of recorded data for low SFD whereas TT+ error of SFD measurement rises to +1.8 while in the growing season 2021, the SFD error measured by TT+ is equal to -1.6 (Fig. 18). The rate of occurred divergence between the two methods is remarkable. The continuous heating system (TDP) has the capability of capturing the SFD rate and pattern near null fluxes since the amount of heat input is sufficient to reach a steady state, yet, this method rise would impact due to the non-stop heat input and thus, underestimates the SFD rate. Using thermal approaches dependent on  $\Delta T_{max}$ , especially TTD method with shorter heat input, generally introduces some error in SFD measurement since detecting accurately the magnitude and time of  $\Delta t_{max}$  occurrence are very difficult. Controlling the time of  $\Delta t_{max}$  occurrence in collected data for two consecutive growing seasons with 10 min heat input revealed the possibility of occurrence of the event at any time within 24 hours

(Fig. 18). Daytime  $\Delta T_{\max}$  might cause significant uncertainties in SFD estimation.

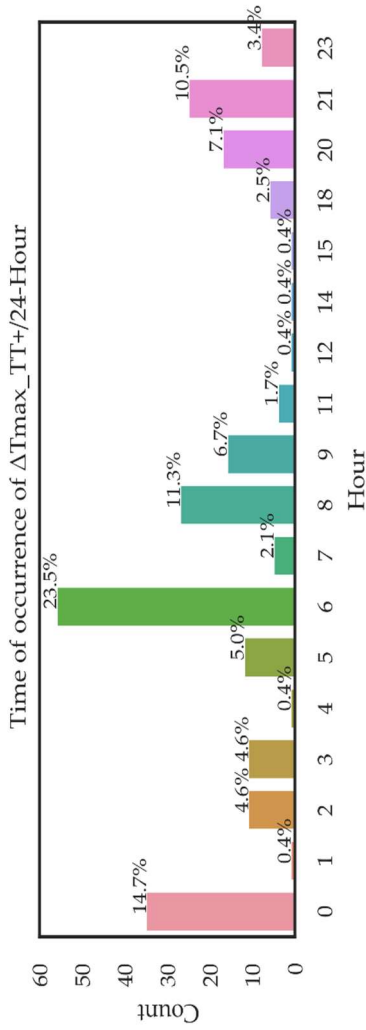


Figure 1. Frequency of the maximal  $\Delta T$  occurrence time for two growing season (2020 and 2021), *Fagus. sylvatica*, MOF study area, Philipps-Universität Marburg.

Multiple  $\Delta T_{\max}$  approaches are now in use to detect correctly the zero-flow conditions including the daily predawn (PD) (Lu et al., 2004), maximum moving window (MW) (Rabbel et al., 2016), double regression (DR) (Lu et al., 2004) and environmental dependent method (ED) (Oishi et al., 2016). Here we applied PD and MW methods to see if the error of SFD measurement decreases (Fig. 19). Even though different approaches were utilized to detect the reasonable time and amount of  $\Delta T_{\max}$ , SFD estimates remain imprecise because of the empirical as well as strong dependency on the  $\Delta T_{\max}$  event. Improvement in SFD measurement using PD and MW approaches in comparison with the TDP system was about 3% which is negligible.

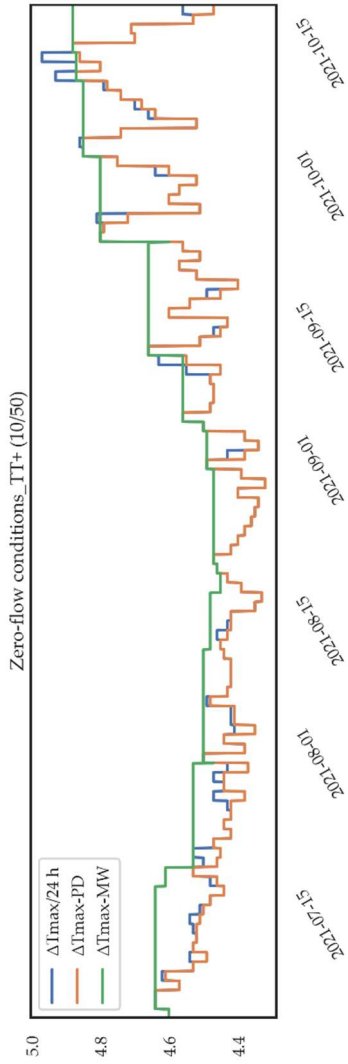


Figure 1. Multiple  $\Delta T_{\max}$  approaches under zero-flux conditions, daily  $\Delta T_{\max}$  ( $\Delta T_{\max}/24 \text{ h}$ ), the daily predawn  $\Delta T_{\max}$  ( $\Delta T_{\max}\text{-PD}$ ), and maximum moving window  $\Delta T_{\max}$  ( $\Delta T_{\max}\text{-MW}$ ).

### **3.8. Comparison of SFD output between TDP and TT+ (10/50) for *P. abies*, *Q. rubra*, and *F. sylvatica***

The last series of graphs below (Fig. 20) is a synthesis of the most used thermal approaches (TDP) versus the TT+ (10/50). Fig. 20 represents three separate wood anatomical features important for the transport of water via the stem and are separated as such according to their anatomical porosity. In Fig. 20, starting from the left side, *P. abies* being non-porous, *Q. rubra* being ring-porous, and *F. sylvatica* being a diffuse-porous medium. This is an important distinction for both thermal dissipation and bound water in the stem.

Although the SFD rate in Fig. 20 for *P. abies* vs *Q. rubra* is in the same range for the TT+ SFD records ( $\approx 0$  to  $1 \text{ l dm}^{-2} \text{ h}^{-1}$ ), the standard variation for *P. abies* is very high (R-value = 0.56 and p-value < 0.001) in comparison to *Q. rubra* (R-value = 0.82 and p-value < 0.001). This may be explained by the different wood anatomy for both species where the small vessels of the *P. abies* trap and bind water which causes an underestimation of  $\Delta T$  under zero-flux with 10 min heat input. Indeed, the stem water content may cause inaccurate estimation of  $\Delta T_{\text{max}}$ , in terms of magnitude and the time of occurrence using empirical thermal approaches. Consequently, this causes a false evaluation of SFD. As displayed in Fig. 20 for *P. abies*, SFD data has a higher standard variation according to the TDP outcomes, the TDP system can overcome the stem water impact with non-stope heat and can neglect the stem water impacts. However, in

oak, the vessels are larger, therefore TT+ with 10 min heating can capture a clear pattern yet the estimation is greater for the SFD amount rather than the TDP. Interestingly, the TDP system is known for underestimating the SFD rate (Vandegheuchte & Steppe, 2013). Therefore, it may be possible that the TT+ is capturing the SFD due to considering the natural temperature gradient. Fig. 20: *F. sylvatica* is showing a very good correlation with TDP system (R-value = 0.89 and p-value < 0.001) since the flux rate is higher than the low flux threshold. In particular, this represents the functionality of the TT+ system to detect SFD using a heating/cooling regime of 10 minutes heating and 50 minutes cooling for different species confirming a low-cost IoT alternative for measuring SFD. In the empirical thermal approaches, TDP and TTD provide relative amounts of SFD rather than absolute (HPV), and consequently comparing these two systems remains a difficult task to summarise.

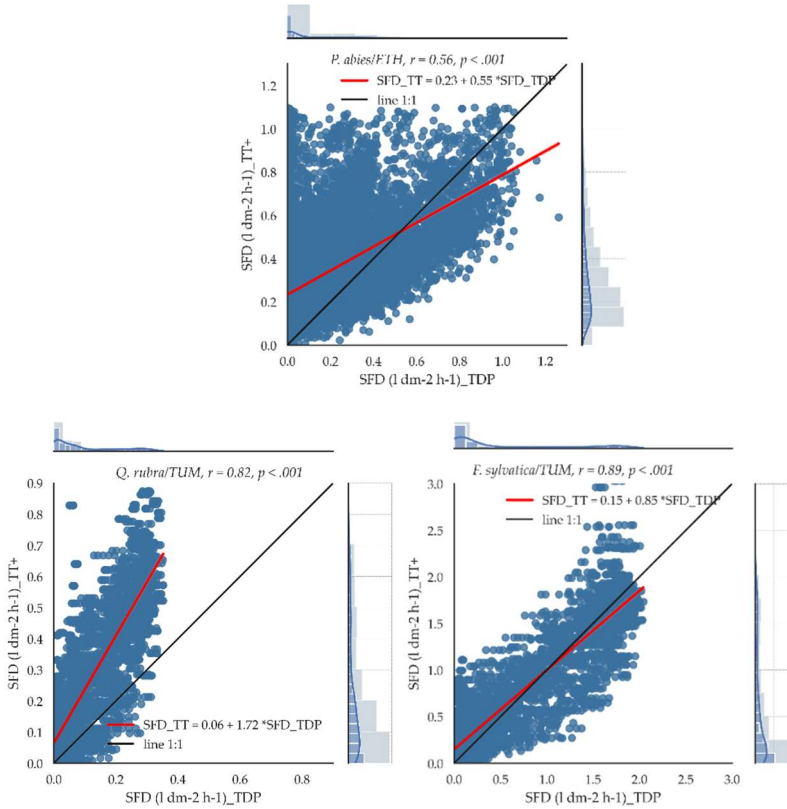


Figure 20. Comparison of SFD output between TDP and TT+ (10/50) for *P. abies*, *Q. rubra*, and *F. sylvatica* from left to the right

### 3.9. Predicting the sap flux density using climatic data

As discussed above, from the different application combinations and scenarios, the empirical thermal approaches did not demonstrate the full capability of measuring SFD. As such, an investigation into other possible factors and approaches affecting SFD prediction was



pursued. Therefore, an analysis of the impact of climatic variables on SFD is discussed below. Climatic variables should naturally impact SFD given the processes of photosynthesis and respiration. Data was obtained from *Fagus sylvatica* for one growing season with the collaboration of Technische Universität München (TUM), Germany. Exploiting air temperature, relative humidity and global radiation, a multivariate linear model approach was used to find the correlation between SFD and these potentially explanatory variables. A statistical description of the variables can be found in Table 3. The statistical approach considers a training set that uses 90% of the datum captured, with a random validation set of 10%.

Table 1: Statistical description for SFD and climatic variables

	Variable	Observations	Minimum	Maximum	Mean	Std. deviation
Training set	SFD ( $\text{l dm}^{-2} \text{h}^{-1}$ )	3518	0	2	0.6	0.7
	Air T ( $^{\circ}\text{C}$ )	3518	9.8	29.9	18	4.1
	RH %	3518	38.7	100	89.9	17.2
	global radiation ( $\text{W/m}^2$ )	3518	6.6	1122	193	238.4
Validation set	SFD ( $\text{l dm}^{-2} \text{h}^{-1}$ )	500	0	2.019	0.613	0.687
	Air T ( $^{\circ}\text{C}$ )	500	10.2	29	18	4.3
	RH %	500	40.5	100	88.8	17.4
	global radiation ( $\text{W/m}^2$ )	500	7.8	1049.8	203.3	247.4

The multivariate linear models report respectable explanations between the independent and dependent variables where SFD prediction is positively correlated with Air T (°C) ( $R^2=0.83$ ) and global radiation ( $W/m^2$ ) ( $R^2=0.77$ ), and negatively with RH% ( $R^2=-0.77$ ). To further validate the impact of the abiotic variables described in Table 3, an ANOVA using Type III error was also performed. Results from the ANOVA suggest that global radiation ( $W/m^2$ ) has the highest weighted impact on sap flux density prediction in comparison with air temperature and RH%. This is supported by the stated F statistic and p-values reported in Table 4. However, each variable is statistically significant for the prediction of SFD in its own right.

Here we have demonstrated the impact of climatic data on SFD by applying Eq. 10 and as such, we suggest that future analysis apply the same approach for further elaboration and validation of abiotic variables on SFD prediction, including longer-term data sets and a broader representation of species. The results for predicted SFD based on Eq 10. Versus SFD applying TDP system are displayed in Fig. 21 including the training and validation set.

$$\text{Equation 10 } SFD (l \text{ dm}^{-2} \text{ h}^{-1}) = 0.31 + 5.63E-02 * \text{Air T } (^\circ\text{C}) - 0.011 * \text{RH } \% + 1.103E-03 * \text{global radiation } (W/m^2)$$

Table 4. Statistical results from ANOVA

Source	DF	Sum of squares	Mean squares	F	Pr > F
Air T (°C)	1	55.66	55.66	<b>708</b>	<b>&lt;0.0001</b>
RH %	1	47.7	47.7	<b>607</b>	<b>&lt;0.0001</b>
global radiation (W/m <sup>2</sup> )	1	139.668	139.668	<b>1776</b>	<b>&lt;0.0001</b>

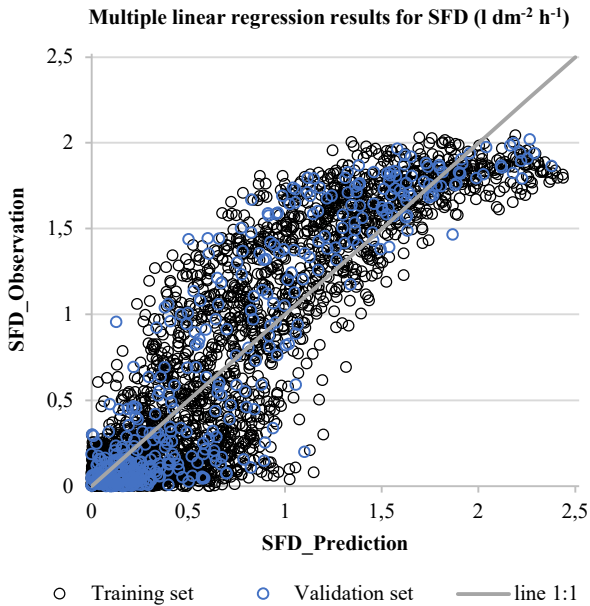


Figure 21. Results from predicted SFD based on abiotic variables versus measured SFD by TDP system.

## 4. Conclusion

Taking advantage of tailored firmware, TT+ platform can easily perform as continuous heating, or a cyclic system with customizable measurement time intervals. Empirical calibration equations were provided for different flow indices using a hydraulic bench filled with pine sawdust in the lab. Using thermal approaches and comparing different sensors and approaches is difficult where limitations arise according to precision limitations for sensors and the subsequent impacts from the surrounding environment and within the trees themselves e.g. physical processes of water transport in stress, climatic events and sensor failure. Nonetheless, comparing TT+ set on the transient regime (10H/50C) performance across different species Norway Spruce, European Beech and Oak in situ with well know thermal approaches (TDP: Continuous Heating and HPV: Heat Pulse Velocity method) proved that the tree talker is capable to measure sap flow with reasonable accuracy (80%) for network-based mass monitoring in remote areas with low power consumption. TreeTalker underestimates approximately 20% midday max sap flow rate and has a weak performance under low flux ( $<0.5 \text{ l dm}^{-2} \text{ h}^{-1}$ ), however, the latter weakness is reported across most thermal approaches. This common weakness may be negated where a TDP approach (Granier) is applied at a higher energy consumption cost. The role of stem water content is not banal and is highly important for capturing accurate temperature differences between the heater and reference probes,  $\Delta T$ .

Using cooling phase data of temperature gradient,  $\Delta T$ , provides better results of the sap flux density estimation, irrespective of the duration of heating/cooling. Flow index based on cooling phase data is independent of  $\Delta T_{max}$  under zero-flux conditions. However, it is limited under the low flux rate. We found that if we can program a tailored firmware, we can capture the full curve of heat dissipation in a living tree with a customizable power input and as such, we can apply the different available equations to evaluate SFD in living trees. Also having the full curve of heat dissipation allows us to apply heat partial differential equations at the microprocessor level to directly provide an output of thermal diffusivity (an indicator of stem water storage) and heat velocity (a direct indicator of sap velocity). TreeTalker, a low-cost IoT-based technology, allows testing and examining different methodologies under different conditions and is emerging as a solution for mass monitoring networks for forests.

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### **3.3 Paper III: Case Study using the TreeTalker device**

**Does tree location along a hillslope affect sap flow rates and stem growth? A case study in a beech forest stand in Central Italy.**

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## **Abstract**

Forest ecosystems play an important role in water regulation through evapotranspiration (ET) fluxes. Variation in forest ET is a complex process due to the influence of numerous variables, such as climatic forcing (e.g., air temperature, air humidity, solar radiation and wind speed), forest structure (e.g., LAI), and soil water availability. The present study aims at understanding and quantifying sap flow and radial growth spatio-temporal variability in response to air temperature and soil moisture dynamics along a steep hillslope in a mountain Mediterranean forest. Sap flow rates and radial increment were measured in a European beech pure stand (*Fagus sylvatica* L.) during the growing season (May-September) in 2021 at three different locations along a hillslope (bottom, mid and top of the hillslope) in the Re della Pietra catchment, Tuscany, central Italy. The monitoring was performed by using 12 TreeTalker devices installed on four trees per hillslope location. The devices measure environmental and tree variables simultaneously with hourly resolution. Soil moisture was measured at three locations along the hillslope at 15 and 35 cm depth through 6 frequency-domain reflectometry probes. The results showed statistically significant differences in the sap flow rates in the three different hillslope locations in May, June, July and September. Soil moisture content showed variability along the hillslope in the same period, while there were not significant differences in air temperature among the three locations. Sap flux density was maximum in the top position in June,

when the soil moisture content showed intermediate values between May (higher values) and July (lower values). Tree radial growth resulted higher in the bottom location where soil moisture content was stable during the vegetative season. This study highlighted that sap flow rate variability is affected by hillslope topography and explained by differences in soil moisture content, while to assess radial growth variability further research is needed.

**Keywords:** sap flow, *Fagus sylvatica*, radial growth, slope position, soil moisture, hillslope

## **1. Introduction**

Forest ecosystems play an important role in the hydrological cycle through water uptake, water storage in plant organs, and evapotranspiration fluxes (ET) (Ellison et al., 2017; Goldstein et al., 1998; Wang et al., 2022). ET is a large component of forest ecosystems' water budget (Oishi et al., 2010) and its spatial variability is influenced by topography, soil moisture, namely water accumulation, and microclimate, such as temperature and solar radiation (Emanuel et al., 2010, 2011; Fan et al., 2017; Renner et al., 2016). Such a strict relationship between ET by forest trees and the hydrological cycle requires methodological approaches to study tree response to environmental conditions at the boundary between the disciplines of tree ecophysiology and soil hydrology (Cocozza & Penna, 2022).

However, identifying the most relevant climatic forcing influencing plant growth and functionality (e.g., photosynthesis, transpiration) is not easy but essential to understand forest characteristics and develop management strategies (Vannoppen et al., 2018). Several questions were proposed and discussed in the discipline of “physiological ecology” and “water requirement” as “efficiency” of the plant systems by Brendel (2021). The knowledge of the responses of forests to environmental changes allows defining the sensitivity and the level of adaptation of forest ecosystems and, in this context, the monitoring of tree functional traits is a useful tool for supporting forest management (Magh et al., 2019)

The Mediterranean region is characterized by historical and recurring issues of aridity and water shortage (La Jeunesse et al., 2016) and current scenarios forecast future lower summer precipitation and prolonged drought periods (Tramblay et al., 2020). More frequent conditions of water shortage might become critical for tree survival and will force species adaptation to water requirements. In this context, forest ecosystems face water management through strategies in water interception due to the heterogeneity of soil-plant water relations, for instance along a hillslope (Jost et al., 2012). In the case of forest trees growing on hillslopes, a better knowledge of water transport in trees, differently located and, consequently, exposed to different water availability due to water storage changes (Singh et al., 2021), can provide practical information on forest functionality through measurements of tree water use (e.g., Alizadeh et al., 2021) and stem increment (e.g., Güneş et al., 2020). Climate and soil properties drive water infiltration, while drainage is influenced by topography (Fan et al., 2017). In fact, soil's hydrological profile is determined by infiltration in the uppermost part of the hillslope and by drainage in the lower part (Stone & Kalisz, 1991). Furthermore, soil properties might change along the slopes, as they are affected by movement and accumulation of soil solution (Tsui et al., 2004). For these reasons, studying plants behaviour along the hillslope is important to assess the responses of trees when subjected to different environmental forcing.

Nowadays, monitoring systems are widely used to achieve measurements of tree functionality (Peters et al., 2021). These monitoring systems benefit from the high efficiency obtained by data acquisition with temporal resolution ranging from minutes to hours to define daily, monthly, or seasonal behaviour processes (van der Maaten et al., 2018). Tree growth and functionality are often measured by instruments, such as dendrometers and sap flow sensors, respectively (Cocozza et al., 2015; Mencuccini et al., 2017). Transpiration can be estimated by empirical or semi-empirical methods using the ‘‘big leaf’’ model (Monteith, 1965) as well as direct methods including gravimetric analysis, heat pulse velocity (HPV), time domain reflectometry (TDR), single leaf and whole plant infrared gas-exchange measurements (Ferrara et al., 2003). The use of the heat pulse method to estimate transpiration through sap flow measurements within the plant stems has been successfully addressed to define the relationship between tree growth and crop yield to soil moisture and climatic forcing variability (Boggs et al., 2021; Cocozza et al., 2015; Giovannelli et al., 2019; Oishi et al., 2010) or to manage crop irrigation (Fernández et al., 2008). The rate of plant transpiration is closely related to biomass accumulation (Wallace et al., 1990) and the relationship between plant growth and hydraulic efficiency (e.g., water consumption) is known as water use efficiency and allows to assess plant responses to environmental changes (Brendel, 2021). The annual stem diameter increment is considered the most suitable proxy of tree growth (Bowman et al.,



2013) and recent findings showed that point dendrometers are important tools to monitor intra-annual stem growth patterns (Cocoza et al., 2016). Through the analysis of dendrometric signals is nowadays possible to extrapolate information about growth rates, phenology, or tree response to the environment (Cocoza et al., 2009, 2012; Giovannelli et al., 2022). Data on tree growth allow predicting the impact of the environment on tree functionality (Cocoza et al., 2018), such as the sensitivity of tree radial growth to soil moisture and air temperature (Magh et al., 2019; Tardif et al., 2001).

In this context, we hypothesize that hillslope topography determines different tree responses in terms of transpiration and growth in relation to tree spatial distribution on the hillslope affected by specific soil moisture dynamics. To test this hypothesis, we compare soil moisture patterns, sap flux, and stem increment trends at three different hillslope locations in a European beech (*Fagus sylvatica* L.) forest stand. For the first time, TreeTalkers (TT), innovative devices that measure hourly and simultaneously tree and environmental variables, were used to assess ecological relationships in forest. Such a tool permits to develop a large-scale, long-term monitoring system, to identify forest dynamics in a changing environment (Oogathoo et al., 2020; Tomelleri et al., 2022).

## 2. Materials and methods

### 2.1 Study area and tree characteristics

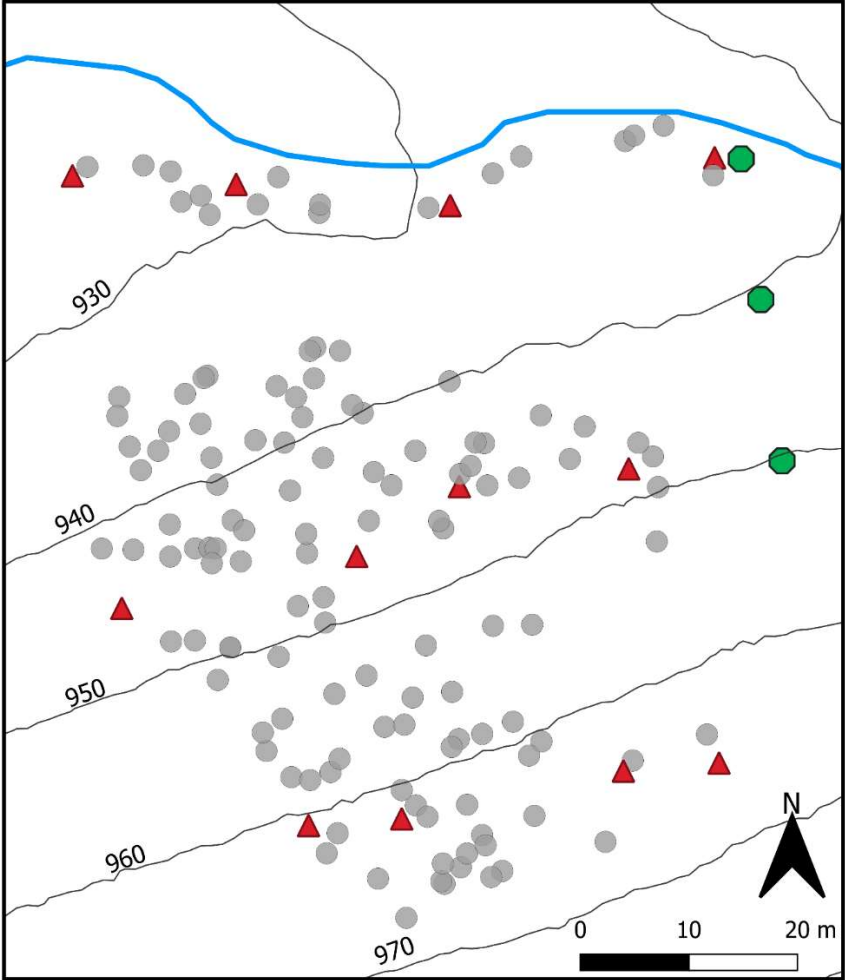
The study has been carried out in a European beech pure stand (*F. sylvatica* L.), historically managed as coppice, for producing charcoal, in the Re della Pietra catchment (11.62874, 43.88785, 950 m asl), Tuscany (central Italy) (Fig. 1). During the 2021 growing season (May-September), precipitation and solar radiation were collected with a weather station Davis Vantage Pro (Vernier, Oregon, USA) to describe the environmental conditions of the study area. The weather station was placed 40 m away from the study site, in a flat area at the top of the study hillslope without trees to avoid canopy interference on meteorological measurements. The weather station measured with a frequency of 5 minutes the following variables: precipitation, wind speed, wind direction, air temperature, air relative humidity and solar radiation. The annual precipitation in 2021 was 928 mm and mean annual temperature was 10.9°C.

Twelve trees were chosen in 3 different slope locations of the hillslope, called bottom, mid and top, along the hillslope (Fig. 2); for each location four trees were considered. The hillslope had a length of 80 m with an average 40% slope (Fig. 2). Trees had a mean DBH of  $36\pm 6$  cm and a height of 18 m at bottom,  $41\pm 8$  cm and 19 m at mid and  $32\pm 5$  cm and 24 m at top location ( $n = 4$ ). Tree age was around 60 years old and the average diameter at breast height was  $29\pm 9$  cm. The mean height of the trees was 19.6 m. Soil texture on the hillslope

was determined in the lab through the analysis of 11 soil samples collected in March 2021 at the bottom location (2 samples at 0-20 cm, 2 samples at 20-40 cm, and 1 sample 40-60 cm depth), and the mid and top locations (1 sample at 0-20 cm, 20-40 cm, and 40-60 cm depth for each location). Sand content in the 11 samples ranged between 57 % and 76 %, and clay content ranged between 4 % and 11 %. Soil texture in all samples resulted in sandy loam, according to the USDA (1999) classification.



Figure 1 - Study site on 9th April 2021. The white containers are for throughfall measurements (data not shown in this study).



- Lecciona stream      ● Trees
- Soil moisture probes    ▲ Trees with TT

Figure 2 - Trees and soil probes distribution along the hillslope.

## **2.2 Soil moisture measurements**

Soil moisture was measured at 15 and 35 cm depth through 6 frequency-domain reflectometry probes (Teros 10, Meter®, Pullman, WA, US) installed in August 2020 at three locations along the hillslope maximum slope, "riparian" (930 m asl), in "lower" (940 m asl) and in "mid"(950 m asl) hillslope (Fig. 2). The probe precision is  $\pm 0.03 \text{ m}^3/\text{m}^3$  VWC (Volumetric Water Content). Two probes were installed a few meters from the stream (we call this location "riparian"), in the lower part of the hillslope after a break in slope, and in the middle part of the hillslope. The linear distance along the slope between the riparian probes and the mid-hillslope probes was approximately 30 m. All the probes were connected to the same datalogger (ZLS6, Meter®, Pullman, WA, US) with a temporal resolution of 5 minutes. No calibration for specific soil was performed and the standard calibration for organic soils recommended by the manufacturer was applied. Since we focus on the dynamics of soil moisture than in the absolute values of soil moisture, the lack of a specific soil calibration does not affect the data interpretation and the results.

## **2.3 Tree parameters measurements**

Sap flux density, radial growth, and air temperature were measured using the TreeTalker (Nature 4.0 SB srl, Italy) device (Valentini et al., 2019). Twelve trees chosen in 3 different slope locations were equipped with TreeTalkers (Nature 4.0, Italy). The TreeTalker (TT)

is a device capable to measure different plant and environmental parameters simultaneously with hourly detail and sending the data in real time to a data logger (TT-Cloud), based on the Internet of Things (IoT) technology (Asgharinia et al., 2022). Data of sap flux density, radial growth and temperature were collected by TT during the 2021 growing season from May to September, from bud breaks to the end of the growing season. TTs were installed on the tree stem facing north, for the sunlight not to influence the measurements.

The TT sap flow density sensor works with the principle of the thermal dissipation method (Granier, 1987). It consists of two probes, a reference and a heated probe, with a cyclic heated system (Do & Rocheteau, 2002) of 10 minutes heating and 50 cooling. The two probes are vertically separated 10 cm and horizontally 2 cm so that the reference probe is not influenced by the heated one. The sap flux density was calculated using the following equation:

$$\text{Sap Flux Density (l dm}^{-2}\text{h}^{-1}\text{)} = 4.79 * \frac{\Delta T_{max} - (\Delta T_{on} - \Delta T_{off})}{\Delta T_{on} - \Delta T_{off}}$$

where:  $\Delta T_{max}$  is the daily maximum temperature gradient between the two probes after the 10 minutes heating;  $\Delta T_{on}$  is temperature difference between the two probes after the heating period;  $\Delta T_{off}$  is the temperature difference between the two probes before the heating period. To ensure the probes were inserted in the sapwood area, wood cores were extracted and the sapwood area of each tree was calculated. For each tree, a wood core reaching the middle of the

plant was extracted, in the part of tree trunk facing East. Directly in the field, the sapwood area was estimated measuring with a ruler the soaked area of the core (Güney, 2018; Long & Dean, 1986). Sapwood area was higher in the trees at bottom than at mid and top locations (reaching the 25%, 14% and 8% of the total basal area, respectively) (data not shown). TTs are equipped also with an infra-red distance sensor (SHARP. Model: GP2Y0A51SK0F). This sensor, called growth sensor, allows monitoring tree radial growth weekly and during the entire growing season by collecting hourly radial variations.

## **2.4 Statistical analysis**

Time series of sap flow density were quite noisy and were smoothed using the moving average method applying a period of 3 hours, to remove off-scale peaks. Afterwards, in order to consider stable signals of TT probes, rainy days were removed from sap flux density time series. The non-parametric Kruskal-Wallis test was used to investigate statistically significant differences in median temperature, sap flow rates, and soil moisture among the 3 different locations and the months of investigation. Afterwards, an LSD post-hoc test was applied to assess significantly different means among locations ( $P < 0.05$  level). Statistical analyses were performed using the `agricolae.R` package (Mendiburu, 2021).

The radial growth was analysed by aggregating the daily data and calculating the week median. Radial increments in the top and the

bottom were reported for two trees per location ( $n = 2$ ), whereas for three trees in the mid ( $n = 3$ ), due to incomplete data acquisition of the sharp sensors. Radial increments, considered as the weekly median values, were interpolated by a modelling approach using Gompertz functions (Cocoza et al. 2016). The Gompertz functions were fitted by using OriginPro v8.0 (OriginLab, Northampton, MA, USA):  $y = a - e^{-k(x-c)}$ ; where  $a$  is the amplitude of the stem increment,  $c$  is the week when the slope of the function changes,  $k$  is rate of change parameter.

### **3. Results**

#### **3.1 Weather data and soil moisture response**

During the 2021 growing season (considered here as May 1<sup>st</sup> – September 30<sup>th</sup>) the precipitation was 284 mm (monthly maximum 113 mm in May and min 19 mm in June) with a mean temperature of 17.9 °C (max 35.1 °C registered 12<sup>th</sup> of August and min 4.7°C registered the 15<sup>th</sup> of May) (Fig. 3). Max precipitation was registered the 26<sup>th</sup> of September (36.55 mm). The day with the maximum VPD resulted the 12<sup>th</sup> of August (2.31 kPa) while the minimum was registered the 12<sup>th</sup> of May (0.097 kPa). In all months between May and October the median air temperature was lower at the bottom and higher at the top locations, indicating a consistent trend in temperature along the hillslope (Fig. 3). However, the differences in



the median temperature were very small (around 0.3 °C) and, at this temporal scale, not significantly different. However, temperature resulted statistically different in the same location in the different months considered, resulting the lowest in all the locations in May and the maximum in July (Fig. 4).

Soil moisture changed among the months in all three different locations ( $p < 0.01$ ), showing a descending trend over time (Fig. 4). In May the soil moisture was the highest registered in all slope locations, with the maximum values at the riparian location corresponding to the rainiest month of the vegetative season (Fig. 4). The lowest values were registered in August at the lower-hillslope location, while in September at the riparian and mid-hillslope locations (Fig. 4). Soil moisture in each month was statistically different among locations ( $p < 0.001$ ) (Fig. 4), although values at the riparian location showed very limited variability in July, August and September.

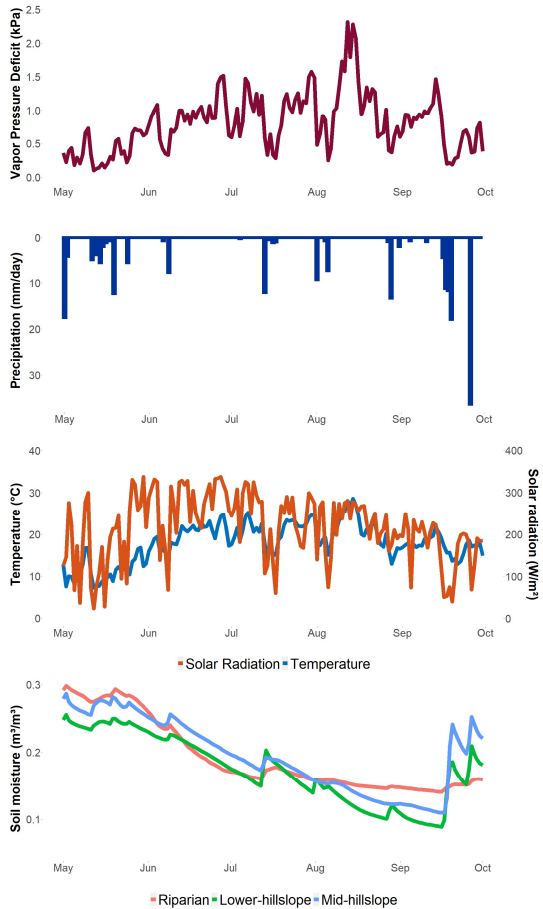


Figure 3 - Daily VPD, Precipitation, Solar radiation and temperature at the site level during the 2021 growing season. Soil moisture content refers to values measured at 15 and 35 cm depth (averaged values) at the tree different locations (Riparian, lower- and mid-hillslope)

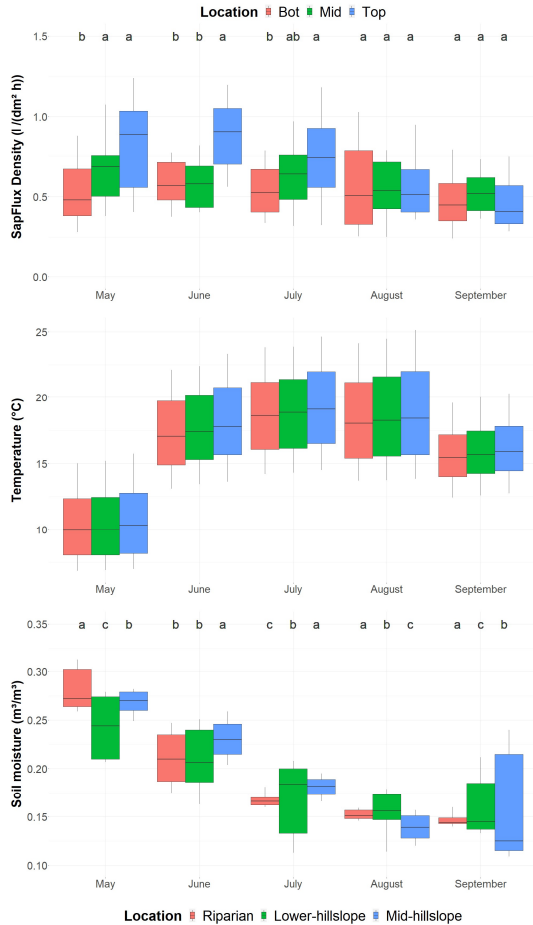


Figure 4 – Monthly sap flux density, temperature (measured directly by the TTs) and soil moisture at three different locations studied during the growing season, from May to September 2021. The boxplot horizontal bar indicates the median, with the lower and upper edges representing the 25% and the 75% quartiles. The highest and the lowest whiskers' values display the 90/10 percentile respectively.

### **3.2 Sap flux density**

Sap flux density was statistically different among locations in May, June, July and September ( $p < 0.05$ ) (Fig. 4). The top location showed significant differences in sap flux density ( $p < 0.05$ ) among the months, while there were not statistically significant differences at bottom and mid. Observing sap flux density trends in two representative months, namely June showed differences among locations whereas no differences in August (Fig.4), the parameter was higher in top locations (Fig. 5) than in August, that showed low values in three locations (Fig. 6)

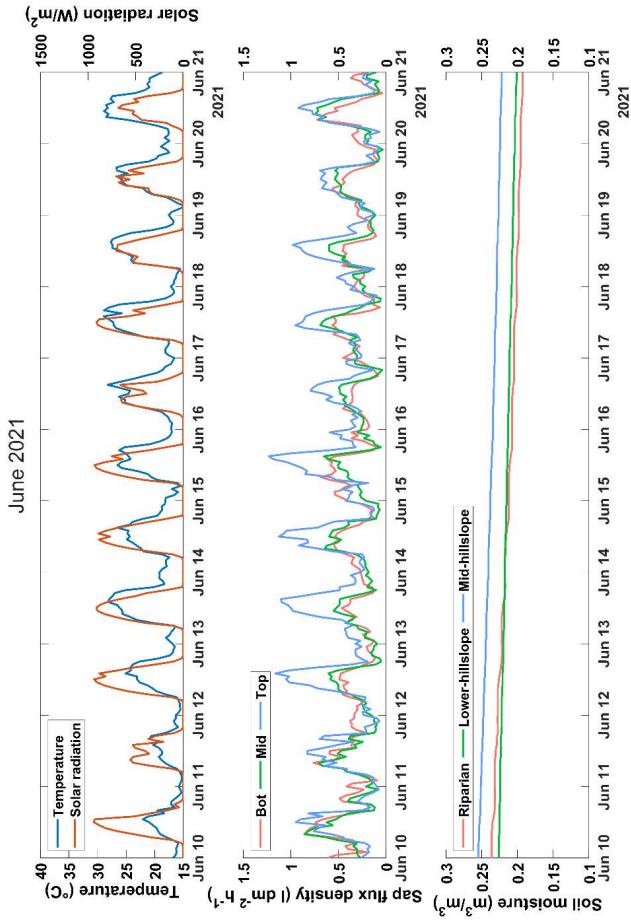


Figure 5 – Air temperature and solar radiation (upper panel), average sap flow (middle panel) and average soil moisture (lower panel) in riparian, lower and mid-hillslope in a representative 10 days-period in June 2021

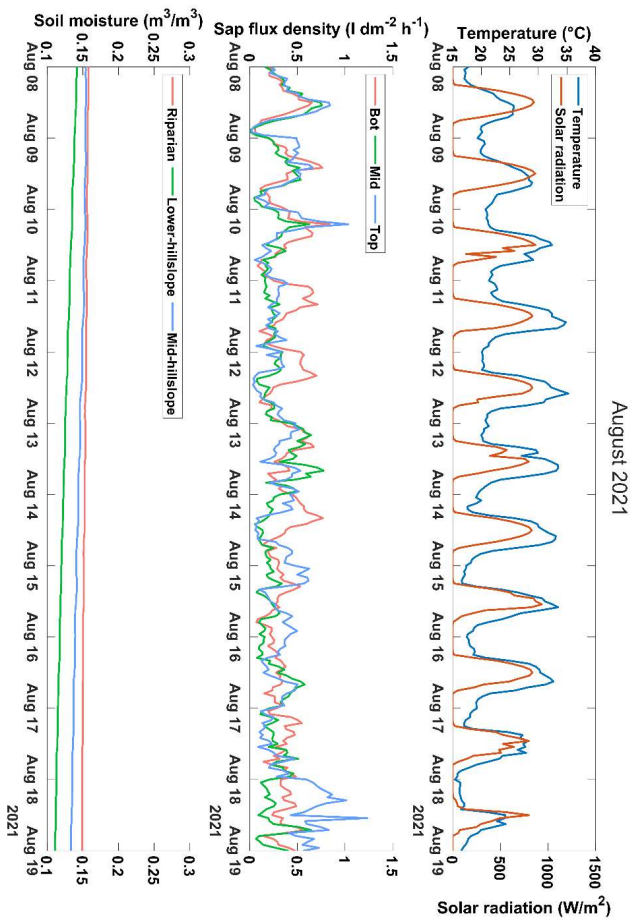
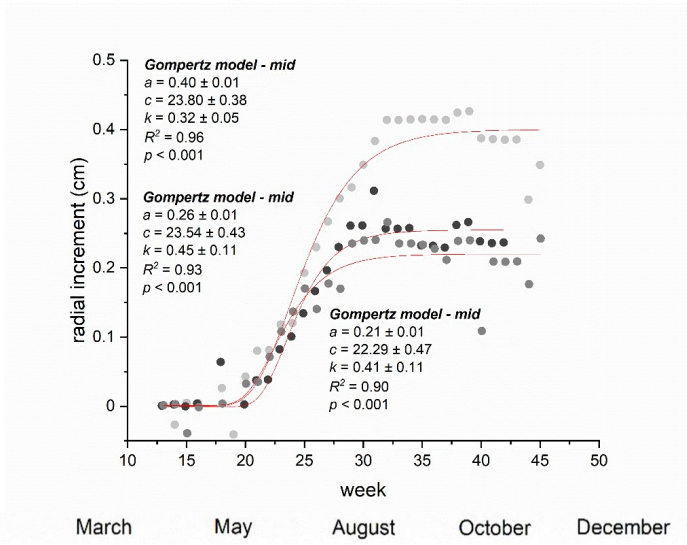
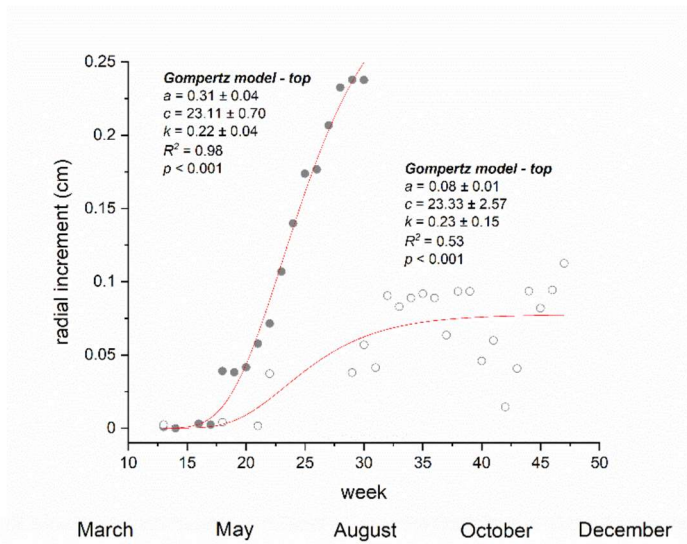


Figure 6- Air temperature and solar radiation (upper panel), average sap flow (middle panel) and average soil moisture (lower panel) in riparian, lower and mid-hillslope in a representative 10 days-period in August 2021

### 3.3 Radial growth

Tree radial increments were differences among the hillslope locations ( $n = 3$ ) (Fig. 7). Radial increment was  $0.38 \pm 0.12$  cm at bottom and  $0.33 \pm 0.08$  cm at mid location, whereas the increment at top location was less, averaging at 0.175 cm in the 2021 growing season (Fig. 7).

Values of Gompertz functions defined: different weekly cumulative increments (“a” values) with higher radial increments in bottom and mid than top locations; the inflection point of functions was in 22th-23th week in three locations (“c” values), representing the period of maximum growth rate; whereas the rate of change parameter (“k” values), that defines the dynamics of growth, defined by the time required for the formation of xylem growth ring in tree, resulted double in mid than top and bottom locations (Fig. 7).





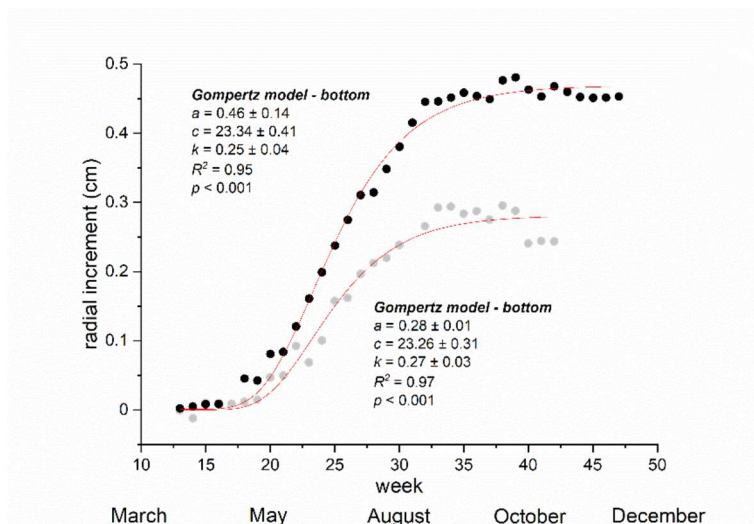


Figure 7 - Radial increment of beech trees in bottom (bot), middle (mid) and top (top) locations along the hillslope in 2021. Gompertz model functions were fitted to the experimental data of each site (p values and R2 are reported per each tree monitored). Red lines are fitting functions, colored dots are trees in hillslope locations.

## 4. Discussion

This study aimed at examining the effects of tree location along a hillslope to define the role of environmental conditions in controlling plant transpiration and stem growth during the growing season. From the leaves emergence recorded the 1<sup>st</sup> of May (data not shown) to June (Fig. 4), high sap flux density especially at top and mid locations was observed (Fig. 4), by suggesting substantial differences in water request by trees between hillslope positions. In our study trees in the top of hillslope might be exposed to site conditions to promptly activate sap flux by considering that water availability is important especially at the beginning of the growing season (according to Rossi et al., 2009). However, other studies showed low sap fluxes at the beginning of the growing season, although they did not register shallow downslope water redistribution through interflow to cause differences in water supply along hillslope (Fabiani et al., 2022; Urban et al., 2015). According to Bronstert et al. (1998), topography affects soil water content because steep hillslopes enhance water drainage, which lowers the soil's capacity to store water, leading to a higher amount of surface runoff and less available water for trees (Shi & Wang, 2020). (Shi & Wang, 2020). Despite soil moisture probes not being positioned exactly adjacent to the TTs transects, this registered the maximum and more stable soil moisture content at the riparian location, which is the measuring site closer to the stream and in the most concave area of the hillslope (Fig. 2 and Fig. 4). This indicates that trees growing in the bottom position had access to a

larger water supply, at least in the investigated depth (35 cm). Soil moisture in the lower-hillslope location was likely higher than in the mid-hillslope location as the lower-hillslope location is characterised by a break in slope causing a relatively less steep slope compared to the mid-hillslope location. The evolution of soil moisture content in June influenced sap flux density of *F. sylvatica* in the three locations, with lower soil water content resulting in lower sap flux density at bottom and mid compared to the top location (Fig. 5). Even if the temperature in the three locations was not significantly different, at the beginning of the growing season, in June, maximum sap flux density and maximum soil water content were registered at the top location (Fig. 4), highlighting the tree ability to use the available water (Krauss et al., 2015). Whereas, low sap flux density at bottom location could highlight well-watered conditions of trees at this location (Manzoni et al., 2013). Lower values of sap flux density were recorded in August, by confirming that low sap flux density values were very dependent on soil moisture and high temperature conditions (Brinkmann et al., 2016; Choat et al., 2012; Köcher et al., 2009). The week from 10<sup>th</sup> to 17<sup>th</sup> of August was the hottest of the year, reaching peaks of more than 35°C in our study site, with the maximum VPD (2.32 kPa) recorded the 12<sup>th</sup> of August. In this week, sap flux density at midday was lower than the other periods considered in all the three locations (Fig. 6). Daily patterns of sap flux density were asynchronized to daily temperature trends, in agreement with Brinkmann et al. (2016) under drought and high

temperatures. These results are in accordance with the physiological strategy of beech trees (isohydric species) that preserve water by reducing water loss through stomatal closure, when air temperatures reach high values and soil moisture becomes less available (Vilagrosa et al., 2013). By observing the trend of sap flux density in the central week of August, two days were characterized by higher values in the bottom location probably for a more persistent water source than the mid and the top level, by suggesting the more suited growing conditions close to the stream (Fig. 6). Moreover, trees at the top location are taller than those at the other locations studied, which reduces their ability to modify hydraulic traits after drought periods (Giles et al., 2022). An effect of slope position on trees transpiration was also reported by Tsuruta et al. (2020), highlighting that the soil moisture content is more stable during dryer months and water availability is more suitable for trees transpiration in the riparian zone (Borchert, 1994; Clark et al., 1999; Morbidelli et al., 2018; Telak et al., 2021).

Tree radial growth was different in the three locations studied, showing higher radial increments of trees at the bottom and lower increments in those at the top location of the hillslope (Fig. 7), even if higher tree diameters are registered at the mid (32 cm, 39 cm, 39 cm, 52 cm) than bottom (33 cm, 33 cm, 46 cm, 31 cm) and top (40 cm, 31 cm, 27 cm, 29 cm) locations. Moreover, at the bottom location trees are more sparse than at mid, resulting in less competition

between trees for light and water supply (Berger et al., 2008; Farrior et al., 2013).

Comparison of the parameters of the Gompertz functions obtained by radial increments revealed variability between each tree, defined by their specific position and confirming the relevance to define the role of tree position to define functional responses. The amplitude of the radial increment, obtained by Gompertz functions, highlighted the correspondence with the tree diameter, with lower values in the top than mid and bottom locations. However, the higher values of the coefficient  $k$  (estimating rate of change parameter) in mid than top and bottom locations might explain a different wood formation behaviour, by suggesting a contribution of early wood tracheids with wide radial dimensions to the final widths of the xylem growth ring, in comparison to cells formed in the second part of the vegetation period, latewood (Gričar et al. 2008). These differences can be explained by differences in soil moisture content, in agreement with various studies on European beech trees which demonstrated that soil water availability is the most important limiting factor of radial growth in temperate climates (Kolář et al., 2016; Lebourgeois et al., 2005), as well as cambial activity (Dittmar et al., 2003; González de Andrés et al., 2018; Gutiérrez, 1988; Vieira et al., 2020). In this case study, the maximum soil water content was not registered in the riparian zone, despite the maximum tree radial growth recorded in this location. Maximum tree radial increment at the bottom location showed the importance of water availability for European beech

xylem expansion especially during dry periods (Prislan et al., 2019), as soil moisture content was less variable in the riparian location during the entire growing season, persisting as well during the dryer months. The maximum sapwood area was recorded at the bottom location by resulting a factor for the tree growth stimulation, as defined by a positive correlation between sapwood and tree growth as reported by Galván et al. (2012) and Vertessy et al. (1995). As an evidence, the tree growth closer to the stream showed an increment of  $0.46 \pm 0.14$  cm and the maximum sapwood area ( $2.54 \text{ dm}^2$  representing the 33% of the tree surface) in comparison to increment of  $0.31 \pm 0.04$  registered in the farther tree at the top of hillslope with a sapwood area of  $0.95 \text{ dm}^2$  (7% of the tree surface).

Finally, we observed lower transpiration fluxes and maximum radial growth at the bottom location compared to the mid and top locations, where soil water storage during the year was higher, confirming the relationship between soil water content, sap flux, and radial growth as investigated by Boggs et al. (2021). Our results suggest that tree transpiration is water-limited at the top location, whereas at the bottom water availability did not represent a limitation for tree functionality (Brinkmann et al., 2016; Magh et al., 2019; Xue et al., 2022). The growing season ended in September when sap flux trends became low and radial growth stopped, as observed by the trees' increment during the last week of September.

## 5. Conclusions

Our results confirm that tree location along a hillslope controls different ecohydrological behaviours driven by water availability and tree water exploitation, which determine ability by trees to manage water and support their growth. The sap flow density of trees growing in the different locations along the hillslope was mostly influenced by soil moisture content. Analogously, radial growth appeared higher at bottom than at top location by reflecting soil moisture variability. These results provide new evidence and contribute to better understand the drivers of tree growth on steep hillslope where water redistribution and availability might become a limiting factor. However, further studies focused on plant functionality and radial increment variability along steep hillslopes are necessary to better understand the role of trees in the water cycle and thus to support sustainable management of water resources in forested area. Although TreeTalkers are still in the initial phase of their technological development (Asgharinia et al. 2022), they have the potential to help to achieve these goals due to their capability to monitor simultaneously and continuously tree and environmental parameters, potentially useful for large-scale forest monitoring.

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## **4. Conclusion**

This thesis proposes a framework about the technology and the usage of TreeTalker device. The steps done in these years starting from automatization of data processing, sensors calibration and ending with the first case study using this technology, enable to begin responding to one of the major societal concerns, namely climate change mitigation. In this context, understanding trees' functionality and identifying trees species that better adapt to environmental changes is crucial, to support forest management and policies setting. To accomplish this goal, forest monitoring requires high resolution data acquisition, resulting in sub daily measurements.

The implementation of the TreeTalker technology in forests ecosystems helps in a punctual data acquisition and storage, permitting to investigate forest adaptation to the changing environment. The ITT-Net represents the first extensive monitoring system using this device, although the usage of the TreeTalker is expanding around Italy and the entire world.

Such low-cost device permits to acquire a large amount of data allowing to develop large databases of tree and environmental factors, potentially acquired worldwide, and stored in a common format, with the advantage of not requiring data harmonization. Furthermore, real-time data transmission has the advantage of allowing quick damage detection and issue resolution.

As per the novelty of the device and the low energy consumption required, sensor technology needs still to be improved, as the

accuracy of data acquisition is not yet optimal, moreover, different species require calibration that is specie-specific, which is currently in progress.

Despite the advancement still needed in the sensor functioning, the TT represents a big step in automatization of large-scale data collection, with the advantage of having all the data gathered simultaneously. The TTs outputs can potentially be combined with other technologies, to have a better picture of forests ecosystems adaptation and setting the first step for modelling trees and forest dynamics. Nowadays, most of large-scale environmental monitoring are done using remote sensed data and it would be important to link above and below canopy measurements. TTs hourly data acquisition provides detailed trees observations in terms of radial increment, sap flow, light transmission trough the canopies, air temperature and humidity, at tree scale. The potential of linking such measurements with remote sensed images, such as Sentinel-2 missions, could help in mapping tree functional traits over large areas. On this matter, preliminary investigations were performed to implement a model linking Sentinel-2 and TreeTalker spectral bands, using a neural network approach, representing the first correlative analysis between below and above canopy reflectance measured by the TTs and Sentinel-2 imagery. The findings of this study, presented in a conference paper of Italian Society of Remote Sensing (AIT) in September 2021, suggest that the below canopy reflectance measured by the TTs can be predicted accurately using Sentinel-2

imagery. Future application of this method include wall-to-wall spatial estimation of tree functional traits recorded by TT devices. If future research will succeed in pursuing this objective, continuous large-scale monitoring of tree functional traits in a simple, highly automated, and cheap way, will be possible. This holds great potential for monitoring forests' health, timely and over large areas, which is crucial to understand the capacity of forests to react to climate change.

Research using the TreeTalker as a monitoring system are still lacking, but in future, still different studies can be done with the TreeTalker devices, taking the advantages of the work done until now. TreeTalker can potentially be part of large scale measuring networks already existing, which have the disadvantage of harmonizing data from different devices and projects, which is time demanding and often difficult. Thanks to the large amount of data acquired, next step would be to assess the responses of trees when under different management systems, after having acquired data of multiple vegetative seasons, to give responses to forests managers to better achieve the scope of understanding the role of forests ecosystems in mitigating climate change and to find the species that better adapt to this changing environment.

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## **Other publications and contributions**

### **1. Papers**

1. Francini S., Zorzi I.\*, Giannetti F., Chianucci F., Travaglini D., Chirici G., Cocozza C. (2021). In situ (TreeTalker) and remotely sensed multispectral imagery (Sentinel-2) integration for continuous forest monitoring: the first step toward wall-to-wall mapping of tree functional traits. In: Dessena M.A., Melis M.T., Rossi P. Planet Care from Space, pp. 108-111, ISBN:978-88-944687-0-0.[DOI](#)

### **2. Conference talks and seminars**

1. Castaldi S., Antonucci S., Asgharina S., Battipaglia G., Beelli Marchesini L., Cavagna M., Chini I., Cocozza C., Gianelle D., La Mantia T., Motisi A., Niccoli F., Pacheco Solana A., Sala G., Santopuoli G., Tonon, G., Tognetti R., Zampedri R., Zorzi I., and Valentini R. -The Italian TREETALKER NETWORK (ITT-Net): continuous large scale monitoring of tree functional traits and vulnerabilities to climate change- EGU General Assembly 2020, Online, 4–8 May 2020, EGU2020-20591, <https://doi.org/10.5194/egusphere-egu2020-20591>, 2020
2. Francini S., Zorzi I., Giannetti F., Chianucci F., Travaglini D., Chirici G., Cocozza C. -In Situ (TREE TALKER) and Remotely Sensed Multispectral Imagery (Sentinel-2) Integration for Continuous Forest Monitoring: The First Step Toward Wall-To-Wall Mapping of Tree Functional Traits

- AIT 2021 X International Conference AIT - “Planet Care from Space” - Virtual Cagliari, 13-15 September 2021
3. Zorzi I., Francini S., D’Amico G., Vangi E., Giannetti F., Travaglini D., Chirici G., Coccozza C. (2019). Analisi e confronto della stagione vegetativa di *Abies alba* e di *Fagus sylvatica* con tecnologia TreeTalker. In: XIII Congresso Nazionale SISEF “Alberi-Foreste-Biodiversità: dal New Green Deal alla Form to Fork Strategy” (Paris P, Calfapietra C, Motta R, Travaglini D, Bucci G eds). Orvieto (TR, Italy) 30 Mag - 2 Giu 2022. Abstract-book, Paper #c13.16.1. [online] URL: <https://congressi.sisef.org/xiii-congresso/>
  4. Coccozza C., Francini S., Zorzi I., Antonucci S., Santopuoli G., Chirici G., Tognetti R. (2019). Continuous large scale monitoring of functional traits in beech trees of mountain forests in Tuscany and Molise. In: XIII Congresso Nazionale SISEF “Alberi-Foreste-Biodiversità: dal New Green Deal alla Form to Fork Strategy” (Paris P, Calfapietra C, Motta R, Travaglini D, Bucci G eds). Orvieto (TR, Italy) 30 Mag - 2 Giu 2022. Abstract-book, Paper #c13.30.85. [online] URL: <https://congressi.sisef.org/xiii-congresso/>
  5. Zorzi I., Fabiani G., Verdone M., Penna D., Coccozza C. (2022). Does tree position along a hillslope affect sap flow rates? A case study in a beech forest in Central Italy. III Convegno AISSA#under40 – La Ricerca Scientifica nel

processo di transizione ecologica in agricoltura, Bolzano 15-16 July 2022.

6. Macchioli Grande M., Verdone M., Borga M., Coccozza C., Dani A., Fabiani G., Gourdol L., Klaus J., Manca di Villahermosa F. S., Massari C., Pfister L., Preti F., Tailliez C., Trucchi P., Zorzi I., Zuecco G., Penna D. (2022). Seasonal meteorological forcing controls runoff generation in a Mediterranean mountain catchment. 12<sup>th</sup> International AIIA Conference, September 19-22, 2022 Palermo, Italy.