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TITLE:

Measurements of CO_2 Fluxes at Non-Ideal Eddy Covariance Sites

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KEYWORDS:

eddy covariance, windthrow, CO_2 fluxes, filtering, measuring site setup, gap filling

SUMMARY:

The presented protocol uses the eddy covariance method at non-typical locations, applicable to all types of short-canopy ecosystems with limited area, on a currently reforested windthrow site in Poland. Details of measuring site setup rules, flux calculations and quality control, and final result analysis, are described.

ABSTRACT:

This protocol is an example of utilizing the eddy covariance (EC) technique to investigate spatially and temporally averaged net CO_2 fluxes (net ecosystem production, NEP), in non-typical ecosystems, on a currently reforested windthrow area in Poland. After a tornado event, a relatively narrow "corridor" was created within surviving forest stands, which complicates some experiments. The application of other measuring techniques, such as the chamber method, is even more difficult under these circumstances, because especially at the beginning, fallen trees and in general great heterogeneity of the site provide a challenging platform to perform flux measurements and then to properly upscale obtained results. In comparison with standard EC measurements carried out in untouched forests, the case of windthrow areas requires special consideration when it comes to the site location and data analysis in order to ensure their representativeness. Therefore, here we present a protocol of real-time, continuous CO_2 flux measurements at a dynamically changing, non-ideal EC site, which includes (1) site location and instrumentation setup, (2) flux computation, (3) rigorous data filtering and quality control, and (4) gap filling and net fluxes partitioning into CO_2 respiration and absorption. The main advantage of the described methodology is that provides a detailed description of the experimental setup and measurement performance from scratch, which can be applied to other spatially limited ecosystems. It can also be viewed as a list of recommendations on how to deal with

unconventional site operation, providing a description for non-specialists. Obtained quality-checked, gap filled, half-hour values of net CO_2, as well as absorption and respiration fluxes, can be finally aggregated into daily, monthly, seasonal or annual totals.

INTRODUCTION:

Nowadays, the most commonly used technique in the atmosphere-land ecosystem carbon dioxide (CO_2) exchange studies is the eddy covariance (EC) technique[1]. The EC method has been used for decades, and comprehensive descriptions of issues concerning all the methodological, technical and practical aspects have already been published[2,3,4]. Compared with other techniques used for similar purposes, the EC method allows for obtaining the spatially and temporally averaged net CO_2 fluxes from automatic, point measurements that consider the contribution of all elements in complicated ecosystems, instead of laborious, manual measurements (e.g., chamber techniques) or the requirement of many samples[1].

Among land ecosystems, forests play the most significant role in C cycling and many scientific activities have focused on investigating their CO_2 cycle, carbon storage in woody biomass and their mutual relationships with changing climatic conditions by both direct measurement or modeling[5]. Many EC sites, including one of the longest flux records[6], were set up above different types of forests[7]. Usually, the site location was carefully chosen before the measurements started, with the goal of the most homogenous and largest area possible. Although, in disturbed forest sites, such as windthrows, the number of EC measuring stations are still insufficient[8,9,10]. One reason is logistical difficulties in measuring site setup and, most of all, a small number of suddenly appearing locations. In order to obtain the most informative results at windthrow areas, it is crucial to start as soon as possible after such an incidental event, which may cause additional problems. In contrast to untouched forest sites, the EC measurements at windthrow sites are more challenging and can deviate from already established procedures[3]. Since some extreme wind phenomena create spatially limited areas, there is a need for a thoughtful measuring station location and careful data processing in order to derive as much reliable flux values as possible. Similar difficulties in EC method application have occurred (e.g., Finish studies performed above a long but narrow lake) where measured CO_2 fluxes required rigorous data filtering[11,12] in order to assure their spatial representativeness.

Hence, the presented protocol is an example of the use of the EC method at non-typical locations, designed not only for windthrow areas, but for all other types of short vegetation with the limited area (e.g., croplands situated between taller vegetation types). The biggest advantage of the proposed methodology is a general description of complicated procedures, requiring advanced knowledge, from the site location choice and instrumentation set up to the final outcome: a complete dataset of high-quality CO_2 fluxes. The technical novelty of the measuring protocol is the use of a unique base construction for the EC system placement (e.g., tripod with a defined height that is a “mini- tower” with an adjustable, electrically operated mast, allowing changing the final height of sensors according to individual needs).

PROTOCOL:

1. Site location and instrumentation setup

1.1. Choose a measuring site location in relatively homogeneous and flat terrain to meet basic requirements of the EC method. Avoid places with complicated landforms (depressions, slopes) or located near aerodynamic obstacles (e.g., surviving tree stands), which can distort the air flow.

1.1.1. Check species composition and plant cover. Choose a place with the most similar characteristics: age and height of the main vegetation type.

1.1.2. If possible, conduct some additional soil investigations, which help to choose homogeneous area. Compare soil types in a few locations (soil profiles), soil carbon and nitrogen content as well as moisture conditions (e.g., using regular grid for soil sampling). Avoid places with outstanding features in comparison with the average values from the soil investigation.

1.2. Before deciding where to place the instruments, investigate prevailing wind directions (ideally for one year before site setup), or analyze data from the nearest meteorological station. If there are some restrictions regarding the extent of the area of interest, choose the location which is within prevailing wind sectors (upwind).

NOTE: In the case of Polish windthrow site, due to the shape of tornado path, it was decided to place the tower in the middle of its width dimension (ca. 400−500 m) and as far from neighboring, few-year-old pine plantation as possible in the east-west direction (ca. 200 m from the tower to their edges), since the prevailing wind direction was from north-west to south-west and from north-east to east (**Figure 1**).

1.3. Decide which EC system to use: open path or closed path (enclosed path = closed path with short intake tube) infrared gas analyzer (or two of them if possible). Each has advantages and disadvantages but in general, both are reliable to be used on a field. Use a three-dimensional (3D) orthogonal sonic anemometer. To use the EC method, high frequency measurements are required — at least 10 Hz in the case of both instruments.

1.3.1. Consider what kind of power supply is the most feasible to be used at the site (is there a power line nearby, solar panels or other power generator?). If there are no limitations, use the closed path (or enclosed) path gas analyzer.

NOTE: An open path system has much lower power consumption, but in harsh environments (very cold weather, icing, rainy locations) it would result in considerable loss of high-quality data.

1.3.2. Follow the rules to position both instruments relative to each other[13]. Avoid mounting any unnecessary elements close to the EC system, which can distort the air flow.

NOTE: An enclosed path analyzer (**Table of Materials**) and a 3D sonic anemometer (**Table of Materials**) were used in this experiment.

1.4. Once the location is chosen, place a tripod with a vertical pole (or another kind of base construction) to mount the EC system on top. Set the height of instruments considering two basic requirements: investigated surface roughness (in simplification the height of existing vegetation) and the area of influence (fetch/footprint — the area "seen" by the EC system)[4].

NOTE: At dynamically developing ecosystems, such as reforested windthrow site Tlen I, the change in instrument placement with time will be required to meet EC method requirements. As an alternative of a base construction for the EC system, an innovative infrastructure (i.e., "mini-tower") was proposed here: an anchor aluminum construction (1.5-m-high rectangular truss (W x L) 1 m x 1.2 m) with a mast (triangular truss 30 cm x 30 cm x 30 cm) moving inside the structure along steel rails, powered by an electric motor.

1.4.1. First, mount both instruments of the EC system on a metal pole attached centrally to the mast. Remember to place the sonic anemometer at a perfectly vertical position. Tilt the gas analyzer slightly to allow rainwater to run off easily.

1.4.2. Elevate instruments to a height twice the canopy height from the soil surface, and at least 1.5−2.0 m above the top of the canopy[4]. Make sure that the base construction is located in a way, which ensures that the investigated area extends at least 100 times the height of a sensor placement in each direction[14].

1.4.3. Remember to install lightning protection for a metal construction.

NOTE: To achieve maximal output from the EC measurement in Polish windthrow site (Tlen I), some compromises were made. The instruments were placed at the height of 3.3 m at the beginning of the experiment.

1.5. For further computation and flux analysis, measure some auxiliary variables at the same time, including at least: air (Ta) and soil (Ts) temperature, relative humidity (RH) of the air, photosynthetic photon flux density (PPFD), incoming solar radiation (Rg) and precipitation (P). Usually, at EC sites a great number of other variables are also obtained.

1.5.1. Place radiation sensors (PPFD and Rg) to the south. Use a horizontal pole to move them away from the tripod. Check the view angle of the sensors and adjust the length of the pole and the mounting height to ensure that only the investigated surface is seen.

1.5.2. Use air temperature and humidity sensors with radiation shields, mounted at a similar height as the EC system.

1.5.3. Install tipping-bucket rain gauges (at least two) in relatively open spaces, near the EC tower, 1 m above the ground level. Bury soil temperature sensors at several different depths (three or more depending on the soil type). Remember to have some repetitions for each depth. Place some sensors at the shallowest possible level.

2. CO_2 flux computation

2.1. Use commercially available, free software (e.g., EddyPro[15]) for EC flux computation that includes correction applications.

NOTE: This software was selected due to its complexity, popularity and user-friendliness and is recommended especially for the non-experts.

2.2. First, create a new project and then in the **project info** tab, specify the raw data file format and choose metadata file. If raw data were obtained as ".ghg" files, the individual metadata file is already embedded, and no further action is required. In other cases, use **alternative file** option and type all information manually.

NOTE: The metadata file specifies the order of measured variables, their units and some additional information needed for flux computation. If any of the setup details or site characteristics change, remember to change it in the metadata section.

2.3. Go to the **flux info** tab, choose the dataset and output directories, specify the raw file name format and check the list of items for flux computation.

2.4. Go to **processing options** tab and choose raw data processing settings.

2.4.1. Choose the method for the correction of anemometers' measurements (**rotation method**), which allows accounting for any misalignment of the sonic anemometer with respect to the local wind streamline[15]. Tick the first planar fit approach[16] (suggested for non-ideal, heterogeneous locations).

2.4.2. Choose the **0-1-2** type of flagging policy[17] (the approach which presents results of a quality check procedure).

2.4.3. Select the preferred footprint method (the area of the influence on measured fluxes) (e.g., the Kljun[18] approach). Leave all other setting unchanged (default options).

NOTE: Here one can choose from the list of options regarding corrections to be applied, fluxes footprint calculation method or the structure of output files. Although, it is suggested not to change standard options during the preliminary run of the selected EC software, except for the ones listed here.

2.5. In case of any problems/questions, use the question mark (?) button next to the option of interest to find out more. Remember that incorrect or missing information in one tab will prevent movement to another.

2.6. Click **Run an Advanced mode** to start fluxes computation at the end. In case of using only default settings click **Run an Express mode**.

3. Filtering and quality control of fluxes

3.1. Avoid data loss by using a regular maintenance plan. According to individual capabilities, clean sensors as frequently as possible using water or mild detergent.

3.2. Carry out calibration of gas analyzers at least once every 6 months using CO_2 standards (0 ppm and at least one other concentration, e.g., 360 ppm). A minimum of 24 h before each calibration, change CO_2 and H_2O absorbing agents (sodium hydroxide coated silica and magnesium perchlorate, respectively) that are present in two small bottles inside the sensor head.

NOTE: The calibration procedure is relatively easy and well described in the gas analyzer manual. In the software dedicated to LI-7200 and LI-7500, there is a tab, which contains all step-by-step guidelines of the whole process. In case of any difficulties, analyzers can always be sent for a factory calibration performed by the producer, but it requires sensor demounting and results in long gaps in the flux dataset.

3.3. Create a common file (e.g., .csv, .xlsx) that contains all results from the flux calculation software and auxiliary measurements. Make sure that corresponding 30-min averages (fluxes and meteorological variables) are measured at the exact same time.

NOTE: To simplify and speed up the filtering procedure, use additional programs (e.g., Matlab or free *R* software), depending on users' skills, rather than work in a spreadsheet.

3.4. Perform all filtering steps described below (sections 3.5-3.7) on data from this file. Use either filtering tools in the spreadsheet (or embedded "if" function) or create custom filtering functions utilizing other software.

3.5. Determine unfavorable weather conditions and instrument malfunctions.

3.5.1. Use instrument's performance indicators to filter out data subjected to errors due to gas analyzer contamination. For an enclosed-path analyzer, check the average signal strength (ASS) value given in the output file from the fluxes' calculation software. Then, mark and discard all fluxes (*co2_flux*) measured below, e.g., ASS = 70% (10% higher threshold than suggested in the instrument's manual).

3.5.2. Optionally, set a constant range for fluxes, which allows exclusion of outliers (e.g., from -15 to 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at Tlen I site). One of the possible ways to remove fluxes outside the normal range is to use a limit of 2−3 standard deviations from the mean flux value, calculated individually for each season.

NOTE: The authors do not strongly advise using an *a priori* range as done in the case of Tlen I site by non-specialist. The statistical approach is much more reliable and objective.

3.5.3. Discard fluxes measured during any rain events (or other type of precipitation); delete fluxes when P ≥ 0.1 mm.

3.6. Account for inappropriate conditions for eddy covariance method application.

3.6.1. Use results of the steady-state test and the well-developed turbulence test[17,19] performed during fluxes computation in the software (see step 2.4.2). Discard flux data with poor quality (CO_2 flag values: qc_co2_flux > 1) in the common results file.

3.6.2. Use the nighttime period indicator (*daytime* = 0) given in the output file to filter out CO_2 fluxes values measured at night. Plot all nighttime CO_2 fluxes against corresponding friction velocity values (u_* measured at the same time) and find the u_* value at which these fluxes stopped increasing.

3.6.3. Mark the obtained value as the friction velocity threshold (u_{*thr}) to be used with the measures of insufficient turbulence conditions. Discard all CO_2 fluxes with corresponding u_* values < u_{*thr} from the dataset.

NOTE: The presented method for u_{*thr} determination is the simplest but also the most subjective. There are few, more precise, complicated and reliable methods to define the friction velocity threshold[21,22] than the simple visual inspection which can be used here. Also, it must be mentioned that at very heterogeneous sites defining u_{*thr} might not be easy. Some other measures must be considered in such cases, which are well described in the literature[3,4].

3.7. Flux spatial representativeness constraints

3.7.1. First, plot the wind rose, obtained from measurements or from the nearest meteorological station, on the map of investigated area. Specify which wind sectors should be excluded from the final analysis (due to the existence of any potential burden or different vegetation type than investigated). Use a custom method or utilize ready functions from other mathematical software (e.g., windRose function in *R* software).

3.7.2. According to the estimation of crosswind integrated footprints chosen during fluxes computation (step 2.4.3), decide which footprint characteristic will be used for further analysis (*x_10%*, *x_30%*, *x_50%*, *x_70%* or *x_90%* level). To simplify, each 30-min footprint value provides information on what is the distance (upwind) to the edge of the area, from which the measured signal (flux) originated with a given probability level.

NOTE: Here footprint values representing 70% (*x_70%*) probability was chosen as the limit, since the highest possible 90% level in spatially limited sites results in going well beyond the area of investigation.

3.7.3. Choose wind direction sectors that are most representative of the measuring site. Do the same with the footprint values, bearing in mind that the furthest distance (the highest footprint value) cannot exceed the area of interest (**Figure 1**). Filter out flux values that do not meet both requirements.

NOTE: Since the windthrow Tlen I site was located between the forest stands that survived the tornado, only two sectors of wind direction were accepted as representative: 30−90° and 210−300°. Thus, all CO_2 fluxes originated from the area beyond these sectors were excluded. Furthermore, the distance to the nearest burden (distorting air flow) or different ecosystem type (with different net CO_2 exchange dynamics) in each direction should be the maximal footprint limit, although, it is recommended to decrease this value. At the centrally located Tlen I site, the distance to the surviving forest's edges was ca. 200−250 m; therefore, the chosen footprint threshold was set to 200 m at most and applied equally in each direction.

4. Gap filling and net flux partitioning into CO_2 respiration and absorption

4.1. Choose the method for quality-checked CO_2 flux gap filling and partitioning into absorption (gross primary production [GPP] fluxes) and respiration (ecosystem respiration [R_{eco}] fluxes) from several commonly used approaches, which include three basic groups: process-based approach[23,24], statistical methods[25,26], and the use of neural networks[27,28].

NOTE: Since the first two groups of methods (process-based and statistical approaches) are widely used among the scientific community, well described and discussed in the literature and in the case of the latter, recommended to be used in a global network of flux measurements sites (FLUXNET) and Integrated Carbon Observation System (ICOS) project (international initiatives aiming at trace gases monitoring, EC data collection and common processing protocols creation), the use of both was recommended here at the beginning.

4.2. As an example of the process-based approach, follow the procedure from the Fluxnet Canada Research Network (FCRN[21,22]).

4.2.1. Select net CO_2 fluxes (NEP) measured during nighttime periods as well as all flux values from outside the growing season. These are assumed to be entirely R_{eco} fluxes.

NOTE: To differentiate between the nighttime and the daytime period, the PPFD threshold value can also be used (e.g., PPFD < 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as a nighttime indicator[29]). Moreover, to estimate when the vegetation period starts and ends, a simple thermal method was used here: when the average daily air (at 2 m height) and soil temperature (at 2 cm depth) were greater than 0 °C, the beginning of the vegetation season was noted and ended when both temperatures fell below 0 °C again. In case of different vegetation species, a different temperature threshold should be used regarding plants physiology. The onset of photosynthetic activity is different for coniferous and deciduous trees, crops and grasses, which comes from the fact, that different vegetation species react differently to air temperature.

4.2.2. Using the temperature (T) of soil, air or the combination of the two, determine the relationship between temperature and R_{eco}. Use any software that allows fitting non-linear functions to the data (e.g., Matlab software). In principal, choose the best fit regression model (use e.g., Akaike information criterion (AIC) to decide on the function which fits best to the data); although in practice, one of the most commonly utilized functions is a Lloyd-Taylor[30] model:

$$R_{eco} = R_{Tref} \cdot e^{E_0\left(\frac{1}{T_{ref}-T_0}-\frac{1}{T-T_0}\right)}$$

where R_{eco} is the ecosystem respiration flux value, R_{Tref} is the respiration rate in a reference temperature, T_{ref} is the reference temperature, T is the measured air or soil temperature, T_0 is the temperature which is a threshold for biological activity to initiate (estimated parameter of the model), and E_0 is the parameter describing activation energy.

NOTE: In the case of FCRN procedure, some of these variables are set in advance: T_{ref} and E_0, which in case of Tlen I windthrow site were equal to 283.25 K and 309 K, respectively. Some studies suggest the use of soil temperature measured at the shallowest depth for the R_{eco} vs. T relationship[25], which for a short vegetation seemed to be the best choice, since a great part of emission comes from the heterogenic respiration from the soil and roots. Unlike in tall forest, the autotrophic respiration of foliage, branches and boles, driven by air temperature, does not play a major role (if present).

4.2.3. Using the obtained R_{eco} vs T regression function, fill the gaps in nighttime and non-growing season NEP fluxes and calculate the function value for missing fluxes using corresponding temperature measurements. Note that in these cases R_{eco} = NEP, and GPP = 0. The same function with daytime temperatures will give daytime R_{eco} fluxes for each half-hour value.

4.2.4. Calculate GPP values according to the equation: GPP = NEP + R_{eco} for each available NEP flux during daytime in the growing season or set to zero during nighttime and the non-growing season. Then, find the relationship between PPFD and GPP fluxes. Use any software that allows fitting non-linear functions to the data. Again, there is one widely used equation to achieve such relationship- rectangular hyperbola of Michaelis-Menten, here in a modified form[26]:

$$GPP = \frac{\alpha \cdot PPFD}{(1-(\frac{PPFD}{2000})+(\alpha \cdot \frac{PPFD}{GPP_{opt}}\;)}$$

where GPP is the 30-min averaged gross primary production flux value, α is the ecosystem quantum yield, and GPP_{opt} is the GPP flux rate at an optimum PPFD (2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

NOTE: Use the obtained function to model GPP values for measured daytime, growing season NEP fluxes values.

4.2.5. At the end of the whole procedure, use modelled GPP and R_{eco} fluxes to calculate missing NEP fluxes values as follows: NEP = GPP - R_{eco}.

NOTE: Some small gaps (a few missing fluxes) can be filled with a simple linear regression function, a moving mean approach or other statistical methods before entering the models. The gaps in ancillary variables (temperature, solar radiation) must be filled before entering the models. Thus, the multiplied measurement of the same or surrogate variables are useful, helping to avoid big gaps in datasets.

4.3. To fill the gaps not only in the CO_2 but also other EC flux values (sensible and latent heat), as well as in the important meteorological elements, use the ReddyProc[25] online tool (available also as an *R* software package).

NOTE: In contrast to the previous method, first missing NEP fluxes are filled and then each half-hour net flux is partitioned into GPP and R_{eco}. The type of model used for R_{eco} fluxes partitioning is the same as in the previous technique.

4.3.1. To use an online tool, prepare data according to the rules concerning their format and order. The data needed include 30-min averages of net CO_2 (NEP), latent heat (LE) and sensible heat (H) fluxes, water vapor deficit (VPD) and friction velocity values calculated using EC measurements, as well as soil or air temperature (T_{air} or T_{soil}), incoming solar radiation (R_g) and relative humidity of the air (RH).

4.3.2. Go to the **Processing page** and fill all needed information regarding the measuring site (name, coordinates, altitude, time zone).

4.3.3. Decide whether to estimate u_* threshold additionally with this software (see steps 3.6.2 and 3.6.3), which method to use and for which period of time: the whole year or separately for each season.

4.3.4. Select one or both methods for net fluxes partitioning (nighttime-[25] or daytime-based[31] and run the computation process.

4.4. Compare obtained results in terms of both method performances in NEP flux gap filling and partitioning by creating artificial gaps in NEP, and check how precisely they were modeled.

4.5. Calculate daily, monthly and annual totals of all gap filled CO_2 fluxes including NEP, GPP, and R_{eco}, on the basis of which the changes of ecosystem functioning can be traced. Use the users' own function to aggregate these fluxes separately into the chosen time domain and sum up all values.

NOTE: At the Tlen I windthrow site, annual totals, as well as monthly fluxes allowed to analyze not only net CO_2 exchange dynamics but also post-disturbance recovery mechanisms of the managed forest.

REPRESENTATIVE RESULTS:

One of the crucial steps in flux filtering and quality control at non-ideal EC sites is the assessment of the measured fluxes' spatial representativeness. The simplest way to perform such analysis, given the fact that calculations were done using commercial, widely applied software, is to include measurements from desired area only, on the basis of wind direction and footprint estimations (see section 3.7). Thus, the wind rose plot, with a chosen wind direction and maximal acceptable extend of fluxes footprint, marked as shaded polygons, on the background of the satellite picture from the Tlen I site, is shown here as a visual representation of the analysis result (**Figure 1**).

In principle, wind speed and trace gas concentration are measured by the eddy covariance system, which are then used to compute net CO_2 exchange fluxes (NEP). Raw flux values have to be then post-processed in order to exclude errors and low-quality data. **Figure 2** shows the results of a filtering procedure on the example of one year of NEP fluxes measurements from the Tlen I windthrow site.

It should be noted that the proposed procedure of flux quality check and assurance resulted in substantial data loss, to a much greater extent than in typical EC sites. The reduction to acceptable NEP fluxes, relative to the previous stage, was similar in sections 3.6 and 3.7, while the smallest number of data points was discarded due to unfavorable weather conditions and instrument malfunctions (section 3.6). The last part of the quality assurance protocol (chosen footprint and wind direction sectors) yielded a final data coverage of only 1/3 of all raw NEP fluxes measured by EC. In general, step 3.7 is the most crucial part of the filtering procedure here, assuring that obtained fluxes represent the gas exchange of the investigated area.

High-quality NEP fluxes can be finally used to derive daily, monthly, seasonal or annual totals. However, they must be gap filled before each action. In **Figure 3**, the relationship between NEP fluxes, gap filled using two different approaches: process-based (FCRN) and statistical method (REddyProc), is shown.

The presented simple linear regression suggests that in general both techniques are comparable (statistically significant regression with r^2=0.89) and thus can be used for NEP fluxes gap filling, giving satisfactorily similar results (the regression line slope equal 0.90, which suggest only 10% difference between gap filled fluxes on average). With only net CO_2 flux values, nothing can be said about individual impacts of absorption (GPP) and respiration (R_{eco}) processes. Therefore, along with gap filling, so-called flux partitioning procedure was realized as well, by use of the same two methods. Daily totals of R_{eco} fluxes are presented in **Figure 4** as examples of two different method performances in net CO_2 fluxes partitioning.

The results of R_{eco} flux computation with two different methods, although the same model of R_{eco} *vs T* was used in both cases, are examples of a potential source of erroneous conclusions regarding a contribution of respiration to the overall NEP fluxes or consequently the absorption rates (GPP fluxes). However, it cannot be clearly indicated which method gives more reliable results without additional analysis in this manner. What can be done, in our opinion, is either plotting measured nighttime fluxes against modeled R_{eco} fluxes to look over the differences, or to compare estimated values with respiration fluxes directly measured with other technique (e.g., chambers). The differences in modeled R_{eco} fluxes between presented approaches may come from the fact, that in one method some parameters are set as constant, while in the other they are estimated. Even the ones, which do not change in both cases (as a reference temperature - T_{ref}), were not the same in given example (in FCRN T_{ref} = 283.25 K, while in REddyProc T_{ref} = 288.15 K). It was done on purpose to make potential users realize that even such slight changes may result in significant discrepancies. The other issue is that a statistical approach is not able to fill big gaps successfully, which in the case of presented non-ideal EC site, where there was only 1/3 of measured fluxes left after filtering and quality check procedure, might be a reason for concern. We do not attempt to provide a "better solution" with this analysis, but rather present options. A more thorough investigation needs to be done in this case.

FIGURE LEGENDS:

Figure 1: Wind rose plot on the background of the Tlen I site area. The blue shaded polygons represent the chosen wind direction and red shaded polygons within them show sectors of a circle with a radius of 200 m (maximal acceptable extend of fluxes footprint).

Figure 2: The course of 30-min averaged NEP fluxes at each step of data filtering (described in the Protocol), on the background of unprocessed, raw NEP fluxes values. The relative number of data points remaining after each stage is given at the top of each plot.

Figure 3: The relationship between NEP fluxes, gap filled with a process-based method (FCRN) and a statistical approach (REddyProc online tool), measured at Tlen I windthrow site.

Figure 4: Daily ecosystem respiration (R_{eco}) fluxes totals obtained from partitioning procedure, performed with a process-based method (FCRN) and a statistical approach (REddyProc online tool) at the Tlen I windthrow site.

DISCUSSION:

This protocol presents the eddy covariance (EC) method to be used at non-ideal sites (here a reforested windthrow site): site location and measuring infrastructure setup, net CO_2 fluxes computation and post-processing, as well as some issues regarding gap filling and fluxes partitioning procedures.

Even though the EC technique is commonly used at many measuring sites around the world, most of them are non-disturbed ecosystems, where the design and the following data processing can be done according to standard solutions (e.g., FLUXNET or ICOS network protocols). Although, in such demanding and often spatially limited areas as windthrow sites, such experiments should be planned and performed with special caution. Additionally, in the long run, measurements at dynamically growing ecosystems would require a change in EC system height in the future, along with new vegetation growth and development. Therefore, we recommend using a unique base construction, which is an innovative "mini-tower" with an electrically operated, extendable mast. This technical solution allows meeting one of the basic requirements of the method itself: the EC system placement in a mixed boundary layer, without the need of reconstruction or instruments demounting, which may result in further data losses in already depleted dataset. Furthermore, the easily moving electrical mast also makes the sensors' maintenance at the site a lot easier (e.g., when one needs to clean the optical path of the analyzer, the whole EC system can be brought down to desired, convenient height). Nevertheless, it must be noted, that increasing the height of the instrument's placement will have consequences in the extension of an area of influence (flux footprint), which will further result in more data being excluded due to an insufficient flux footprint. In the worst-case scenario, the measured fluxes would probably no longer be representative for the investigated area or even the EC method requirements would not be met anymore.

The site location in a relatively homogeneous and flat terrain, as described in the Protocol, is the most desired option. Under such conditions, advection issues are generally neglected. However, if the area of interest is located on a hilly terrain, it must be taken into account in the measured flux analysis, which implies more advanced knowledge to be gained.

The suggested software (EddyPro) for flux calculation from the raw, high frequency data, is a free, complex and user-friendly tool, designed for EC flux computation. All embedded equations and corrections have the scientific background and corresponding references to the methods used are given[15]. Moreover, it is constantly adjusted and developed by specialists-scientists in order to implement the most current state of knowledge.

Once temporally averaged CO_2 fluxes are computed, they need to be carefully processed in order to assure their high quality and representativeness. One of the prosaic sources of errors are disturbances in instruments' operation: precipitation, pollen, dirt, ice deposition on gas analyzer window (open-path analyzer) or inside intake tube (enclosed- and closed-path analyzers), which affect CO_2 fluxes measurements. Such events can also disrupt wind speed measurement to some extent (sonic anemometer). Thus, in this protocol, subsequent stages of NEP fluxes filtering were presented, in which the last step is of the biggest importance for the non-ideal, spatially limited sites. Even though the number of data points, after accounting for representative wind direction sectors and footprint, was very small (**Figure 2**), it must be remembered that it is crucial not to include "false" signals, coming from different areas than the ones we are interested in. In contrast to the first two steps, the above-mentioned flux filtering procedure (mainly wind direction constraints) is not commonly used in EC forest sites, since the undisturbed site location is usually chosen in a way to ensure the best representative area possible. Windthrow sites, on the other hand, appear as a result of unpredictable phenomena; therefore, some compromises have to be made in order to carry out EC measurements at these scientifically valuable areas. Unlike in this study, proposed footprint limits can have different values in different wind directions. It is also worth mentioning that there are other kinds of flux representativeness estimations than the one presented here (e.g., 2D footprint climatology approach[32], which is free to use online and gives more complex results). In such complicated sites, this approach can be even more helpful in specifying the area of the greatest influence on the measured fluxes. However, to simplify post-processing of fluxes, calculated using chosen commercial software, it was decided to use only information given in its output files.

The weakest point of the protocol is the gap filling and flux partitioning description. The two suggested methods were individually developed by other specialists before and only implemented here as proposed techniques. What is more, the FCRN method requires much more contribution from the user since there is no ready tool to perform this procedure. The comparative analysis of corresponding gap filled (NEP) and partitioned fluxes (GPP and R_{eco}), which might have been of a greater interest among potential users, require a more thorough investigation in order to be fully applicable (**Figure 3** and **Figure 4**).

There is still room for improvement regarding both the technical details of EC measurements and data processing presented in this protocol. One potential possibility is the fusion of processed-based and statistical method for data gap filling and partitioning (e.g., ReddyProc method for gap filling and then FCRN for fluxes partitioning), according to individual needs, or simply the use of neural networks approach.

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DISCLOSURES:

The authors would like to mention, that presented protocol is mostly a simplification of a well-known and widely described issues regarding EC measurements. All sufficient references were given when required. Our main aim was to promote the use of this method, as well as our new and unique adjustable, electrically operated mast for EC measurements, among non-specialists with a step-by-step approach. We hope, that it makes it easier to realize and imagine that however strict requirements need to be met, EC technique can be satisfactorily applied also in non-typical, spatially limited ecosystems. With already broad literature concerning EC theory and methodology, presented protocol can potentially also be an encouragement to further knowledge acquisition on this subject.

REFERENCES:

1. Baldocchi, D. Measuring fluxes of trace gases and energy between ecosystems and the atmosphere – the state and future of the eddy covariance method. *Global Change Biology*. **20**, 3600–3609 (2014)

2. Aubinet, M. et al. Estimates of the annual net carbon and water exchange of European forests: the EUROFLUX methodology. *Advances in Ecological Research*. **30**, 113–174 (2000).

3. Aubinet, M., Vesala, T., Papale, D. (Eds.). Eddy Covariance. A practical guide to measurements and Data Analysis. *Springer, Dordrecht, Heidelberg, London, New York* (2012).

4. Burba, G. Eddy Covariance Method for: Scientific, Industrial, Agricultural, and Regulatory Applications. A Field Book on Measuring Ecosystem Gas Exchange and Areal Emission Rates. *LI-COR Biosciences, Lincoln, Nebraska* (2013).

5. Pan, Y. et al. A Large and Persistent Carbon Sink in the World's Forests. *Science*. **333**, 988–993 (2011).

6. Wofsy, S.C. et al. Net exchange of CO_2 in a midlatitude forest. *Science.* 260, **5112**, 1314–1317 (1993.)

7. Luyssaert, S. et al. CO_2 balance of boreal, temperate, and tropical forests derived from a global database. *Global Change Biology*. **13**, 2509–2537 (2007).

8. Knohl, A. et al. Carbon dioxide exchange of a Russian boreal forest after disturbance by wind throw. *Global Change Biology*. **8**, 231–246 (2002).
9. Lindauer, M. et al. Net ecosystem exchange over a non-cleared wind-throw-disturbed upland spruce forest-Measurements and simulations. *Agricultural and Forest Meteorology.* **197**, 219–234 (2014).
10. Schulze, E.D. et al. Productivity of forests in the Eurosiberian boreal region and their potential to act as a carbon sink – a synthesis. *Global Change Biology*. **5**, 703–722 (1999).
11. Mammarella, I. et al. Carbon dioxide and energy fluxes over a small boreal lake in Southern Finland. *Journal of Geophysical Research-Biogeosciences*. **120**, 1296–1314 (2015).
12. Vesala, T. et al. Eddy covariance measurements of carbon exchange and latent and sensible heat fluxes over a boreal lake for a full open water period. *Journal of Geophysical Research-Biogeosciences*. **111**, D11101, 1-12 (2006).
13. Burba, G., Anderson, D. A brief practical guide to Eddy Covariance Flux Measurements. Principles and workflow examples for scientific and industrial applications. *LI-COR Biosciences, Lincoln, Nebraska* (2010).
14. Businger, J. Evaluation of the accuracy with which dry deposition could be measured with current micrometeorological techniques. *Journal of Applied Meteorology and Climatology*. **25**, 1100–1124 (1986).
15. Eddy Pro Software Instruction Manual. LI-COR Biosciences, Lincoln, Nebraska (2017, February 8). https://www.licor.com/documents/1ium2zmwm6hl36yz9bu4.
16. Wilczak, J. M., S. P. Oncley, S. A. Stage. Sonic anemometer tilt correction algorithms. *Boundary-Layer Meteorology*. **99**, 127–150 (2001).
17. Foken, T. et al. Post-field quality control, in: Lee, X. (Eds.), Handbook of Micrometeorology: A Guide for Surface Flux Measurements. *Kluwer Academic, Dordrecht*. (2004).
18. Kljun, N., Rotach, M.W., Schmid, H.P. A three-dimensional backward Lagrangian footprint model for a wide range of boundary-layer stratifications. *Boundary Layer Meteorology*. **103**, 205–226 (2002).
19. Foken, T., Wichura, B. Tools for quality assessment of surface-based flux measurements. *Agricultural and Forest Meteorology*. **78**, 83–105 (1996).
20. Mauder, M., Foken, T. Impact of post-field data processing on eddy covariance flux estimates and energy balance closure. *Meteorologische Zeitschrift*. **15**, 597–609 (2006).
21. Gu, L. et al. Objective threshold determination for nighttime eddy flux filtering. *Agricultural and Forest Meteorology*. **128** (3–4), 179–197 (2005).
22. Papale, D. et al. Towards a standardized processing of Net Ecosystem Exchange measured with eddy covariance technique: algorithms and uncertainty estimation. *Biogeosciences*. **3** (4), 571–583 (2006).
23. Barr, A.G. et al. Interannual variability in the leaf area index of a boreal aspen-hazelnut forest in relation to net ecosystem production. *Agricultural and Forest Meteorology*. **126**, 237–255 (2004).
24. Krishnan, P., Black, T.A., Jassal, R.S., Chen, B., Nesic, Z. Interannual variability of the carbon balance of three different-aged Douglas-fir stands in the Pacific Northwest. *Journal of Geophysical Research*. **114**, G04011, 1–18 (2009).

25. Reichstein, M. et al. On the separation of net ecosystem exchange into assimilation and ecosystem respiration: Review and improved algorithm. *Global Change Biology*. **11**, 1424–1439 (2005).

26. Falge, E. et al. Gap filling strategies for defensible annual sums of net ecosystem exchange. *Agricultural and Forest Meteorology*. **107**, 43–69 (2001).

27. Ooba, M., Hirano, T., Mogami, J.I., Hirata, R., Fujinuma, Y., Comparisons of gap-filling methods for carbon flux dataset: A combination of a genetic algorithm and an artificial neural network. *Ecological Modelling*. **198**, 473–486 (2006)

28. Papale, D., Valentini, R., A new assessment of European forests carbon exchanges by eddy fluxes and artificial neural network spatialization. *Global Change Biology*. **9**, 525–535 (2003).

29. Baldocchi, D.D., Vogel, C.A., Hall, B. Seasonal variation of carbon dioxide exchange rates above and below a boreal jack pine forest. *Agricultural and Forest Meteorology*. **83**, 147–170 (1997).

30. Lloyd, J., Taylor, J. On the Temperature Dependence of Soil Respiration. *Functional Ecology*. **8**, 315–323 (1994).

31. Lasslop, G. et al. Separation of net ecosystem exchange into assimilation and respiration using a light response curve approach: critical issues and global evaluation. *Global Change Biology*. **16**, 187–208 (2010).

32. Kljun, N., Calanca, P., Rotach, M. W., and Schmid, H. P. A simple two-dimensional parameterisation for Flux Footprint Prediction (FFP), *Geoscientific Model Development*. **8**, 3695–3713 (2015).

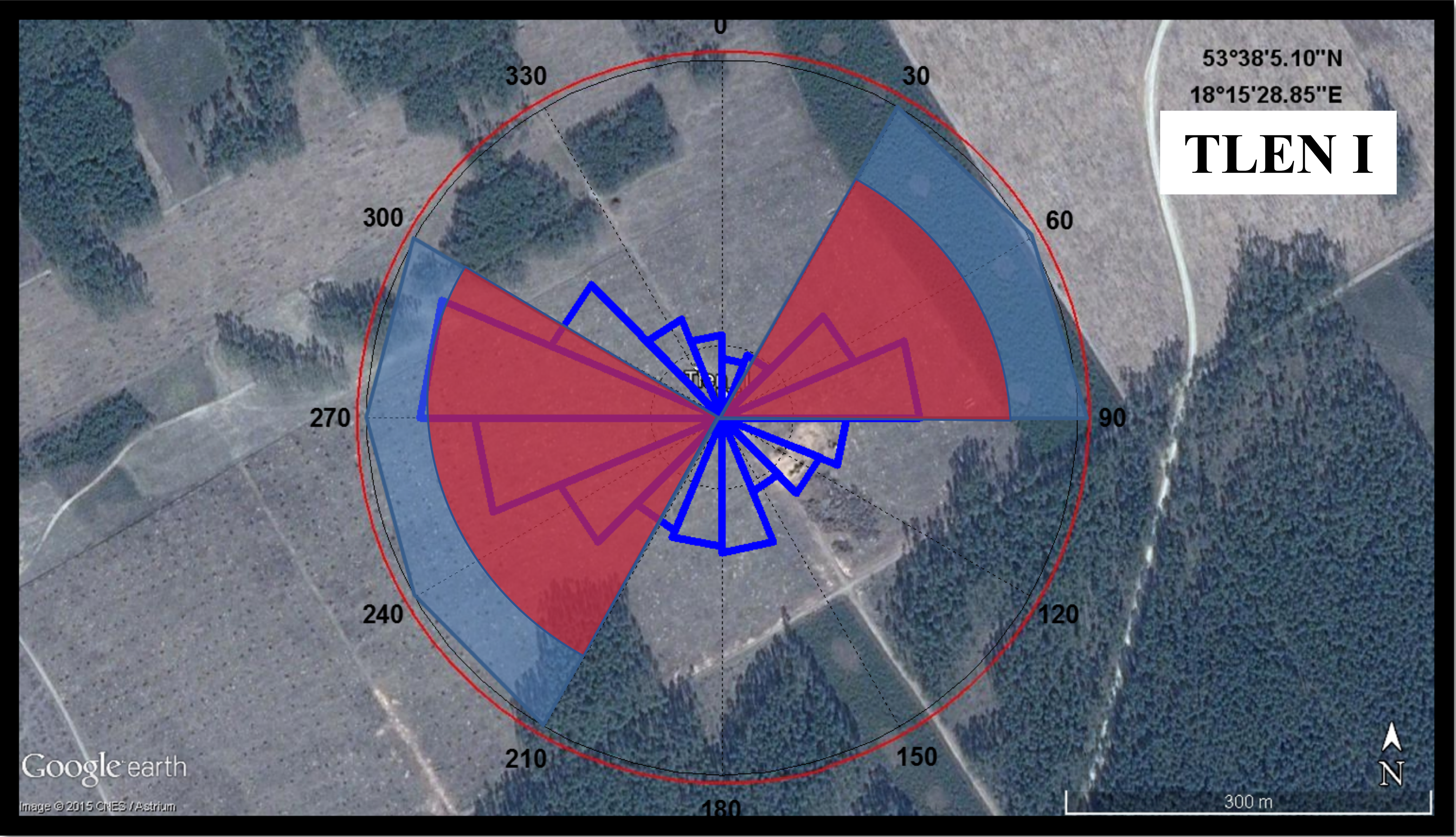

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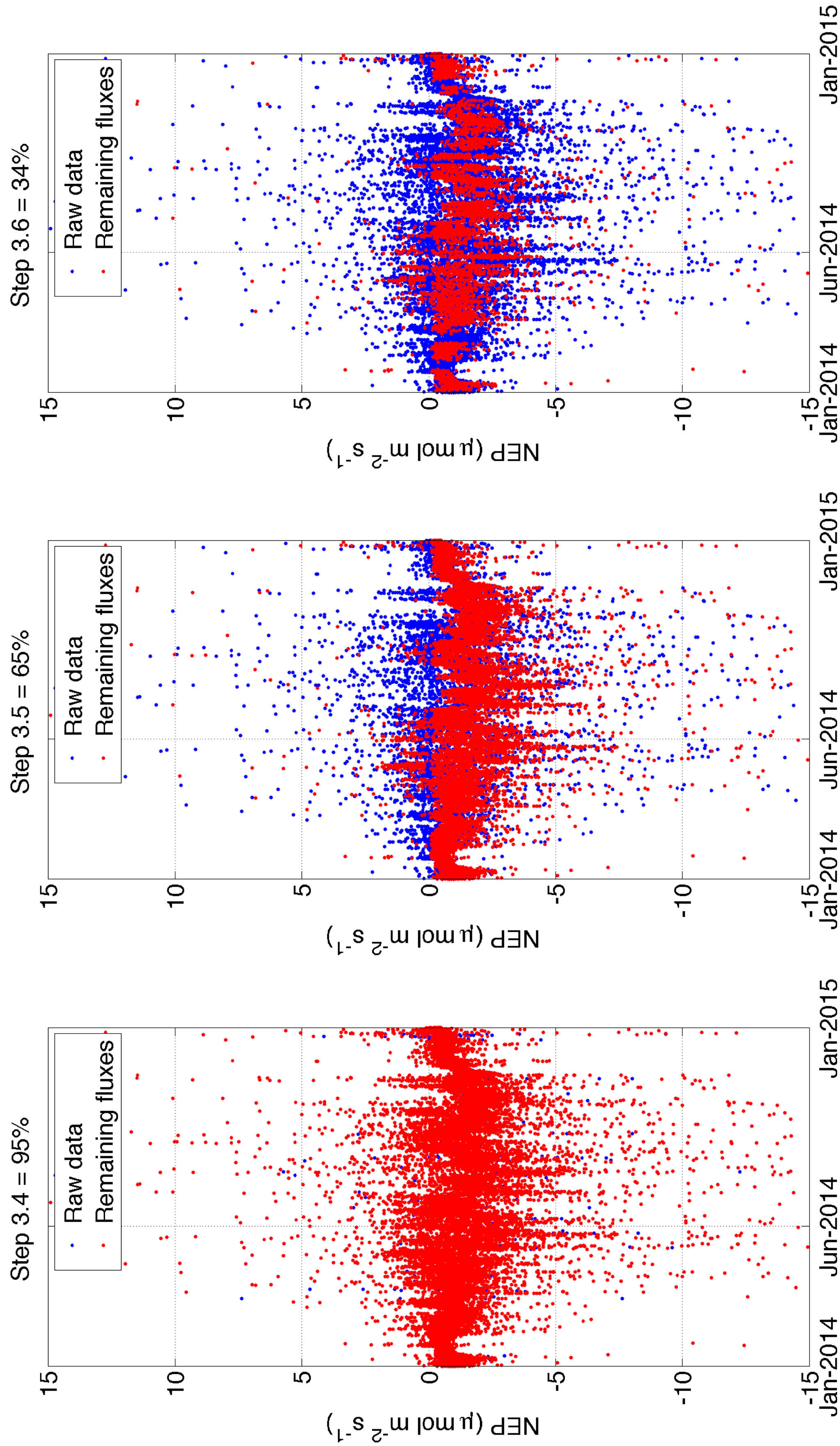
Figure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Step 3.5 = 65%
Step 3.6 = 34%
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-5
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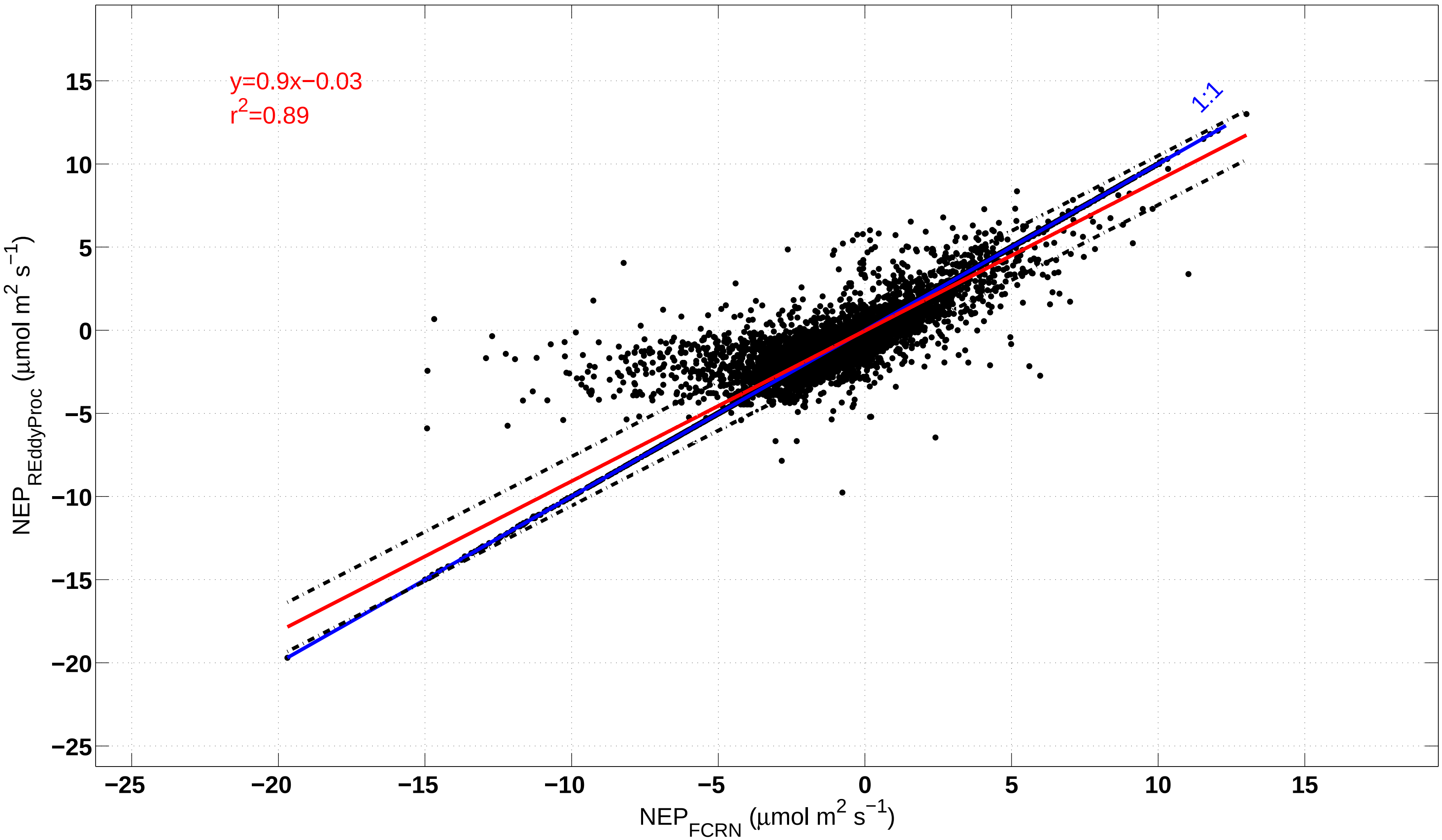
-15
Jan-2014
Jun-2014
Jan-2015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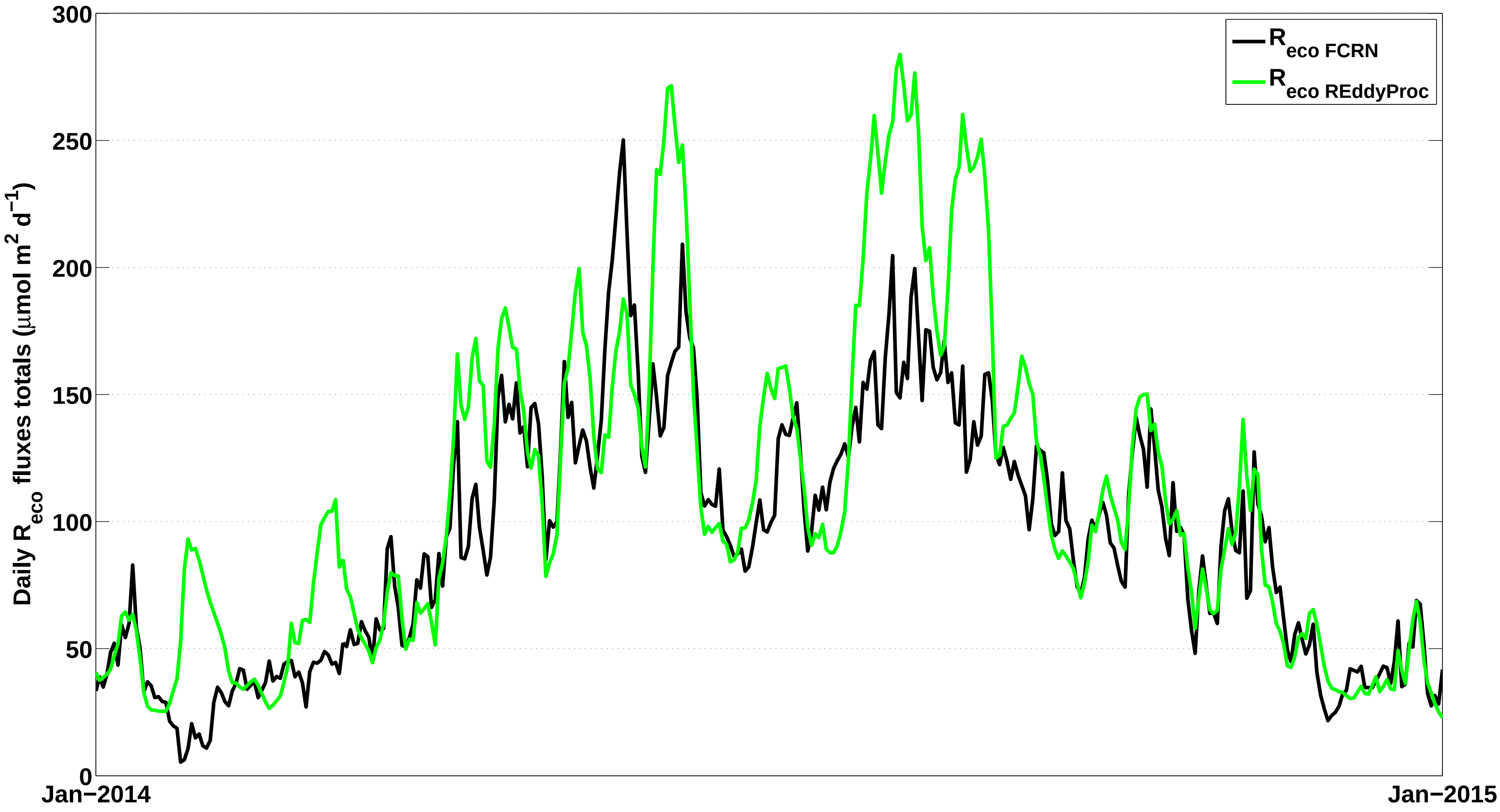
NEP FCRN (µmol m2 s−1)



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Jan−2014
Jan−2015
Daily R eco fluxes totals (μmol m2 d−1)
R eco FCRN
R eco REddyProc

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Adjustable mast with metal rails and electric engine (24 V)	maszty.net	-	Alternative basic construction. To be designed and made by professionals
EddyPro	LI-COR, Inc.	ver. 6.2.0.	Free commercial software for fluxes calculation. Available on a website: https://www.licor.com/env/products/eddy_covariance/software.html, on request
Enclosed-path infrared gas analyzer	LI-COR, Inc.	LI-7200	One of two instruments of the eddy covariance system (EC) used for CO2 fluxes measurements. Other types of fast analyzers (>10Hz sampling frequency) can be used
REddyProc	-	-	Free software for EC fluxes gap filling and partitioning. Available on Max Planck Institute for Biogeochmistry: https://www.bgc-jena.mpg.de/bgi/index.php/Services/REddyProcWeb. Both online tool and *R* package are provided.
Short aluminum tower base with concrete foundation	maszty.net	-	Alternative basic construction (pioneering solution). To be designed and made by professionals
Sonic anemometer	Gill Instruments	Gill Windmaster	One of two instruments of the eddy covariance system (EC) used for wind speed measurements. Other types of three-dimensional sonic anemometers can be used
Stainless-steel tripod	Campbel Scientific, Inc.	CM110 10 ft	The basic construction for eddy covariance (EC) system. Can be constructed by yourself-materials to be found in a hardware store

Sunshine sensor	Delta-T Devices Ltd.	BF5	One of the exemplary instruments for photosynthetic photon flux density measurements (PPFD). To be bought from several commercial companies. Remember to place it above the canopy, far from reflective surfaces.
Thermistors	Campbel Scientific, Inc.	T107	One of the exemplary instruments for soil temperature measurements. To be bought from several commercial companies. It is advisable to have a profile of soil temperature
Thermohygrometer	Vaisala Oyj	HMP155	One of the exemplary instruments for air temperature and humidity measurements. To be bought from several commercial companies. Remember to place it inside radiation shield at similar height as the EC system.

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Responses to Editor's comment

We have addressed all the Editor's comment and suggestion in the revised version of the manuscript. Here we are presenting how we have solved the most important issues raised by the Editor in the order of their appearance in the former manuscript. After applying all suggested edits, we have highlighted (2.75 pages at most) these steps of the Protocol which are meant to be recorded, as required. We would also like to mention, that in order to comply with some of the Editor's comments/suggestions we had to add two more references. Therefore, in the current version of the manuscript there are 32 references and the former numbering has changed accordingly. The structure of the Protocol has slightly changed as well.

Editor: Please revise the Protocol text to avoid the use of any personal **pronouns** (e.g., "we", "you", "our" etc.).

Authors: We have revised the Protocol thoroughly and there are no personal pronouns used in the current version of the manuscript anymore.

Editor: "*1.5. At dynamically developing ecosystems, such as reforested windthrow site Tlen I, the change in instrument placement with time will be required to meet EC method requirements. As an alternative of a base construction for the EC system, an innovative infrastructure, i.e., "mini-tower" was proposed here: an anchor aluminum construction (1.5-m-high rectangular truss (W x L) 1 x 1.2 m) with a mast (triangular truss 30 x 30 x 30 cm) moving inside the structure along steel rails, powered by an electric motor.*"- Please write the text in the imperative tense. Any text that cannot be written in the imperative tense may be added as a "NOTE".

Authors: This section is given as a NOTE, not a step, in the revised version of the manuscript. The whole step: "*Site location and instrumentation setup*" has been reorganized in order to contain only imperative tenses ad required.

Editor: JoVE policy states that the video narrative is objective and not biased towards a particular product featured in the video. The goal of this policy is to focus on the science rather than to present a technique as an advertisement for a specific item. To this end, we ask that you please reduce the number of instances of "EddyPro" within your text. The term may be introduced but please use it infrequently and when directly relevant. Otherwise, please refer to the term using generic language.

Authors: We have complied with this suggestion and "EddyPro" is mentioned once in the Protocol and then in Discussion always as an example. In the main body, we now use the term "EC fluxes calculation software" or similar.

Editor: "Once all previous details are correctly entered, go to "Processing options" tab and check or choose raw data processing settings"- Please specify which method is selected. Or Please describe how.

Authors: In this step (currently 2.4.) the Editor has mentioned several times to be more specific on which methods was used or how was it performed. All needed details are now given in the respective sub-steps

Editor: In regard to step headings: "*Unfavorable weather conditions and instruments malfunctions*"; "*Appropriate conditions for eddy covariance method application*"- Please rephrase it to provide information what are being done in the following steps.

Authors: As suggested by the Editor these heading has been edited in order to reflect the actions which are included in each step. Currently, they are: "Determining unfavorable weather conditions and instruments malfunctions" and "Accounting for inappropriate conditions for eddy covariance method application", respectively.

Editor: *"Use instrument's performance indicators to filter out data subjected to errors due to gas analyzer contamination. For LI-7200 enclosed-path analyzer, check the average signal strength (ASS) value given in the output file from the EddyPro software and discard all fluxes measured below e.g., ASS = 70 % (10 % percent higher threshold than suggested in the instrument's manual)"* - Is this performed with the software? Please describe how to filter out data.

Author: To make it clear how was the filtering procedure performed we have added a few sub-steps before going into details, which are currently included in the step 3.3. as follows:

"*3.3. Create a common file (e.g.: .csv, .xlsx), which contains all results from fluxes calculations software and auxiliary measurements. Make sure that corresponding 30-min averages (fluxes and meteorological variables) were measured at the exact same time!*

NOTE: To simplify and speed up filtering procedure it is advisable to use additional programs e.g. Matlab or free R software, depending on users' skills, rather than work in MS Excel file.

3.3.1. Perform all filtering steps described below (3.4-3.6) on data from this file. Use either filtering tools in Excel (or embedded "if" function) or create your own filtering function utilizing other software (Matlab, R statistical software)."

Editor: Please update section/step number.

Author: All references to the section/steps numbers in the text were updated according to the new Protocol structure.

Editor: Referring to the sentence: "If the commonly used flag system 0-1-218 (high, intermediate and poor quality, respectively) was chosen, discard fluxes data with poor quality (flag values >1)"- >1 or ≥ 1? In the response letter, it is ≥ 1.

Author: In the last Rebuttal Letter we have explained: "AD 7: We admit, that it was simply a typing mistake, and it should have been: "discard fluxes data with poor quality (flag values >1)" instead of : "discard fluxes data with poor quality (flag values ≥ 1), which means that qc=0 and qc=1 were accepted.". We have checked this sentence and given information is the same as marked in red here.

Editor: "*There are few methods to define its limit value[19,20]. After defining u_{*thr}, either for the whole measuring period or individually for each season, remove all CO2 fluxes with corresponding u_* values (calculated together with fluxes in EddyPro software for each averaging interval) < u_{*thr} from the dataset*"- This is too vague to film. Please specify how to define u_{*thr} is calculated here.

Authors: We have revised this step and currently, this information is given in a NOTE and a simple approach on how to obtain u_{*thr} is given there instead as follows:

"*3.5.2. Use nighttime period indicator (daytime = 0), given in the output file, to filter out CO2 fluxes values measured at night.*

3.5.3. Plot all nighttime CO2 fluxes against corresponding friction velocity values (u measured at the same time) and find the u* value at which these fluxes stopped increasing.*

*3.5.4. Mark obtained value as friction velocity threshold (u*thr) to be further used as on the measures of insufficient turbulence conditions.*

3.5.5. Discard all CO2 fluxes with corresponding u values < u*thr from the dataset.*"

Editor: Regarding former step 3.3.1 (currently 3.6.1): "3.3.1. First, plot the wind rose, obtained on the basis of your own measurements or from the nearest meteorological station, on the map of investigated area (…)" - Is this done with a software? Please specify.

Authors: To clarify this issue we have added a one-sentence explanation here: "*Use your own method here or utilize ready functions from mathematical software (e.g. windRose function in R software)*"

Editor: Referring to the sentence: "*Choose method for quality-checked CO_2 fluxes gap filling and partitioning into absorption (Gross Primary Production- GPP) and respiration (Ecosystem Respiration- R_{eco}) from several approaches commonly used by environmental researchers.*" Are the GPP and Reco the methods selected here? Please make it clear.

Author: We have explained that GPP and R_{eco} are not METHODS but FLUXES values, which are the results of using gap filling and partitioning methods (FCRN and REddyProc).

Editor: *"Since the first two methods are widely used among scientific community, well described and discussed in the literature and in the case of the later, recommended to be used in FLUXNET and ICOS networks"* - It is unclear what the first two methods refer to. Please clarify; Please spell FLUXNET and ICOS out.

Authors: We are now specifying which methods were meant here (in the brackets). All abbreviations were spelled out.

Editor: Regarding the sentence: "*4.2. As an example of process-based approach, use the procedure from the Fluxnet Canada Research Network (FCRN[21,22]), following several steps:*" - Please specify how to carry out the following steps (4.2.1-4.2.5). Is a software used? Software steps must be more explicitly explained ('click', 'select', etc.). Please add more specific details (e.g., button clicks or menu selections for software actions, numerical values for settings, etc.).

Authors: In the following sub-steps of step 4.2 we have mentioned: "*Use any software which allows fitting non-linear functions to the data (e.g. Matlab software).*" Moreover, currently in the Discussion, this inconvenience was additionally stressed out as follows: "*The weakest point of the protocol is the gap filling and fluxes partitioning description. The two suggested methods were individually developed by other specialists before and only implemented here as proposed techniques. What is more, FCRN method requires much more contribution from the user since there is no ready tool to perform this procedure, unlike in the case of REddyProc software.*"

Editor: Does reference 23 contain the weblink? If so, please remove the weblink.

Author: We have moved the weblink to the References (currently reference 25) as suggested.

Editor: In reference to: "*4.5. Calculate daily, monthly and annual totals of all gap filled CO_2 fluxes: NEP, GPP, and R_{eco}, on the basis of which the changes of ecosystem functioning can be traced.*" - Please describe how these are calculated.

Author: This procedure is not included in any of the presented tools. It was performed with Matlab software, but such calculations can be made in any suitable way. Therefore, we have given such a suggestion here: "*Use your own function to aggregate these fluxes separately into the chosen time domain and sum up all values.*"

Editor: Please include all the figure Legends together at the end of the Representative Results in the manuscript text.

Authors: All Figures' legends were moved to the end of the Representative Results as suggested. Figure 2 (and its legend) was additionally edited in order to update steps numbers.

Editor: Regarding references to Figures in the Discussion: Please note that Figure 2 appears after Figure 3 and Figure 4 in the text. Please number the figures in order of appearance.

Authors: This comment refers to the appearances of references to respective Figures in the Discussion part, therefore we did not change Figures' numbers there. The order of Figures was already properly set in the Results part.