bio-protocol

Forest GPP Calculation Using Sap Flow and Water Use Efficiency Measurements

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[Abstract] This is a protocol to evaluate gross primary productivity (GPP) of a forest stand based on the measurements of tree's sap flow (SF), ^{13}C derived water use efficiency (WUE), and meteorological (met) data. GPP was calculated from WUE and stomatal conductance (g_s), the later obtained from SF up-scaled from sampled trees to stand level on a daily time-scale and met data. WUE is obtained from ^{13}C measurements in dated tree-ring wood and/or foliage samples. This protocol is based on the recently published study of Klein *et al.*, 2016.
Keywords: Trees, Ecosystem carbon flux, Transpiration, Stable isotopes

[Background] Forests play a major role in the terrestrial carbon cycle through CO_2 assimilation and respiration, as well as on the Earth climate by influencing atmosphere CO_2 concentration (Luyssaert *et al.*, 2007; Bonan, 2008; Canadell and Raupach, 2008; Reichstein *et al.*, 2013). Gross primary productivity (GPP), plants' carbon uptake through photosynthesis, is the ultimate source of organic material in land biosphere in general and in food production in particular.

GPP at the ecosystem scale is mainly derived using the eddy covariance (EC) technique, as the difference between EC-measured net ecosystem CO_2 exchange (NEE) and the daily inferred ecosystem respiration (Re). The latter one is obtained by extrapolating measured night-time NEE, which equals to ecosystem respiration, Re, to daytime based on empirical equations of Re response to temperature and soil humidity (Aubinet *et al.*, 2000; Baldocchi, 2003; Reichstein *et al.*, 2005; Grunzweig *et al.*, 2009). However, the EC approach has several limitations and uncertainties. Its application is limited to relatively large homogeneous and flat terrains. EC technique is critically dependent on factors such as the system deployment in field (*e.g.*, height above the canopy), on atmospheric conditions (*e.g.*, turbulence conditions, advections) (Aubinet *et al.*, 2000), on corrections applied to data processing programs and algorithms. A common way to assess EC measurements reliability is through the evaluation of the 'energy closure' over the measured ecosystem. This test indicates in most sites an energy gap of 20% or more (Foken, 2008). Empirical extrapolation of the night-time NEE measurement to approximate daytime Re has by itself significant uncertainties (*e.g.*, Van Gorsel *et al.*, 2009). NEE measurements provide a whole ecosystem flux only, thus explicit carbon uptake by trees cannot be distinguished from others ecosystem layers. EC measurements are also relatively expensive.

The current protocol describes an alternative method for calculation of forest trees GPP based on measurements of air temperature and humidity, trees' sap flow (SF) rate and intrinsic water use

efficiency (WUE$_i$, the ratio A/g$_s$), where A is the rate of photosynthetic CO_2 assimilation and g$_s$ is stomatal conductance. This protocol is based on the recently published study of Klein *et al.*, 2016. WUE$_i$ is a parameter characterizing plant species, with a seasonal variation (Seibt *et al.*, 2008; Klein *et al.*, 2013 and 2016). It can be calculated from the carbon isotope ratio (δ^{13}C) in the assimilated carbon of plant tissues (Farquhar and Richards, 1984). This is combined with sap flow measurements, which can be obtained easier and at a lower cost than EC measurements, and often have less spatial limitations (independent on ecosystem footprint, topography, homogeneity, *etc.*), and enable GPP estimation in complex ecosystems. The method is applicable to calculate GPP of woody vegetation and can be considered as total ecosystem GPP if the contribution of understory layer to the total ecosystem GPP can be neglected (*e.g.*, dry environments) or independently assessed. Note that using this approach can also be applied to archived SF and ^{13}C data to reconstruct past variations in GPP, as long as these data are available.

Materials and Reagents

1. Forest or woody stand, which can be assumed to be homogenous to facilitate scaling up to stand level
   Note: If stand is composed of considerably different plots and species, the method can be applied separately for each plot. The measurements are conducted on living trees. No reagents are used in this procedure.

Equipment

1. Tape measure of 2-3 m or caliper for tree diameter at breast height (approximately at 1.3 m, DBH, cm) distribution measurements
2. A total station theodolite or at least 30-50 m long tape-measure and compass for establishing a sample plot(s) with known area in order to get stand density
3. Sap flow sensors of any suitable type (*e.g.*, EMS Brno, model: THB sensors; or ICT International, model: HFD8-100; or UP, model: TDP sensors, *etc.*)
   Note: The type of sensors depends on tree diameter, financial possibilities, power supply options etc. The most simple, TDP sensors can be manufactured in the institute workshop (see description, e.g., in Lu et al., 2004).
4. Dendrometers of any type for obtaining of seasonal DBH growth curve (*e.g.*, EMS Brno, model: DRL26C or Natkon, model: Point Dendrometers [Oetwil am See, Switzerland])
5. Thermometer and air moisture meter or automatic meteorological station for continuous data recording
6. Datalogger for storing sap flow and met' data (if no own logger is provided by SF and met' sensors suppliers), *e.g.*, (Campbell Scientific, model: CR1000). Most of factory-made sap flow sensors are supplied with own loggers (*e.g.*, EMS Brno, Czech Republic)

7. Incremental borer. Short, 5.15'' borers would usually fit for obtaining wood formed in the recent decade or period of interest

8. Equipment for tree rings analysis

   Note: Best would be professional dendrometry desk station. Microtome can also help for intra-annual slicing. But the wider the tree-rings are, the easier it is to slice them with scalpel alone.

9. Laboratory for $\delta^{13}C$ analysis

   Note: This can be done in house isotope ratio mass spectrometry, or by commercial IRMS service labs.

Procedure

Note: The technology of SF measurements and up-scaling from measuring point to tree is not discussed in this protocol. The theory and practice of SF measurements is discussed, e.g., in Čermák et al., 2004; Lu et al., 2004; Tatarinov et al., 2005, as well as in user guides of particular SF sensors. Furthermore, it is assumed that continuous data of SF of single trees in L h^{-1} $tree^{-1}$ are available. Similarly, the protocol does not discuss $\delta^{13}C$ isotopic analysis of wood material.

1. Select representative sample plot, measure its area and DBH of all sample trees within the plot. For small trees or shrubs instead of breast height it is possible to measure diameter at lower level, *e.g.*, at 10 cm. In any case, diameter should be measured and SF sensors should be installed below the first living branch (for shrubs, when branching starts from the ground, each branch should be considered as separate stem). The plot size depends on woody plant species variability, stand density and DBH variability, with more sparse and variable stands requiring larger plot area. We recommend that the plot should include about 50-150 trees in order to get reliable DBH distribution. For multi-species stands the researcher should first evaluate the possible contribution of each species to the total stand GPP (*e.g.*, its proportion of the total stand basal area/LAI/crown area, taking into account, if possible, its photosynthesis rate relatively to other species, on the basis of data from literature or chamber measurements) before deciding how to distribute sensors among species (taking into account financial limitations of total amount of sensors). However, this is a special topic beyond the scope of this protocol. For more details about sampling size see, *e.g.*, Kish, 1965.

2. Select sample trees for SF measurement within the plot according to DBH distribution. Sample trees should be representative of the stand and include the entire DBH range. The total number of sample trees depends on the DBH range, stand species composition, desired precision and financial constraints. J. Cermak proposed to select sample trees using so called quantiles of total method, when total set of trees at sample plot is sorted according to a certain biometrical parameter B (usually DBH or basal area) in ascending order and cumulative B is calculated simultaneously. See details on sampling strategy, *e.g.,* in Čermák *et al.*, 2004 and 2014. Good description of sampling strategy is also given in ICT booklet Sap flow installation scenarios.

Install SF sensors on selected sample trees. Installation procedure is described in user guides of particular sensors. See more information, *e.g.*, in Čermák *et al.*, 2004 and 2014; Paudel *et al.*, 2013.

3. Install dendrometers on the trees which will be used for $\delta^{13}C$ sampling. The number of trees for $\delta^{13}C$ sampling depends on the level of variance in the plot. For statistical reasons, n = 30 is usually used. But often not all SF sensors are functional and hence we ended up using a lower number for our analysis. But 30 trees is a good recommendation. Optimally, one should use the same trees as for SF measurements, and these must represent the plot. If possible, stem growth should be monitored in all study trees. This is feasible using simple girth-tapes which hare monitored at least once a week.

4. Measure SF and met data continuously (1/2 hourly) during the study period. Meteorological data (air temperature, T_a, and moisture, RH) should be best measured in the middle of the canopy foliage layer.

5. Take plant material samples for ^{13}C analysis

   a. For further GPP calculation, SF and WUE_i values for the same time periods are required. Consequently, the time of formation of the plant organs samples for ^{13}C analysis should be known at the best time resolution (monthly or weekly, then the values should be interpolated in order to get daily values for the whole period of analysis), as it determines the time resolution of WUE_i calculation.

   b. Time resolution can be obtained in several ways.

      i. Wood samples should be taken from tree rings. Identifying the period of formation of specific tree-ring sub-sections could be allowed by calibration to an empirical stem growth curve from contemporary dendrometer data. Points along this curve (y-axis) were then applied to the observed growth curve to extract the estimated date of formation (x-axis) (Klein *et al.*, 2016, see Figure 1).

      ii. In some ecosystems, the period of stem growth can be lagged after the period of foliage growth. For periods when no stem growth occurs, but foliage or shoots growth takes place, leaf or shoot samples should be taken (see Figure 1). Leaf or needle samples can be dated in the same way as tree ring samples, by means of regular measurements of needles or leaves length (see Klein *et al.*, 2005).

      iii. If there is a dormancy period (*e.g.*, winter in boreal zone), no sampling is necessary, as no GPP occurred.
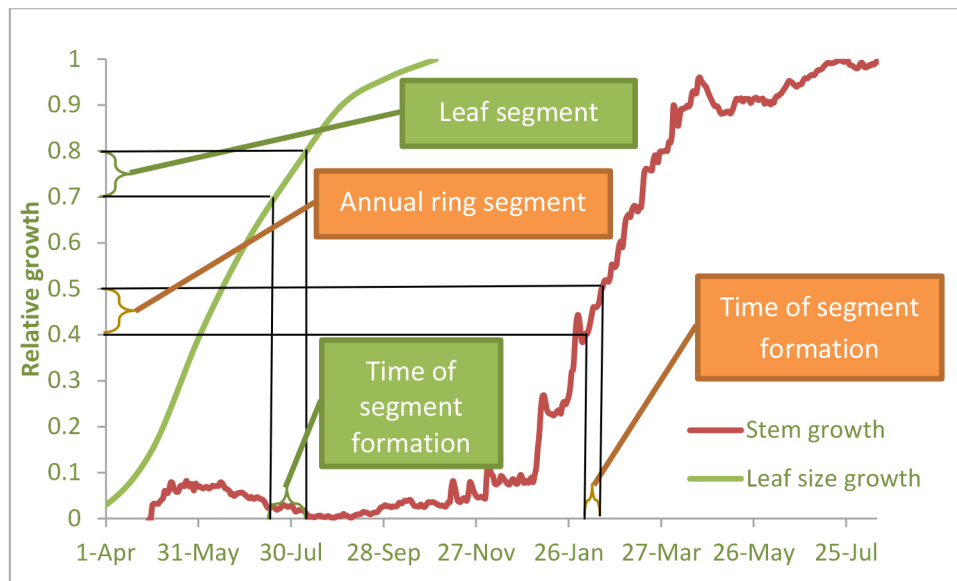
Figure 1. Scheme of high-resolution ring width development and leaf/needle elongation with time, and the sample slices dating. Example from Yatir semi-arid pine forest, Israel (see Maseyk *et al.*, 2008)

6. ^{13}C analysis should be made on the plant samples (tree ring sections and/or leaf samples) and attributed to particular time intervals.

Data analysis

1. The estimation of GPP is based on the well-established physiological relationship: $WUE_i = A/g_s$ (μM CO_2 mol^{-1} H_2O). Assuming that for a good approximation, stand assimilation - A_{stand} = GPP (in reality, A = GPP - R_L; where R_L is daytime leaf respiration and is usually a minor component of Re) and GPP is usually estimated using nighttime EC measurements extrapolated to the daytime (Reichstein *et al.*, 2005). We use the following main estimate for GPP at time period t:

GPP (t) = WUE_i(t)/g_s(t) (1)

which can be solved by obtaining independent estimates of WUE_i and g_s.

2. WUE_i is estimated from $\delta^{13}C$ measurements of organic material averaged from all samples taken at the same growing time interval t, using the following equation (adapted from Farquhar and Richards 1984; Seibt *et al.,* 2008):

$WUE_i(t) = C_a(t)/r \times \{[b - \Delta - pr \times (\Gamma^*/C_a(t))]/[b - a + (b - a_m) \times (g_s/(r \times g_i))]\}$ (2)

where,

C_a is the atmospheric CO_2 concentration in ppm (continuously measured on site, as it is not varying or taken as period average from the nearby station where it is measured),

r is the ratio of the diffusivities of CO_2 and water vapor in air (1.6),

a, a_m, b and p_r are the leaf-level discriminations against ^{13}C in the diffusion through the stomata (4.4‰), during dissolution and liquid phase diffusion (1.8‰), in biochemical CO_2 fixation (29‰), and in photo-respiratory CO_2 release (8‰), respectively,

Δ is the tree discrimination against ^{13}C,

Γ^* is the temperature-dependent CO_2 compensation point of *ca.* 30-45 ppm at our site (Maseyk *et al.,* 2008),

g_s/g_i is the ratio between stomatal and internal conductance to CO_2 respectively (0.5 according to Maseyk *et al.,* 2011). The values above are valid for plants in general, but they can vary, and the best approach would be to test it for the species of study or use best estimate for the most phylogenetically close species. The tree discrimination against ^{13}C (Δ) is calculated as follows:

$$\Delta = (\delta^{13}C_a - \delta^{13}C_o)/(1 + \delta^{13}C_o) \quad (3)$$

where, $\delta^{13}C = (\delta^{13}C/\delta^{12}C)_{sample}/(\delta^{13}C/\delta^{12}C)_{reference} - 1$ and the reference is Vienna-PDB (Coplen, 1994), and subscripts *a* and *o* stand for atmospheric air (at annual resolution) and the organic material (tree-ring or needle), respectively. WUE_i from Eq.3 is representative for the period of $\delta^{13}C$ signal deposition (depending on time resolution of plant sample formation dating; C_a should be averaged for the same period). WUE_i values for intermediate days between $\delta^{13}C$ measurements could be interpolated.

3. Stomatal conductance $g_s(t)$ for Eq.1 (as daytime average) can be calculated from measured stand transpiration and continuously monitored VPD(t) (average daytime, daily) using the general relationship (Beer *et al.,* 2009):

$$g_s(t) = T(t)/VPD(t) \quad (4)$$

T(t) was measured as sap flow (SF) of individual trees integrated over the daily cycle (to overcome possible time offsets between SF and T, which can reach up to a few hours, *e.g.,* under dry conditions) and up-scaled to stand level as described below. Vapor pressure deficit (VPD) can be calculated as follows (see, *e.g.*, Monteith and Unsworth, 2007):

$$VPD(t) = \left(1 - \frac{RH}{100}\right) \times 610.7 \times 10^{\frac{7.5 T_a}{(237.3 + T_a)}} \quad (5)$$

where, VPD(t) is in Pascals and RH (in %) and T_a (in °C) are air humidity and temperature, respectively. VPD is daytime averaged since CO_2 uptake is restricted to daytime hours only. If other conditions preventing CO_2 uptake for the particular species/region are known (too high or

too low VPD or air temperature *etc.*), the VPD records corresponding to such conditions should be also excluded from averaging.

As mentioned above, we assume that although a time offset between SF and T of up to few hours can occur on the daily scale, stand transpiration total T is equal to total daily stand sap flow total (SF_{stand}). As GPP is restricted to daytime, T should be restricted to same period, thus since water storage depleting and refilling acquired daily at a tree level, but night time T is negligible it is assumed that daily SF equals daytime T. Several possible SF up-scaling procedures from tree to stand level are available. (1) Dependence of tree SF on DBH can be derived by means of linear or nonlinear regression (where SF(t) is expressed in L tree^{-1} day^{-1}) for each particular day and then

$$T(t) = SF_{stand}(t) = \sum_k SF(t, D_k) \times n_k \quad (6)$$

where,

T(t) is expressed in mm day^{-1},

D_k and n_k are mean DBH of DBH class *k* and stocking density (trees ha^{-1}) of trees of this diameter class (Čermák *et al.*, 2004). (2) Another option is to calculate sap flux density per unit sapwood cross-section area averaged among sample trees, $\overline{SFD(t)}$ (in cm^3 cm^{-2} day^{-1}) and then multiply it by total stand sapwood area ($\sum SWA$, in cm^2 ha^{-1}, see Klein *et al.*, 2014):

$$SF_{stand}(t) = \sum SWA \times \overline{SFD(t)} \quad (7)$$

In addition, an evaluation of sapwood depth of the particular tree species is required, which can be measured separately or taken from the literature (see, *e.g.*, Cermak and Nadezhdina, 1998; Gebauer *et al.*, 2008). Then *SWA* of a particular tree can be calculated as follows:

$$SWA_{tree} = \frac{\pi}{4}((BH - 2d_b)^2 - (DBH - 2 \times (d_b + d_{SW}))^2) \quad (8)$$

where, d_b and d_{SW} are bark and sapwood widths, respectively. Then stand *SWA* can be calculated as the sum of sapwood areas of all trees on the sample plot divided by plot area.

We recommend to use the first method if there is expressed dependence of SFD on DBH and the second one if such dependence is missing (Tatarinov *et al.*, 2015).

Finally, substituting WUE_i from Eq.2 and g_s calculated in daily scale by Eq.4 using Eqs. 5-8 into Eq.1 gives us daily GPP total.

The total analysis scheme is presented in Figure 2. An example of the protocol application is described by Klein *et al.*, 2016.
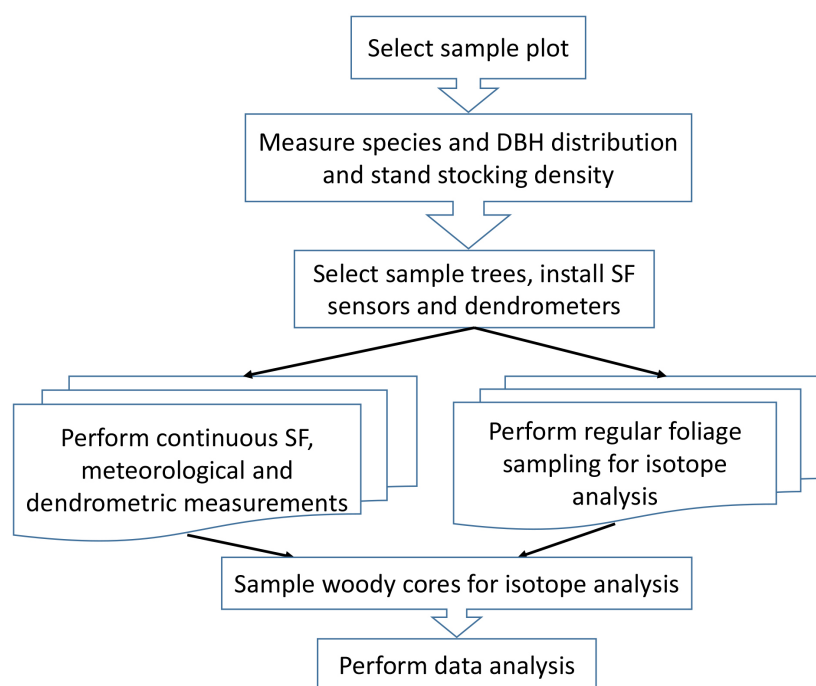
Figure 2. Diagram of the protocol of forest GPP Calculation Using Sap Flow and Water Use Efficiency

Notes

1. Weaknesses and uncertainties

    a. Sap flow measurements have their own sources of uncertainties related, *e.g.*, to sapwood depth, SF radial profile, SF variation around stem, changing of sapwood physical properties with shrinkage/swelling *etc.* Preliminary information of sapwood depth is usually necessary for sensors installation. These data are generally species-specific and can be found in the literature. The problems and tips of sap flow measurements are discussed, *e.g.*, in Čermák *et al.*, 2004 and 2014; Paudel *et al.*, 2013.

    b. The method is applicable for plants where sap flow sensors can be applied, *i.e.*, mainly woody plants. Generally, it could be fully grown trees, but also young trees or shrubs, but this may require particular type of sensors (see, *e.g.*, at http://emsbrno.cz/p.axd/en/Sap.Flow.small.stems.html). The method is not applicable for grasslands and small shrubs. Whereas in the forests with negligible understory layer the method should give total ecosystem GPP evaluation, in the presence of significant understory layer the result should be interpreted as GPP of trees only.

    c. Another limitation of the method concerns the time of formation of plant material used for the ^{13}C analysis (which depends on the temporal resolution of measurement plus the slicing resolution and using standard techniques is weekly at best). The method is inapplicable for periods with no detectable growth of woody tissues or foliage observed.

d. Additional source of uncertainty is the variability of T_a and *RH* within canopy, both, vertically and radially (with the distance from leaf surface), which influences VPD estimation. Generally, VPD at the leaf surface should be somewhat lower than VPD in the canopy air, which could lead to a certain overestimation of g_s and consequently to the underestimation of GPP.

e. Finally, the possible errors due to approximation of GPP = A_{stand} should be assessed (which usually are negligible).

Acknowledgments

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