Field indicators and monitoring protocols to assess forest ecosystem services (FES): climate regulation, water cycle regulation / water provision, and biodiversity

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Field indicators and monitoring protocols to assess forest ecosystem services (FES): climate regulation, water cycle regulation/water provision, and biodiversity

LIFE CLIMARK project report

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Introduction

Multifunctional management of forest ecosystems requires the availability of protocols and methods to assess the achievement of the target objectives and evaluate potential non-desired impacts. Data collection through these methods and their analysis will allow to verify empirically the sustainability of forest management and the consequences of newly implemented management strategies. Therefore, it emerges the necessity to define standard methods to measure and monitor basic forest ecological functions through a set of selected indicators and measurements. Indicators are quantitative or semiquantitative variables to quantify at stand or landscape levels short- and long-term effects of forest management on key ecosystem services components such as C stocks, water balance or biodiversity and their resistance and resilience in front to climate change factors (e.g. wildfire, drought or pests). An indicator should be a measure based on verifiable data that conveys information about more than itself (Biodiversity Indicators Partnership, 2011). They can be used for diagnoses and for checking the effectiveness of forest management practices, which may help to choose suitable policies as well as to forecast future trends in forest functions. Therefore, successful indicators should provide information that support sound decision making and connect policy to science (Biodiversity Indicators Partnership, 2011).

This guide is intended to provide guidelines for recording several measurements and indicators with the objective to detect changes in the forest functionality, focused on carbon sequestration, water use efficiency and biodiversity. The method employed in a specific project will depend upon a number of factors, such as the objectives of the survey, the study scale, the forest type, and the resources available. Expected users include forestry managers (i.e. individual owners, companies and public administration) and scientists studying the environmental and management change effects on forests functioning. The value of measurements is limited by the method used and the sampling design chosen. Spatial and temporal variabilities require explicit attention to accurately quantify the property we are measuring. Users should adapt the recommendations to the specific circumstances and taking into account the local knowledge of forest conditions and management practices. As far as possible, two types of methods are proposed: 1) soft methods, of easy implementation, and 2) hard methods. Soft methods will allow to obtain relatively rapid information of the indicator variable range, while hard methods will serve to validate soft methods results in specific stands and check the temporal and spatial generalisation. The methods proposed can be complementary to better estimate an ecosystem services component.

References

Biodiversity Indicators Partnership. 2011. Guidance for National Biodiversity Indicator Development and Use. UNEP World Conservation Monitoring Centre, Cambridge, UK, 40pp

MacDicken KG, Sola P, Hall JE, Sabogal C, Tadoum M, de Wasseige C. 2015. Global progress toward sustainable forest management. *For Ecol Manag* 352: 47-56

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A. ES: Climate regulation - forest carbon cycle

Carbon sequestration has become a key issue for managers and researchers, as provides a mechanism that contributes to mitigate the effects of global anthropogenic CO_2 emissions. Indeed, it has been estimated that 166 Gt (34%) of the 480Gt of C emitted by anthropogenic activities (fossil fuel and land-use change related emissions) since the start of the industrial revolution had been absorbed by forest ecosystems until year 2000 (House et al, 2002), illustrating the potential role of forests in the mitigating strategies. Carbon accumulates in forests by absorbing atmospheric CO_2-C and assimilating it into the biomass. Then, it is transferred to the soil through the deadwood, the leaf- and root-litter and rhizodeposits. In the soil, organic matter is partially mineralised returning its C to the atmosphere, but a fraction remains in soil, where can be stabilised for several decades or even centuries.

The highest total forest C stocks are found in the oldest forest stands (Harmon et al, 1990; Smithwick et al, 2002), and forest thinning usually leads to a decline in C stocks, particularly in the aboveground biomass (Ruiz-Peinado et al, 2013; Marchi et al, 2018) and forest floor (Nave et al, 2010). So, one of the main challenges that is currently under debate (Bellassen & Luyssaert, 2014) is how to manage forests for C sequestration/conservation while maintaining or improving other ecosystem services (e.g. biodiversity conservation, water regulation, etc). The decision making should be supported by empirical data on the response of forest C pools to different management options under a range of different conditions.

The dominant C pools in forests are the aboveground biomass and soils, including organic layers, while deadwood (i.e. standing dead and fallen trees) and litter generally represents a little portion (about 6-8% each, Ravindranath & Ostwald, 2008) of the total forest C stock. Because the estimation of changes of all C pools requires an important economic and human effort, the goal should be to maximise the cost-effectiveness of the C inventory, that is to obtain the highest accuracy for the available resources (Ravindranath & Ostwald, 2008). When resources are limited, we should prioritise the most relevant pools, and choose less-demanding methods that provide an indication of the spatial or temporal trends.

Indicators to assess the impact of forestry on this ES are:

A.1. Indicator: Aerial Carbon Stock
A.2. Indicator: Soil Carbon Stock
A.3. Indicator: Carbon sink capacity
A.4. Indicator: Risk of Carbon emission
A.5. Indicator: Carbon Stock in products (substitution)

References

Bellassen V, Luyssaert S. 2014. Carbon sequestration: managing forests in uncertain times. Science 506: 153-155

Harmon ME, Ferrell WK, Franklin JF. 1990. Effects on carbon storage of conversion of old-growth forests to young forests. Science 247: 699-702

House JI, Prentice IC, Le Queré C. 2002. Maximum impacts of future reforestation or deforestation on atmospheric CO2. Global Change Biol 8: 1047-1052

Marchi M, Paletto A, Cantiani P, Bianchetto E, De Meo I. 2018. Comparing thinning system effects on ecosystem services provision in artificial black pine (Pinus nigra J. F. Arnold) forests. Forests 9: 188

Nave LE, Vance ED, Swanston CW, Curtis PS. 2010. Harvest impacts on soil carbon storage in temperate forests. For Ecol Manag 259: 857-866

Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Canadell JG, Ciais P, Jackson RB, Pacala SW, McGuire AD, Piao S, Rautiainen A, Sitch S, Hayes D. 2011. A large and persistent carbon sink in the world's forests. Science 333: 988-993

Ravindranath NH, Ostwald M. 2008. Carbon inventory methods handbook for greenhouse gas inventory, carbon mitigation and roundwood production projects. Advances in Global Change Research 29, Springer

Ruiz-Peinado R, Bravo-Oviedo A, López-Senespleda E, Montero G, Río M. 2013. Do thinnings influence biomass and soil carbón stocks in Mediterranean maritime pinewoods? Eur J For Res 132: 253-262

Smithwick EAH, Harmon ME, Remillard SM, Acker SA, Franklin JF. 2002. Potential upper bounds of carbon stores in forests of the Pacific Northwest. Ecol Appl. 12: 1303-1317

A.1 Indicator: Aerial Carbon Stock

A.1.1. C stock in living trees

Method

A.2.1 Non-destructive method using measured dimensions obtained at individual tree level (soft method)

The proposed method estimates the biomass of each individual from its estimated volume using species-specific allometries calibrated at the individual level (*). The method consists on:

1. to measure tree diameter and height. In adult forests, diameter will be measured at breast height (1,3m). Height will only be measured in adult stands, measuring the height of at least one representative tree for each Diameter Class. In regeneration phases where trees are < CD 5 at breast height, basal diameter (at 5cm from soil) will be measured. In regeneration phases of resprouting species, mean diameter will be measured at 5cm for the 3 biggest stems of each resprout. A minimum fixed diameter can also be established in homogeneus resprouting stands, to set the diameter that a stem must accomplish to be measured.
2. to apply species-specific allometric regressions to get individual biomass.
3. to sum up all the individual biomass in the sampling unit

(*) Species specific bulk density values and allometric regressions are available in the literature (e.g. Montero, 2005; Cáceres? et al. in preparation and references therein).

Plot arrangement

A fixed-area plot or fixed band transect can be used to estimate tree diameter in the target area. If the spatial variability is high a band transect can be more cost-efficient than a two-dimension plot. The sample unit depends on the size of the dominant species and it usually varies between 20-100 m^2. The number of units per target area depends on their variability and the required precision of the estimates. The sample unit can be placed randomly or systematically in the target area.

References

Cotillas. M. 2016. Aboveground and belowground biomes allocation paterns in two Mediterranean oaks with contrastin leaf hàbit: an insight into carbon stock in Young oak coppices.

Ibáñez JJ, Vayreda J, Gracia C (2002) Metodología complementaria al Inventario Forestal Nacional en Cataluña. En: Bravo F, del Río M, del Peso C (eds) El Inventario Forestal Nacional. Elemento clave para la gestión forestal sostenible. Ed. Ministerio de Medio Ambiente y Fundación General de la Universidad de Valladolid, Madrid, pp 67–77

Montero. G, et al.2005. Producción de biomasa y fijación de CO2 por los bosques espanyoles. [Madrid]: INIA

A.1.2. Indicator: C stock in shrubland and understory woody vegetation

Shrub-like plants constitute a major component of many terrestrial ecosystems. They account for the major part of the total aboveground C stocks in shrublands, encroached grasslands and woodlands. Woody understory can represent an important part of aboveground forest biomass in Mediterranean and temperate forests, specially under high light conditions and low stand density. In arid regions, fine branches and leaves of shrubs and small trees are the only forage available during most of the year. Finally, shrub-like plants are quantitatively and qualitatively a major component of the surface fuel of many fire-prone ecosystems and, as understory fuel layer, they contribute with their heat realease to the initiation and sustenance of crown fires.

Methods

While forest inventories are routinely made around the world, standard methods for measuring shrub or understory wood biomass are not well stablished. Aboveground of woody shrub-like formations can be assessed by direct or indirect methods (Ravindranath NH, Ostwald M 2010). Destructive sampling is the most precise method to estimate aboveground plant biomass. However, when the forest component assessed is larger (e.g. woodlands or shrublands) or highly variable in the space, harvesting techniques are time consuming and expensive (Bonham 2013 and references therein). Moreover, destructive methods are not appropriate when the study goal requires repeated sampling over time in the same unit. In contrast, predicting woody biomass from plant dimensions using allometric regressions are easier than destructive methods. These allometric methods are advantageous in that they are no destructive and can be applied to new sites by recording only plant dimensions. However, allometric regressions for shrub-like species are not common. Another important shortcoming is that allometric regressions can be site- and species specific. Moreover, it often occurs that species-specific equations are lacking for some of the species found in the target site. This is usually solved using allometries calibrated for morphologically or functionally similar species groups.

A.1.2.1. Destructive sampling (hard method)

This method requires an estimation of the variability in the target area (see Plot arrangement below) and a destructive collection of systematically placed representative units.
This destructive method involves:

1. to clip and collect to ground level all the plant material per species from small systematically located sample units
2. to separate in the field the clipped materials into different fractions per species. The fractions depend on the objectives of the study but for the water content correction it is necessary to weigh separately homogeneous fractions. leaves and branches with diameter < 0.6 cm; branches between 0.6 and 2.5 cm; trunks >5 cm diameter) per live and dead material and per species.
3. to take to the laboratory representative subsamples of each species and material fractions to obtain the fresh-to-dry ratio per each material and species by weighting the fresh sample and re-weighting after oven drying at 80 °C until constant weight.

2 b and 3. Alternatively, all the material can be separated per species and weighted in the lab after drying it at 80 ºC to constant weight.

4. to summing up the dry weight of all the materials per species in the sample unit.

Plot arrangement

The sample unit can be a circular or rectangular plot. The size depends on the dominant species and should be less than 20 m^2. The number of units per target area depends on the variability of the target area and the precision of the estimates required. If the variability is high

across destructive sampled microplots, this method may require many microplots to accurately estimate the biomass.
The sample unit can be placed randomly or systematically in the target area. In high variable areas, to reduce the cost, sampling units can be placed randomly in more homogeneous subareas. These subareas can be established after a visual estimation of cover and height and plant composition. The results derived from the destructive sampling units in each subareas can be easily upscaled using the proportion of each in the target area.
Timing of sampling
Before, just after management and several years after depending on the objectives of the study.

A.1.2.2. Non-destructive method using measured dimensions obtained at individual plant level (soft method)

The proposed method estimates the biomass of each individual from the estimated volume of each individual using species-specific bulk density values or allometries calibrated at the individual level (*). The method consists on:

1. to measure maximum and orthogonal crown diameters and maximum height of all the individuals in a sampling unit.
2. to get the cylindroid volume per each species individual.
3. to apply species-specific bulk density values or allometric regressions to get individual biomass (**).
4. to sum up all the individual biomass in the sampling unit.

(*) Species specific bulk density values and allometric regressions are available in the literature (e.g. De Cáceres et al. in preparation and references therein). If allometric relations are inexistent for one specific species, it is possible to apply a plant-type regression (see De Cáceres et al. in preparation) or to obtain specific regressions from the field. In the last case, the volume to biomass regressions can be derived, from about 20 individuals per species, measuring the same dimensions as above and cutting and weighting each individual separately. See A3.1. to convert fresh weight into dry weight. Size of sampled individuals should range between the minimum and maximum size of the species in the target area.
(**) The Medfuel package for R by De Cáceres (2018) facilitate the calculations of individual biomass using allometric regressions of species or species groups. Maximum and orthogonal diameters and height are required inputs for each species individual.

Plot arrangement
For non-destructive methods, a fixed-area plot or fixed band transect (narrow rectangular plot) can be used to estimate the cover and height of plants in the target area. If the spatial variability is high a band transect can be more cost-efficient than a two-dimension plot to estimate plant dimensions. The sample unit depends on the size of the dominant species and it usually varies between 20-100 m^2 for non-destructive methods. The number of units per target area depends on their variability and the required precision of the estimates. The sample unit can be placed randomly or systematically in the target area.

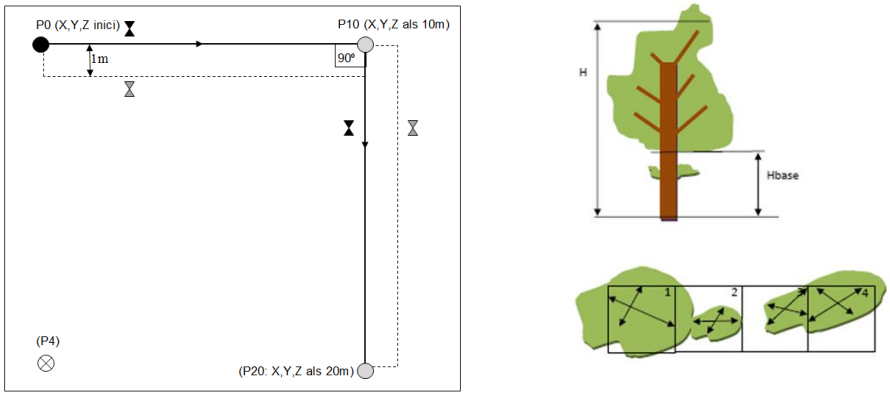

Figure 1. Two 90º-angle belt transects of 10 m length and 1 m width (20 m^2) (left) where to tall the height and crown dimensions of all shrub-like individuals (Right: H, height; Hbase, crown base height; crown diameters in the first four belt-transect meters). P0. Starting point; P10 and P20, reference points at 10 m and 20 m; X, Technicians.

Timing of sampling

Before, just after management and several years after depending on the objectives of the study.

A.1.2.3 Non-destructive method using visual dimension estimates obtained at stand level (soft method)

This method uses empirical relations to predict total woody biomass from visual estimates of total cover and mean height of woody species at the stand level. The biomass is derived using species-specific bulk density values or allometries calibrated at the individual level. This is an efficient method for estimating biomass of small trees and shrubs. The main disadvantages are the high variability and the difficulty to estimate the mean height by species at the plot level.

Specifically, this method consists on:

1. To visual estimate the cover and height of the woody species in the sampling unit.
2. to apply species-specific bulk density values or allometric regressions to get species biomass (*).
3. to sum up all the individual biomass in the sampling unit.

(*) The Medfuel package for R by De Cáceres (2018) facilitate the calculations of species biomass at plot level using allometric regressions of species or species groups. Mean cover and height are required inputs for each species in the sampling unit.

Timing of sampling

Before, just after management and several years after depending on the objectives of the study.

References

Bonham Ch. D. 2013. Measurements for terrestrial vegetation. John Wiley & Sons, 2º Ed. Oxford. UK. 246pp

De Cáceres M, Casals P, Gabriel E, Castro X. (in prep.). Allometric equations to predict stand fuel load in Mediterranean shrublands from individual or species-level data

Ravindranath NH, Ostwald M 2010. Carbon inventory methods: handbook for greenhouse gas inventory, carbon mitigation and roundwood production projects. Advances in global change research, vol 29. Springer, Heidelberg, p 308

A.2. Indicator: Soil Carbon Stock

A.2.1. C stock in soil organic horizons

The forest floor is composed by the organic layers consisting of undecomposed (litter) to completely humified organic matter that accumulates above the mineral soil in most forested ecosystems. Organic forest floor can be separated into different layers according to its decomposition status. Three organic layers are usually considered: The L (or Oi) corresponding to recently fallen leaves and fine branches (<1 year old); the F (Oe) layer corresponding to fragmented and moderately decomposed leaves and fine branches; and the H (Oa) layer, corresponding to highly decomposed organic material, in which it is difficult to recognize the original tissues and structures. Collecting separate samples of each organic layer (L, F, H) will allow a better understanding of the organic matter dynamics and increase the precision of C stocks estimates. The ability to separate each organic layer comes with practice but, even for experienced technicians, sometimes is difficult to completely separate F from L or H in the field. Therefore, it is recommended to sieve each layer in the laboratory to better classified organic florr into each layer.

The magnitude of C stocks in the forest floor is variable and may depend on the litterfall (Díaz-Pinés et al, 2011) and the litter quality (i.e. decay rates) (Vesterdal et al, 2008). But these organic layers can accumulate a remarkable percentage of soil organic C, and also can account for a significant portion of the total soil C accrual in the short and medium term. Given the high spatial variability of the forest floor organic matter accumulation, a relatively large number of samples or measurements are required to estimate the C pools with accuracy.

Sampling frequency

Given the high variability, and the large amount of C compared to the magnitude of changes, detecting changes in C stocks is challenging in the monitoring schemes. Hence, this should be sampled at least every 10 years, along with mineral soil sampling.

Plot arrangement

Fixed area plots of 100-800 m^2, depending of the heterogeneity observed.

Field sampling

Seasonal cycles of litterfall makes the amount of forest floor organic material, particularly the L layer, highly variable, hence for monitoring changes in forest floor C stocks all samplings should occur at the same time of year, preferably in late summer (Burton & Pregitzer, 2008). Samplings are made using frames of a specific size. The variance decreases as the area sampled increases (Fons et al, 1997). So the recommendation is using frames of at least 30 x 30 cm (Burton & Pregitzer, 2008).

Given the inherent high variability of the soil organic layers, at least 15 samples per plot should be taken to integrate all the variability. This size may be higher in forests with higher spatial variability. A systematic sampling design is recommended over a random sampling because it ensures a better coverage of this variability (Fons et al, 1997). In mature boreal forests, Kristensen et al (2015) found that a size of between 10 and 20 measurements in an area of 2000 m^2 provide unbiased estimates of C stocks with an accuracy of ±0.5 kg C m^{-2} (in sites where forest floor C stocks ranged between 1.7 and 3.3 kg C m^{-2}).

Following current trends in mitigation-related literature we have also included in the indicator "Soil C stocks" dead woody material lying on the forest floor. Generally, dead woody debris (DWD) is not a large C pool, but in some cases can be relevant, particularly in mature and old-growth forests. It is sensitive to forest management (Paletto et al, 2014) and hence highly variable. For instance, Ruiz-Peinado et al (2013) found that heavily thinned plots had stored 30% less C in dead woody debris than non-thinned control plots in Mediterranean pine forests.

To quantify the C stocks in downed woody debris (DWD) (i.e. downed trees and large branches), the variables of interest are total volume and mass of pieces of different size and decay classes. This, together with their C concentration will allow to estimate total DWD C stocks.

For sampling purposes, dead woody debris can be separated between coarse woody debris (CWD) and fine woody debris (FWD). A diameter of 10 cm and length of 1 m of dead trunk and branches can be used as a threshold between both debris types.

Sampling frequency

Every 5 years, unless we suspect a change in the woody debris density due to wind/snow damage or changes in forest management.

Methods

A.2.1.1. Systematic sampling of forest organic layer mass (hard method)

The proposed method consists in locating about 15 microsites in a systematic located at the intersections of a regular grid. Each microsite should be separated at least by about 3 m. Any site with detected disturbance (e.g. by wild boars) in the organic layer should be avoided. Each microsite should be marked adequately or coordinates recorded with a sub-metric GPS for future samplings.

In each microsite, L, F and H layers inside a frame of 30 cm diameter will be collected.

Prior to sampling, any live plant material inside the frame should be removed and discarded. Samples should then be oven-dried at 60ºC until constant weight.

Because it is not easy to discern the F and H layers in the field, we propose to sieve the H layer with a mesh size of 4 mm and consider the organic material above this size as F fraction. Fragmented, semidecomposed leaves and wood material eventually collected as L fraction should be also added to this F fraction. After drying and sieving, the three fractions (L, F and H) samples should be weighed, ground and analysed for organic C.

Figure 2. Circular frame (25 cm diameter) to sample forest floor organic layers

Calculation of C stocks

Carbon content in the soil organic layer (g m^{-2}) at each plot can be calculated multiplying the sample mass (kg) by its C concentration (g kg^{-1}) of each samples and dividing by the sample area (m^2). Calculate the mean value of all samples in the plot to estimate the C stocks of the plot.

A.2.1.2. Organic layer thickness to estimate C stocks of forest organic layers (soft method)

Alternatively, samplings can be done by taking multiple measures of the organic layer thickness. The measurement of thickness allows obtaining high resolution data at a relatively low cost. These measurements provide an indicator of C stocks, but they are not a direct measure of the C pool. But organic layer thickness is usually correlated with C stocks (Kristensen et al, 2015; Schulp et al, 2008).

Measurements in a grid design are preferable over linear transects to avoid the effect of the anisotropy (i.e. differences in the spatial structure in any direction) (Kristensen et al, 2015). The number of measurements should be at least 50, but higher numbers should be desirable in heterogeneous plots. At each point, the nearest 0.5 cm in the thickness should be recorded. The distance between measurements should be at least 3 m.

Recording the thickness of organic horizons together with sampling for C analyses would allow to find the relationship between C stocks and the thickness of the organic layers. This would allow to reduce the number of samples needed for C analysis, to have a better estimation of the spatial variability and, in turn would simplify and reduce the costs of future samplings (Kristensen et al, 2015). However, no relationships between organic layer thickness and mass is currently available for Mediterranean forests. In addition, the foreseeable high variability in bulk density of organic layers in managed or highly antrophic Mediterranean forests may constraint the applicability of this method.

References

Burton AJ, Pregitzer KS. 2008. Measuring Forest Floor, Mineral Soil, and Root Carbon Stocks. In: Hoover CM (Ed.) Field Measurements for Forest Carbon Monitoring. A Landscape Scale Approach. Springer

Díaz-Pinés E, Rubio A, van Miegroet H, Montes F, Benito M. 2011. Does tree species composition control soil organic carbon pools in Mediterranean mountain forests? For Ecol Manag 262: 1895-1904

Fons J, Sauras T, Romanyà J, Vallejo VR. 1997. Sampling strategies in forest soils. Ann For Sci 54: 493-499

Kristensen T, Ohlson M, Bolstad P, Nagy Z. 2015. Spatial variability of organic layer thickness and carbon stocks in mature boreal forest stands–implications and suggestions for sampling designs. Environ Monit Assess 187: 521

Schulp CJE, Nabuurs GJ, Verburg PH, de Waal RW. 2008. Effect of tree species on carbon stocks in forest floor and mineral soil and implications for soil carbon inventories. For Ecol Manag 256: 482-490

Vesterdal L, Schmidt IK, Callesen I, Nilsson LO, Gundersen P. 2008. Carbon and nitrogen in forest floor and mineral soil under six common European tree species. For Ecol Manag 255: 35-48

A.2.2. C stock in mineral soil

Mineral soil is one of the largest organic C pools in forests and a substantial fraction of it is relatively stable in the short term. Several studies indicate that the rate of C accumulation in mineral is slow, compared with the C accretion in the biomass, because only a small fraction of plant-derived C is stabilised in the soil (e.g. Richter et al, 1999). Short-term changes in soil C occur in the relatively small light fraction, so detecting temporal changes in the bulk soil C is difficult. Hence monitoring bulk soil C stocks is only useful in long-term monitoring schemes.

Surface layers are more C-enriched than subsurface soil. As topsoil also contain most of the root system, it is also the most sensitive to changes in the C inputs and outputs caused by forest management. Moreover, organic C in subsurface soil is typically more stabilised and hence less prone to be lost after environmental or management changes. So sampling the topsoil could be enough to get an indication of changes in C stocks into the soil. However, if we want to quantify the actual whole-soil C stocks, sampling subsurface soil layers is necessary.

Sampling frequency for monitoring

For bulk soil organic C, Smith (2004) indicated that assuming a detection power of 3%, the effect of a 15% increase in C inputs will require 10.1 years to demonstrate a change in soil organic C in forest soils. In soils where increase of C inputs is less than 15%, detecting such changes will require a huge number of samples and long time between samplings (Smith, 2004), which is usually impracticable. Hence, for monitoring changes in bulk mineral soil organic C, it should be sampled at least every 10 years.

Plot arrangement

Ellert et al (2002) indicated that, instead of sampling on large plots, for detecting temporal changes in C storage, collecting volumetric soil cores from microsites is more effective.

Methods

A.2.2.1. Systematic core sampling of top soil for C stock assessment (hard method)

This method proposes to take up soil samples in 15 permanent sampling microsites systematically distributed in a fixed-area plot of about 100-800 m^2. I would be worth sampling at the same microsites where forest floor material is sampled. Each microsite should be marked adequately or coordinates recorded with a sub-metric GPS for future samplings.

Fig.3. Mineral top soil sample

Field sampling and measurements

In each microsite, a soil core of 5 x 5 cm and at least 20 cm depth is taken up and divided by depths at the following intervals: 0-5 cm, 5-15 cm and >15 cm. Samples are dried (60ºC for 48 h) and 2-mm sieved. Fine earth is fine grounded and organic C and total N analysed. If we are not interested to assess spatial C variability, samples from several cores of the same depth can be composited for chemical analysis.

Calculation C stocks

Soil organic carbon stocks (SOCS, kg m^{-2}) are calculated as the sum of the content of each soil layer, using the organic C concentration, the measured bulk density and the stoniness of each sample, as follows:

$$SOCS = \sum_{n}^{i=1} OC_i \times (BD_i - CP_i) \times th_i$$

where *i* represents each sampled layer, *OC* is the organic C concentration (%) in the fine earth (< 2 mm), *BD* is the bulk density (kg m^{-3}), *CP* are the coarse-particle (> 2mm) content (kg m^{-3}), *th* is the thickness (m) of each layer.

The organic C stocks of the plot is expressed as the mean SOCS of the four microsites (in Kg m^{-2}), usually referred to a specific depth.

A.2.2.2. Whole-profile soil C stocks (hard method)

When the quantification of whole-profile soil C stocks is necessary, a soil profile should be opened in order to take representative samples of the mineral soil horizons. Soil profile description is also important to estimate water retention and availability. If the objective is to know the C stocks in the stand, it is necessary to determine the representability of the profile described. Because the high variability of organic and top mineral soil horizons, C stocks of these horizons should be better estimated using the previous methodologies (see A6 and A7.1).

The localization of the profile is subjective, but it must be apparently representative in terms of tree density and vigour, surface stoniness and stand slope and aspect. The profile should be open until bedrock or at least 1-m depth.

Soil profile should be described and sampled by trained personal. Horizon boundaries are defined by changes in soil penetrability, texture, structure, colour, and density of roots and stones. A metalic core (5 diameter x 5 depth) is used to estimate the bulk density of each horizon in the opened profile, which is needed to calculate the C stocks. Representative soil samples of each horizon should be taken to the laboratory for soil analysis. It is also needed to estimate the percentage of the soil volume that the coarse fragments and rocks occupy in order to correct the total profile C stocks.

The representability of the soil profile described in the landscape or stand can be assessed tacking soil samples at different depths, and comparing horizons in those cores to the profile description.

Fig. 4. Soil profile

Calculation C stocks

Soil organic carbon stocks (SOCS, kg m^{-2}) are calculated as the sum of the content of each soil horizon, using the organic C concentration, the measured bulk density and the stoniness of each sample (see A7.1 section).

References

Ellert BH, Janzen HH, Entz T. 2002. Assessment of a method to measure temporal change in soil carbon storage. Soil Sci Soc Am J 66: 1687-1695

Post WM, Izaurralde RC, Mann LK, Bliss N. 2001. Monitoring and verifying changes of organic carbon in soil. Climatic Change 51: 73-99

Richter DD, Markewitz D, Trumbore SE, Wells CG. 1999. Rapid accumulation and turnover of soil carbon in a re-establishing forest. Nature 400: 56-58

Smith P. 2004. How long before a change of soil organic carbon can be detected? Global Change Biol 10: 1878-1883

A.2.3. C stock in litter – dead fine woody debris (DFWD)

A.2.3.1. Method to estimate dead fine woody debris (DFWD) stocks (soft method)

Plot arrangement and measurements

Fine woody debris is estimated in 4 subplots of 2 x 2 m per plot. The location of these subplots are chosen randomly within the plots. All downed and dead woody debris between 2.5 cm and 10 cm in diameter within each subplot (e.g. Ruiz-Peinado et al, 2013), is collected and weighed in the field using a dynamometer. Representative subsamples are taken to estimate a conversion factor to calculate the dry weight in the lab (until constant weight at 80 ºC). Carbon content was analysed using these dry subsamples.

Carbon stocks of fine woody debris per subplot is calculated using the dry-to-fresh weight factor on the field weight and the C content per unit of dry weight.

A.2.4. C stock in dead wood – dead coarse woody debris (DCWD)

A.2.4.1 Method to estimate dead coarse woody debris (DCWD) stocks (soft method)

Plot arrangement

Fixed-area plots. The minimum required area depends on the DCWD density, but should be at least about 625 m^2 (e.g. 25 x 25 m quadrat). In open woodlands, with low DWD density, may require 2500 m^2 plots to have a precise estimation (Woldendorp et al 2004). Narrow rectangular plots (i.e. belt transects) are also adequate, analogous to fixed-area plots. It is particularly worth using this type of plots when DWD is sampled together with SDT.

Field sampling and measurements

At each plot we should measure all downed trees and branches with a minimum size of 10 cm diameter and 1 m length. Fallen trees are considered DCWD when the angle of lean from the vertical is more than 45º (Curtis, 2008). Branches above the 7-cm-diameter threshold size should be considered as separate pieces and measured similarly. If accuracy is important, we will measure the length and the diameter at the base, at the midpoint and at the upper end of each piece to calculate the three cross-sectional areas. A less-accurate alternative is measuring only the base and the upper end diameter. When the pieces are asymmetrical about the central axis, the maximum and minimum diameter should be recorded, to obtain a mean diameter and obtain a more accurate estimation of cross-sectional areas.

Each piece should be assigned to a one of the following decay classes (Kenshaw et al 2016):

Table 1: Decay classes and their characteristics (Di Cosmo et al, 2013)

Decay Class	Characteristics
1. Recently dead	Bark still attached. Small branches (diameter < 3 cm) present. Wood consistency intact. Fungus mycelium absent or poorly developed.
2. Weakly decayed	Bark is loose but not fragmented. Small branches are only partially present. Wood consistency intact but fungus mycelium under bark well developed. Presence of rotten areas but narrower than 3 cm
3. Medium decayed	Bark is fragmented. Small branches absent. Wood consistency reduced but log still has a hard core. Rotten areas wider than 3 cm.
4. Very decayed	Bark and small branches absent. Rotten throughout the log. Wood consistency compromised and the log is irregularly shaped under the effects of its own weight
5. Almost decomposed	Bark and small branches absent. Wood consistency is lost (dust). Rotten areas are large, the log is fragmented in sections and may be mossy.

Volume calculation

Volume of each DCWD piece is calculated according to the Newton's equation (Kershaw et al 2016):

$$V = \frac{L}{6}(A_b + 4A_m + A_u)$$

where L is the piece length, A_b is the cross-sectional area at the base, A_m is the cross-sectional area at the longitudinal midpoint and A_u is the cross-sectional area at the upper end.

In case of having only the A_b and A_u, volume is calculated according to the Conic-paraboloid equation, introduced by Fraver et al. (2007):

$$V = \frac{L}{12}\left(5A_b + 5A_u + 2\sqrt{A_b A_u}\right)$$

Carbon stocks estimation

Since the density of wood of DCWD by species and decay classes can be laborious and time consuming, we can use published density values for conifers and broadleaves, such as those provided by Di Cosmo et al (2013) (Table 5.2).

Table 2. Mean density and C concentration of DWD by decay classes provided by Di Cosmo et al (2013) and Harmon et al. (2013) respectively

Decay classes	Density (kg m^{-3})		C concentration (%)	
	Conifers	Broadleaves	Conifers	Broadleaves
1	411.5	513.0	49.3	47.9
2	389.8	468.6	49.6	47.7
3	342.0	444.3	50.4	47.9
4	295.4	344.0	52.1	47.4
5	265.9	255.1	53.6	46.2

The volume of each piece and their estimated density for each decay class and species allow to calculate their mass. This should be converted to C stock (Kg C) at each piece applying the C concentration measured at the corresponding decay class and species. Total C stocks should be expressed in Mg ha^{-1}, by dividing total C stocks by surface area of the plot.

A.2.4.2. Alternative method to accurate estimation of woody density and C stocks of coarse debris (hard method)

For an accurate estimation, wood density of each decay class and species can be estimated in the lab. Given that heartwood to sapwood proportions change with log size, taking samples of at least two diameter classes (10-20 cm and >20 cm) will increase the precision of the estimate. Four representative samples of each recorded decay type, species and size should be taken to estimate their C content and density.

Wood samples should be oven-dried at 65ºC, weighed and its volume estimated by the water displacement method. Then, a representative dried subsample should be ground and analysed for C concentration. It is assumed the density and C concentration for each decay type and species is relatively constant. So, once we have densities and C concentrations of each species and decay type, it can be applied for all the plots and samplings.

References

Curtis PS. 2008. Estimating Aboveground Carbon in Live and Standing Dead Trees. In: Hoover CM (Ed.) Field Measurements for Forest Carbon Monitoring. A Landscape-Scale Approach. Springer, pp 39-44

Di Cosmo L, Gasparini P, Paletto A, Nocetti M. 2013. Deadwood basic density values for national-level carbon stock estimates in Italy. For Ecol Manag 295: 51-58

Fraver S, Ringvall A, Johnson BG. 2007. Refining volume estimates of down woody debris. Can J For Res 37: 627-633

Kershaw Jr. JA, Ducey MJ, Beers TW, Husch B. 2016. Forest Mensuration. Fifth Edition. Willey Blackwell

Paletto A, De Meo I, Cantiani P, Ferretti F. 2014. Effects of forest management on the amount of deadwood in Mediterranean oak ecosystems. Ann For Sci 71: 791-800

Ruiz-Peinado R, Bravo-Oviedo A, López-Senespleda E, Montero G, Río M. 2013. Do thinnings influence biomass and soil carbon stocks in Mediterranean maritime pinewoods? Eur J For Res 132: 253-262

Woldendorp G, Keenan RJ, Barry S, Spencer RD. 2004. Analysis of sampling methods for coarse woody debris. For Ecol Manag 198: 133-148

A3. Indicator: Carbon Sink capacity

Not applicable at this stage – Developed in Action D2

A.3.1. Tree growth in the last 10 years

A.3.2 Cork growth in the last 5 years

A.4. Indicator: Risk of Carbon emission

Not applicable at this stage – developed in Action C5

A.4.1. Annual probability of fire

A.4.2. Fire intensity

A.5. Indicator: Carbon stock in products

Not applicable at this stage – developed in Action D3

C.5.1. C Stock in harvested wood

C.5.2. C Stock in harvested biomass/firewood

C.5.3. Carbon Stock in harvested Cork

B. ES: Water cycle regulation and water provision

The ongoing global warming is expected to affect terrestrial ecosystems by influencing the frequency, severity and spatial extreme weather events ((IPCC, 2014). Extreme events can modify runoff and streamflow of forested landscapes, and affect the balance between blue water (the water ultimately going to streams and lakes) and green water (the part that flows through the plant before returning to the atmosphere, hence contributing to vegetation growth). However, large uncertainties exist on how forests will respond to such changes. In addition, forest stand simulations suggest that management, particularly moderate or heavy thinning intensities, can mitigate climate change effects on stand productivity and tree water status (Ameztegui et al., 2017).

The assessment of forest water balance can be obtained using a range of approaches differing in the target objective, such as watershed, stand or tree levels, the spatial and temporal resolution and the amount of information required.

Indicators to assess the impact of forestry on this ES are:
B.1. Indicator: Water use efficiency (WUE)
B.2. Indicator: Water quantity (provision)
B.3. Indicator: Water quality

B.1. Indicator: Water use efficiency (WUE)

Both the exchange of water and CO_2 in the leaves occurs through the stomata. So the uptake of CO_2 inevitably leads to loss of water vapour. Water use efficiency (WUE) refers to the amount of water lost during the fixation of CO2 in photosynthesis. So, it is defined as the ratio between carbon gain by photosynthesis and water loss in transpiration. However, in order to be comparable, the ratio between photosynthesis and leaf conductance for water vapour (intrinsic water-use efficiency, iWUE) is used instead.

The C-isotope approach to estimate the iWUE relies on the fact that photosynthetic enzymes discriminate against the heavier C isotope (^{13}C) during the photosynthesis, resulting in a depletion of ^{13}C in leaves compared with that in the atmosphere (Ca). But such discrimination against ^{13}C depends on the CO_2 concentration in the stomatal cavity (Ci). When this is low relative to that in the atmosphere, the ability to discriminate against it declines, resulting in greater proportion of ^{13}C fixed. Physiological measurements showed that Ci/Ca ratio decreases and iWUE increases with decreasing water availability as a result of stomatal closure (Lambers et al., 2008). Therefore, $\delta^{13}C$ is lower in drought conditions as a result of decreased gs and consequently Ci.

Changes in the CO_2 concentration in the stomatal cavity can be caused by the changes in both photosynthetic activity and stomatal conductance. On the other hand, assuming the same water source, the $\delta^{18}O$ is related to the evaporative loss of water, with an ^{18}O enrichment of leaf water with transpiration, which is unaffected by photosynthetic activity.

Variations in $\delta^{13}C$ can be also caused by fluctuations in other environmental factors such as light and nutrient availability (Ehleringer et al., 1986).

B.1.1. Water Use Efficiency at tree level

Methods

B.1.1.1. Tree WUE estimates based on tree-ring stable isotopes analysis

Trees rings contain a long-term archive of growth rates. Together with tree ring growth, the variation in stable isotopes composition is used for a retrospective analysis to assess the responses of trees to environmental variability or changes in water competition due to management practices. The simultaneous analysis of the two stables isotopes ($\delta^{13}C$ and $\delta^{18}O$) on the same sample annual growth rings and the mechanistic interpretation (i.e. dual isotope approach, Roden and Farquhar 2012) allows to differentiate between the effects of the photosynthetic activity (A) and stomatal conductance (gs) on WUEi. For instance, an increase in $\delta^{18}O$ over time is an indication of decreasing gs. The decreasing $\delta^{13}C$ (increasing of A/gs) would then be the result of this decreasing in gs and not an increasing A. The absence of an increase in diameter growth is consistent with a lack of increasing photosynthesis.

Plot arrangement

Fixed area plots with environmental conditions as homogeneous as possible.

Timing of sampling

Measurements will be taken at the end of the project to retrospectively analyses basal area increments and obtain stable isotopes ratios of specific tree rings.

Field sampling and sample processing

Wood core samples are usually collected during the growth dormancy. Samplings should be always done on individuals of similar size and crown status to avoid size-related differences in the isotopic response to climate conditions (Brienen et al, 2017). Sampling 4 – 5 trees of each species, age and crown status is generally enough to capture a representative isotope record of a site (Leavitt, 2010). Sampling is done by extracting cores at a specific height (e.g. breast height, or tree basis) with an increment borer (diameter 5 mm). It is recommended to take two cores per each tree. Cores are prepared following standard dendrochronological techniques (Stokes and Smiley 1968). Cores were after air dried in the laboratory. Tree rings width is measured to the nearest 0.01 mm using a LINTAB measuring system coupled to TSAP tree-ring software (Frank Rinn, Heidelberg, Germany). All cores were visually cross-dated by comparing characteristic narrow rings present in all trees to detect any false or missing rings. Within each class, cross-dating was validated using the COFECHA software (Holmes 1983), which calculates cross-correlations amongst individual series of tree growth.

For isotopic analysis wood cores are carefully separated into sections corresponding to specific growth years using a razor blade under stereomicroscope. The whole ring can be used in the analyses, but early and late wood can also be separeted for specific growth research questions. Ring wood is grounded with a mill. Cellulose extraction from tree rings is recommended for isotope analyses for determining climate signals (Ferrio and Voltas 2005, Battipaglia et al. 2008). However, it has also disadvantages such as the labour intensive and costly laboratory procedure, which may limit the number of samples that can be processed. In addition, larger samples are also required. Recent studies have shown that the use of whole-wood isotope values is justified for ecophysiological and dendrochronological studies that analyze the response of trees to environmental changes recorded within the sapwood, i.e. in a relatively short-term period (Weight et al. 2015).

For $\delta^{13}C$ and $\delta^{18}O$, ground samples are analysed using a Continous Flow Isotopic Ratio Mass Spectrometer (IRMS).

Calculation of iWUE:

For comparative studies, it is recommended to calculate the intrinsic WUE, which excludes the effect of differences in water vapour pressure on transpiration rates (Pérez-Harguindeguy et al, 2016). The interpretation of isotope changes on photosynthesis rates (A) vs. stomatal conductance (g) can be infered from the conceptual model of Scheidegger et al., (2000).

B.1.2. Water Use Efficiency at stand level

A way of estimating water balance at stand level is by using process-based models that, given some meteorological, edaphic and vegetation data, are able to predict temporal variations of soil moisture and plant drought stress (e.g., Granier et al., 2007; Lafontet al., 2012; Ruffault et al., 2013). When coupled with climatic projections, mechanistic models allow to anticipate future drought stress impacts and the potential effects of different forest management options on the water balance of the forest stand.

Methods

B.1.2.1. Medfate water balance simulation

The "medfate" model is a process-based model on forest inventory plots can be used to obtain species-specific estimates of drought stress at landscape and regional scales. Details of the design and specific formulation of the "medfate" water balance model is given in De Caceres et al. (2015). The model is implemented in C++ and is executed from an interface written in R language. An R package is available upon request to Miquel De Cáceres. The state base of the model is adapted for its use in combination with forest inventory data, whereas the complexity in terms of processes is kept very simple to reduce the number of parameters and facilitate its application to different areas. The water balance model predicts temporal variations in soil water content and assess drought stress for plants in a forest stand. The model calculates water balance on a daily basis. Soil is represented using two layers – topsoil and subsoil – and the model keeps track of the proportion of moisture relative to field capacity for each layer. Soil water holding capacity includes the effects of rock fragment content. Vegetation is represented as a set of plant cohorts having different height, root distribution, species identity and leaf area index (LAI; i.e., the one-side area of leaves corresponding to the cohort per unit of stand surface area). The root system of each cohort is described by the vertical distribution of its fine root biomass. The minor fraction of root mass located below soil depth isredistributed within the existing layers and the proportion of fine roots in each soil layer is assumed proportional to the amount ofwater extracted from it.

References

Ameztegui, A., Cabon, A., De Cáceres, M., Coll, L., 2017. Managing stand density to enhance the adaptability of Scots pine stands to climate change: A modelling approach. Ecol. Modell. 356, 141–150.

Battipaglia G, Jäggi M, Saurer M, Siegwolf RTW, Cotrufo MF. 2008. Climatic sensitivity of 18O in the wood and cellulose of tree rings: results from a mixed stand of Acer pseudoplatanus L. and Fagus sylvatica L. Palaeogeograph Palaeoclimatol Palaeoecol. 261:193-202.

Brienen RJW, Gloor E, Clerici S, Newton R, Arppe L, Boom A, Bottrell S, Callaghan M, Heaton T, Helama S, Helle G, Leng MJ, Mielikäinen K, Oinonen M, Timonen M. 2017. Tree height strongly affects estimates of water-use efficiency responses to climate and CO2 using isotopes. Nat Commun 8: 288

De Cáceres, M., Martínez-Vilalta, J., Coll, L., Llorens, P., Casals, P., Poyatos, R., Pausas, J.G., Brotons, L., 2015. Coupling a water balance model with forest inventory data to predict drought stress: the role of forest structural changes vs. climate changes. Agric. For. Meteorol. 213, 77–90.

Farquhar GD, O'Leary MH, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intracellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9: 121-137

Ferrio JP, Voltas J. 2005. Carbon and oxygen isotope ratios in wood constituents of Pinus halepensis as indicators of precipitation, temperature and vapour pressure deficit. Tellus. 57:164-173.

Holmes RL 1983. Computer-assisted quality control in treering dating and measurement. Tree ring Bulleting. 43:69-75.

IPCC, 2014. Climate Change 2014: Impacts, Adaptation and Vulnerabilities. Working Group II contribution to the Fifth Assessment report of the Intergovernmental Panel on Climate Change. UK and New York, NY, USA.

Leavitt SW. 2010. Tree-ring C-H-O isotope variability and sampling. Sci Total Environ 408: 5244-5253

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter I, Wright IJ, Ray P, Enrico L, Pausas JG, de Vos AC, Buchmann N, Funes G, Quétier F, Hodgson JG, Thompson K, Morgan HD, ter Steege H, van der Heijden MGA, Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino S, Cornelissen JHC. 2016. New handbook for standardised measurement of plant functional traits. Aust J Bot 61: 167-234

Roden JS, Farquhar GD. 2012. A controlled test of the dual-isotope approach for the interpretation of stable carbon and oxygen isotope ratio variation in tree rings. Tree Physiol. 32:490-503.

Scheidegger Y, Saurer M, Bahn M, Siegwolf R. 2000. Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. Oecologia. 125:350-357.

Stokes MA, Smiley TL. 1968. An introduction to tree-ring dating. University of Chicago Press, Chicago, Illinois, USA

Weigt RB, Bräunlich S, Zimmermann L, Saurer M, Grams TE, Dietrich HP, Siegwolf RTW, Nikolova PS. 2015. Comparison of δ18O and δ13C values between treering whole wood and cellulose in five species growing under two different site conditions. Rapid Communications in Mass Spectrometry. 29: 2233-2244.

B.2. Indicator: Water quantity (provision)

B.2.1. Water infiltration + Runoff at stand level

B.2.1.1. Medfate water balance simulation – Infiltration + Runoff (see B.1.2.1)

B.2.2. Water infiltration + Runoff at micro-watershed level

Not applicable at this stage of the project – developed in Action D2

B.2.2.1. Water runoff and infiltration monitoring (using control sites or twin watersheds)
B.2.2.2. Water runoff and infiltration monitoring (monitoring changes in long data series)

B.3. Indicator: Water quality

B.3.1. Risk of forest loss - Assessed through indicator C.3

C. ES: Biodiversity

Biodiversity refers to the total specific, taxonomic or genetic richness contained in the nature or a part of it (Wilson, 1988). Biodiversity complements the concept of diversity (more focused to understand ecosystem dynamics –Margalef, 1997) and refers to the stock of available genetic material that is processed by ecosystem functioning and returned, modified or not, to the store of biodiversity (Margalef R, 1997). Although the debate still exist, species biodiversity or richness are frequently considered as desirable property of any ecosystem based on the "insurance" effect of a potential replacement of one species and its function by another species in high species-rich communities. Alternative to species richness, functional biodiversity focuses on the diversity of potential responses to different ecosystem perturbations or stresses.

Indicators to assess the impact of forestry on this ES are:
C.1. Indicator: Real biodiversity
C.2. Indicator: Potential biodiversity
C.3. Indicator: Risk of forest loss

C.1. Indicator: Real biodiversity

C.1.1. Animal biodiversity

C.1.2. Plant biodiversity

Plant biodiversity description requires three considerations: capacity to identify plant species; how to measure species abundance; the basis of sampling. For specific purposes, working at species level is not necessary and, therefore, grouping species into functionally similar groups that share ecological traits and play similar roles in the community may be more suitable to describe vegetation for management purposes.

Plot size and arrangement

The basis of sampling for physiognomic or floristic description is rarely discussed by it is a major criticism of the whole approach (Kent, 2012). The description is usually made in an area that the researcher considers to be typical or representative of the total stand.

Plot size depends on the type of vegetation, the size of the plants and their arrangement pattern (e.g. clustered, random or regurlar). The plot size varies from one type of vegetation to another and it is not easy to determine the minimal area to be sampled to record all the plants in the stand. The minimum plot size could be determined by methods to assess the species–area curves (see method C2.2.). Stohlgren et al. (1995) accurately analyses different methods and potential problems. Alternatively, Table 3 can be used as a reference of the optimum size for different vegetation types, but it should not be universally appropriate for every situation.

Table 3. Suggested quadrat sizs for certain vegetation types for recording plant composition (Kent, 2012).

Vegetation type	Quadrat size
Bryophyte and lichen communities	0.5 x 0.5 m
Grasslands, dwarf heaths	1 x 1 m – 2 x 2 m
Shrubby heaths, tall herbs and grasslands	2 x 2 m – 4 x 4 m
Scrub, woodland shrubs, small trees	10 x 10 m
Woodland canopies	20 x 20 m – 50 x 50 m

Timing of sampling

Before, just after management and several years after depending on the objectives of the study.

Methods

C.1.2.1. Braun-Blanquet plant inventory

The methodology of the Braun-Blanket school of vegetation classification is the most common approach to inventory vascular plant biodiversity in Europe.

This method consists on listing all the vascular taxons in the plot and record a visual estimation of the cover of each species. Cover is defined as the percentage area of the ground that is occupied by the aboveground parts of each species when viewed from above. Multiple layering of vegetation results in total percentage values over 100%. The cover is recorded in six or seven categorical classes (Table *).

Table 4. Braun-Blanket cover scales.

Value	Cover
r	rare species
+	<1%
1	1-5%
2	6-25%
3	26-50%
4	51-75%
5	75-100%

C.1.2.2. Modified-Whittaker plot for plant richness assessment

The Modified-Whittaker plot is a vegetation sampling design that can be used for assessing plant communities' richness at multiple scales, from 1 m^2 to 1000 m^2. The plot contains nested subplots of three different sizes (figure *): ten subplots of 1 m2; two of 10 m2 and one of 100 m2. See description and setting up details at ***. In brief, after setting up the plots, all plant species are recorded in each subplot.

This approach helps to find minimum plot size and allow to record with high confidence all the plant species in the plot. The nested design of plots with different areas allows to estimate the species-area curves and determine the minimum area size for species richness estimations.

The shape of the plot can be rectangular or square quadrats. Rectangular plots are placed parallel to the major environmental gradient of a vegetation type and encompass more heterogeneity and recover greater species richness than rectangular.

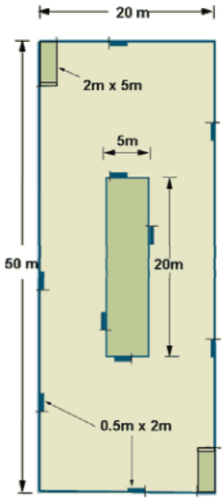

Fig. 5. Modified-Whittaker plot for species-area curves estimation.

C.1.3. Soil biodiversity

Forest soils contain a vast array of biota that have an essential role on soil functioning and quality (Doran & Jones, 1997). The simplest way to characterize soil biodiversity is to divide the biota into classic wide groups (Binkley & Fisher, 2013 and references therein): Macrofauna (>2mm) are edaphic species large enough to disrupt soil structure through their activity and include small mammals, earthworms, termites, ants, and other arthropods; Mesofauna (0.1-2 mm) are those soil-dwelling organisms large enough to escape the surface tension of water and move freely in the soil. They include some molluscs, mites, woodlice and other arthropods. Microfauna (<0.1 mm) are usually confined in water films on soil particles. Measuring soil fauna populations is challenging and requires sound experimental and sampling designs, as well as personnel with skills in arthropod identification. Finally, microorganisms, which include archaea, bacteria and fungi, are the most diverse group of organisms.

Plot size and arrangement

****

Timing of sampling

Due to seasonal cycles of the faunal activity, it is recommendable taking samples several times over long periods of time (i.e. the entire growing season). And comparison between communities should be made on the basis of samples collected in the same seasons (Uetz & Unzicker, 1976) and with the same sampling effort (i.e. number of traps, number of trapping days per season).

To assess the effect of forest management, soil biodiversity should be sampled just before and a few years after the management treatment.

Methods

C.1.3.1. Pit-fall for litter and topsoil macrofauna

Pitfall traps are trapping pits for ground-dwelling arthropods. This is a passive method, and probably has a bias towards surface-active taxa (i.e. Orthoptera, Blattaria, Diptera, Araneae, Formicidae, Collembola, Hemiptera, Coleoptera, and other Himenoptera), underestimating the abundance of less-active taxa (Acariformes, insect larvae, Psocoptera…) (Sabu & Shiju, 2010). About 10 pitfalls should be installed along a 40 m transect at roughly 4-m intervals (Swift & Bignell, 2001). Glass jars of about 10-15 cm in diameter are adequate. The depth of the jars is not critical, but the open top must be exactly at the same level of the ground surface. A mesh placed close to the open top of the jar will allow to cover the trap with a layer of litter. Each trap should contain a killing/preserving agent such as water saturated with salt, soapy water, ethyl alcohol, diluted formaldehyde, ethylene glycol or benzoic acid (Yi et al, 2012). It should be taken into account that the use of volatile compounds like alcohol can actively attract certain species like molluscs so, in this case could be controversial as a standard passive sampling method (Yi et al, 2012). Pitfall traps may also be baited with various substances, especially if we are interested in particular groups of arthropods. A sloped cover placed few centimetres above the ground surface is recommendable to keep rain out, as well as to prevent desiccation, scavenging and bycatch.

Pitfall traps should be installed preferably during the afternoon and early evening, and should be left at least 24h (Swift & Bignell, 2001) to avoid biases in the catches due to day-night cycles of faunal activity.

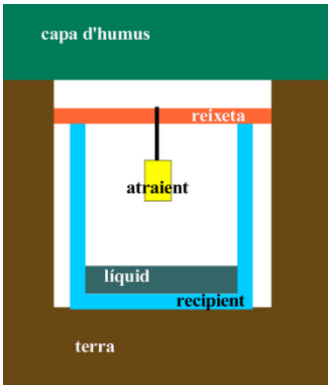

Fig. 6: Schematic view of a pitfall trap for trapping ground-dwelling arthropods

C.1.3.2. Berlese funnel for sampling organic or mineral soil mesofauna

Berlese funnels are used to extract meso- and micro-fauna inhabiting mineral soil and organic layers that move actively throughout the soil column. The method implies field collection of soil samples and faunal extraction in the laboratory. Once collected, soil samples should be processed quickly to avoid the death of specimens (Yi et al 2012).

Samples are collected along a 40 m transect, with a separation of 4-10 m. For studying fauna in the mineral soil, we can collect soil cores of known volume with a metal ring. When the objective is studying the fauna inhabiting the organic layer, we can use 50 x 50 cm frames to collect all the surface organic material inside, which should then be sieved with a 1.5 cm mesh (Sabu & Shiju, 2010). The collected material is then placed in funnels and left at room temperature for 5-10 days. For organic layer samples, use funnels of about 30 cm diameter with a 4-6 mm mesh screen fitted inside (Sabu & Shiju, 2010). For mineral soil samples, smaller funnels of about 10-15 cm diameter are adequate, with a 3 mm mesh screen fitted inside. Then, a temperature/desiccation gradient is applied with a source of heating with a light bulb above each funnel (Domingo-Quero & Alonso-Zaragaza). The heating causes a movement of living arthropods from the warmer side to the cooler side and finally to the collection jar, which should contain a killing/preserving agent, such as 75% ethanol.

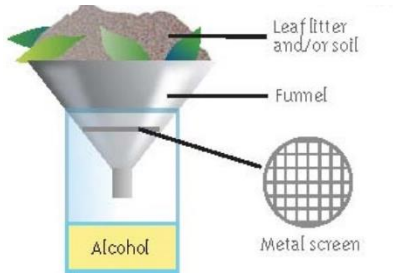

Fig. 7. Schematic view of a Berlese funnel for trapping soil and litter micro- and mesofauna. Image taken from: https://www.soils4teachers.org/lessons-and-activities

C.1.3.2 Microbial functional diversity

Microbial communities (i.e. archaea, bacteria and fungi) are the most diverse group of organisms and play a key role in the ecosystem functions, including the biogeochemical cycling. Its diversity is huge, with thousands of species per gram of soil (Roesch et al, 2007). However, there is a recurrent debate about how microbial diversity shapes soil functions, a relationship that is not fully understood. Generally, is assumed that most of microorganisms are functionally redundant, as many processes are carried out by most of microbes. Probably a large number of species is important for maintaining stable processes in changing environments (Nannipieri et al, 2003), but a reduction in microbial diversity has usually little effect on some functions, such as the estimation of soil organic matter mineralisation based on total CO_2 release. Although this redundancy, some specialised functions appear to be limited to a few specific groups of microorganisms. For instance, the degradation of recalcitrant organic matter (Tardy et al, 2015) and, related to this, the priming of soil organic matter mineralisation (Garcia-Pausas & Paterson, 2011) depends on the structure of microbial populations. Also pesticide mineralisation (Singh et al, 2014) are affected by the decrease of microbial diversity. So, the assessment of functional

diversity of the microbial communities can be more indicative than taxonomic diversity when the interest relies on soil ecosystem function.

The assessment of soil functional diversity consists on measuring the response of soil respiration to the application of a set of organic substrates. An easy method to measure the functional diversity is the MicroResp™ technique (James Hutton Ltd, Aberdeen, UK), by which the physiological profiles of soil microbial communities are characterised (Campbell et al, 2003). This is a colorimetric method in which soil is incubated for 6 hours in deepwell microtiter plates after the application a set of organic substrates, which includes carbohydrates, aminoacids, amines, organic acids and phenols. During the incubation, 96-well deepwell plates are assembled to 96-well microplates containing agar with a pH indicator dye. The carbon source utilisation is indicated by the colour development of the indicator, measured in a microplate reader, subtracting the response to the addition of deionised water only. Then the Shannon-Weaver index is calculated by using the CO_2 response to the addition of the different C substrates:

$$H' = -\sum p_i \times \ln (p_i)$$

where p_i is the ratio between the respiration response to the addition of a particular C substrate and the sum of responses to all C substrates (Zak et al, 1994).

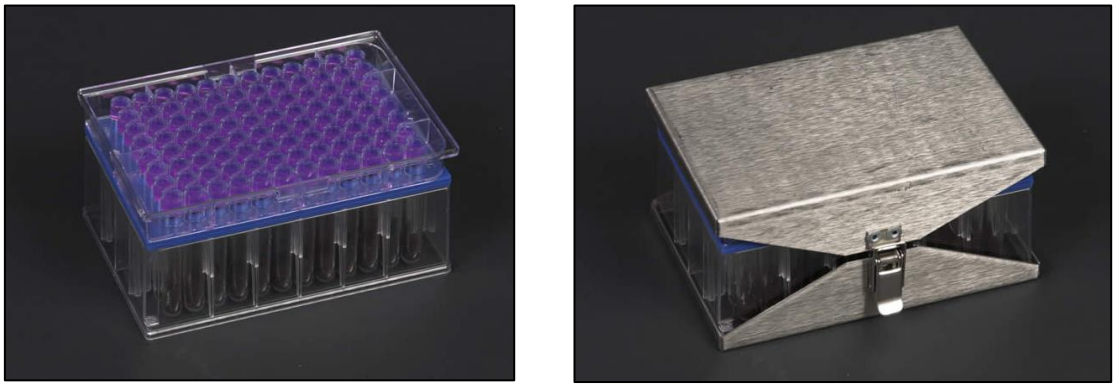

Figure 8: MicroResp™ device showing the assemblage of deepwell plate and the microplate (left). A rubber gasket in the middle ensures adequate seal between both plates, while allowing connection between each well of each plate to its corresponding well of the other plate. A metal clamp holds the system together ensuring the sealing during the incubation (right). Images taken from MicroResp™ website (https://www.microresp.com/MicroRespEquipment.html).

MicroResp technique has been used not only for mineral soils, but also for forest litter layers (McIntosh et al, 2013) and organic peaty soils (Curlevski et al, 2011). However, the low density of organic material, the large size of its particles and its inherent heterogeneity of forest floor material can present a challenge for the MicroResp technique. Furthermore, sieving the organic material with 2-mm mesh could reduce the representativeness of the sample, as a large fraction of forest floor material is in >2 mm particles and is removed in the sieving. In these cases, 24-well deep well plates and microplates can be used (Swallow & Quideau, 2015). The bigger wells of these plates allow bigger samples, which increases the likelihood of capturing a wider range of microbial habitats of the organic horizons and minimises the effect of the 2-mm sieving.

References

Binkley D, Fisher RF. 2013. Ecology and management of forest soils, 4th ed. Wiley-Blackwell, West Sussex, UK

Campbell CD, Chapman SJ, Cameron CM, Davidson MS, Potts JM. 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities using whole soil. Applied & Environmental Microbiology 69: 3593-3599

Curlevski NJA, Artz RRE, Anderson IC, Cairney JWG. 2011. Response of soil microbial communities to management strategies for enhancing Scots pine (Pinus sylvestris) establishment on heather (Calluna vulgaris) moorland. Plant & Soil 339: 413-424

Domingo-Quero T, Alonso-Zaragaza MA. 2010. Chapter 9: Soil and litter sampling, including MSS. Abc Taxa 8: 173-212

Doran JC, Jones A (eds.). 1997. Methods of assessing soil quality. Soil Science Society of America, WI

Garcia-Pausas J, Paterson E. 2011. Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. Soil Biology & Biochemistry 43: 1705-1713

McIntosh ACS, Macdonald SE, Quideau SA. 2013. Linkages between the forest floor microbial community and resource heterogeneity within mature lodgepole pine forests. Soil Biology & Biochemistry 63: 61-72

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G. 2003. Microbial diversity and soil functions. European Journal of Soil Science 54: 655-670

Sabu TK, Shiju RT. 2010. Efficacy of pitfall trapping, Winkler and Berlese extraction methods for measuring ground-dwelling arthropods in moist deciduous forests in the Western Ghats. Journal of Insect Science 10:98

Roesch LFW, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Kent AD, Daroub SH, Camargo FAO, Farmerie WG, Triplett EW. 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. The ISME Journal 1: 283-290

Singh BK, Quince C, Macdonald CA, Khachane A, Thomas N, Al-Soud WA, Sorensen SJ, He Z, White D, Sinclair A, Crooks B, Zhou J, Campbell CD. 2014. Loss of microbial diversity is coincident with reductions in some specialized functions. Environmental Microbiology 16: 2408-2420

Swallow MJB, Quideau SA. 2015. A method for determining community level physiological profiles of organic soil horizons. Soil Science Society of America Journal 79: 536-542

Swift M, Bignell D. 2001. Standard methods for assessment of soil biodiversity and land use practice. International Centre for Research in Agroforestry. ASB Lecture Note 6B, Bogor, Indonesia

Tardy V, Spor A, Mathieu O, Lévèque J, Terrat S, Plassart P, Regnier T, Bardgett RD, van der Putten WH, Roggero PP, Seddaiu G, Bagella S, Lemanceau P, Ranjard L, Maron PA. 2015. Shifts in microbial diversity through land use intensity as drivers of carbon mineralization in soil. Soil Biology & Biochemistry 90: 204-2013

Uetz GW, Unzicker JD. 1976. Pitfall trapping in ecological studies of wandering spiders. Journal of Arachnology 3: 101-111

Yi Z, Jinchao F, Dayuan X, Weiguo S, Axmacher JC. 2012. A comparison of terrestrial arthropod sampling methods. Journal of Resources and Ecology 3: 174-182

Zak JC, Willig MR, Moorhead DL, Wildman HG. 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biology & Biochemistry 26: 1101-1108

C.2. Indicator: Potential biodiversity

Even though the existent biological links within an ecosystem is highly complex, it is possible to identify the key variables and dynamics mostly influencing forest biodiversity. A great number of these variables (forest structural treats) can be modified by forest management. This idea is the basis for the development of a Potential Biodiversity Index (PBI) which can be easily applied by foresters and that it is based in forest structural parameters that are key to host biodiversity. This index is an indirect valuation of the biodiversity a forest stand can host (potential)

C.2.1. Forest structural parameters

Methods

C.2.1.1. The Potential Biodiversity Index (PBI)

The potential biodiversity index (IBP) is a proxy indicator of the capacity of a forest to host biodiversity, based on the field assessment of 10 key factors. The index came originally from France, where, since 2008, the CNPF and INRAE have been working with it as part of the implementation of the French national biodiversity strategy (Larrieu and Gonin, 2008; Larrieu et al., 2012). Since 2011, they have had a version adapted to Mediterranean conditions, which has

already been used in different regions (Gonin, Larrieu and Deconchat, 2017). In parallel, in Catalonia, the Forest Ownership Centre (CPF) began work in 2010 on a biodiversity index (Fernández et al., 2013), to bring to a close a decade of study into forest management and biodiversity in the framework of the Biodiversity Monitoring Programme for the Forests of Catalonia – BIBOCAT. After a period of testing and improvement, the first version of the IBP for Catalonia, compatible with international Mediterranean IBP standards, was finalised in 2019: the IBP_Cat (Baiges et al., 2019), coordinated by the CPF and the CNPF, and agreed with the different agents involved in the management and conservation of forests in Catalonia.

The IBP involves observation of 10 key factors (Figure 9): the first seven factors, which we can call stand factors (stand IBP), are those where management has most impact. They refer to key elements, present in mature forests, which have been found to be the attributes that contribute most to greater diversity: large trees, dead wood, forest microhabitats, clearings... If these elements can be maintained in managed forests, their ability to host biodiversity is greatly increased, especially for those species that depend on them at some point in their life cycle. The last three are the context factors (context IBP): the continued existence of the forest, i.e. the age of the site as undisturbed forest land and the presence of aquatic and rocky environments in the stand, which have their own specific diversity.

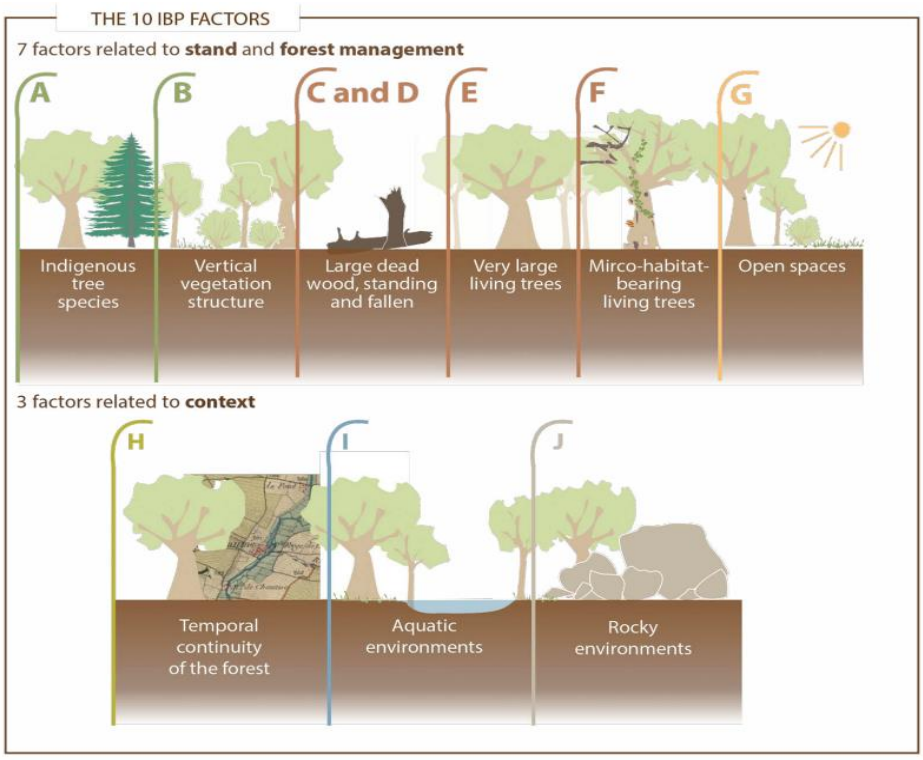

Figure 9. Factors considered in the IBP methodology. Source: Emberger et al. (2013)

For each of these 10 factors are defined different states (between little and very favorable to host biodiversity) and is given to each one numerical value (from 0 to 5). The application of the IBP is to evaluate in the field in which of these states falls the studied stand, for each factor, until you get a final overall score. The results are given in the form of absolute value of IBP, disaggregated into IBP_rodal (first 7 factors) and IBP_context (last 3 factors) and in percentage. Templates and field measuremt protocols can be found here: https://cpf.gencat.cat/ca/cpf_03_linies_actuacio/cpf_transferencia_coneixement/Index-Biodiversitat-Potencial/

References
Brotons, L., 2007. Biodiversidad en mosaicos forestales mediterráneos: el papel de la heterogeneidad y del contexto paisajístico. EN: Camprodon, J. y Plana, E. 2007. Conservación de la biodiversidad, fauna vertebrada y gestión forestal. Pp 137-146
Emberger C., Larrieu L., Gonin P., 2013. Dix facteurs clés pour la diversité des espèces en forêt. Comprendre l'index de Biodiversité Potentielle (IBP). Document technique. Paris: Institut pour le dévelopement forestier, mars 2013, 56p
Kraus, D., Bütler, R., Krumm, F.,Lachat, T., Larrieu, L ., Mergner, U., Paillet, Y., Rydkvist, T., Schuck, A., and Winter, S., European Forest Institute, 2016. Catalogue of tree microhàbitats. Reference field list.
Minuartia, 2013. Disseny d'un índex d'avaluació del potencial dels boscos per a la diversitat. Centre de la Propietat Forestal. Informe inèdit.
Piqué, M., Vericat, P., Cervera, T., Baiges, T., Farriol, R., 2011. Tipologies forestals arbrades. Sèrie: Orientacions de gestió forestal sostenible per a Catalunya (ORGEST). Centre de la Propietat Forestal. Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Barcelona. 341 p.

C.3. Indicator: Risk of forest loss

Functional diversity is defined as those components of diversity that influence how an ecosystem operates or functions (Tilman, 2001). Methods for measuring FD are based on species groups (Functional types) on the basis of selected traits or characteristics.

C3.1. Forest persistence (resilience and resistance)

Methods

C.3.1.1. The persistence index (PI)

The persistence index (PI) described in Sánchez-Pinillos et al. (2016) is a quantitative measure that allows to assess the capacity of a vegetation community to maintain their functions and services after disturbances. The index is based on the diversity and evenness of plant traits present in the community that confer to the species resilience and/or resistance to selected stressors. It is a useful tool to guide ecosystem management decisions in a context of changing climate and uncertain disturbance regimes. The index assess the persistence of specific ecosystem properties and services selected by the user through the value, range and relative abundance of species functional traits in a given stand.

To calculate the PI, the required inputs are: (1) the set of species that are relevant for the persistence of the desired community state and/or function; (2) the main disturbances threatening the community's state and/or function; (3) the response traits that confer species with the ability to resist or recover from these disturbances; and (4) the abundance of species in the community.

References
Kent, M. 2012. Vegetation description and data analysis. A practical approach. Second Ed. Wiley-Blakwell. UK.
Margalef R. Our biosphere. Excellence in Ecology, 10. O. Kinne (Ed). Ecology institute, Oldendorf. Germany.
Sánchez-Pinillos, M., Coll, L., De Cáceres, M., Ameztegui, A., 2016. Assessing the persistence capacity of communities facing natural disturbances on the basis of species response traits. Ecol. Indic. 66, 76–85.
Stohlgren, T.J., M.B. Falkner, L.D. Schell. 1995. A Modified-Whittaker Nested Vegetation Sampling Method. Vegetatio 117(2):113-121.
Wilson E.O. (Ed). 1988. Biodiversity. Nat. Acad. Press, Washington D.C.