Supporting Information S1 Site characteristics and data management

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Systematic reporting of basic site information of the studied system is necessary to ensure that studies are repeatable and to enable data re-use across studies, syntheses, modelling, and upscaling (Haddaway & Verhoeven, 2015; Gerstner et al., 2017). In climate change research, there are experiments *sensu stricto* and natural experiments in space (i.e. gradient studies) and time (i.e. observational studies). This chapter on reporting and documentation is applicable to all these types of studies (if not otherwise specified). In addition, many of the principles discussed in this chapter are not exclusively relevant for climate-change studies, but also apply to global-change studies in general.

Surprisingly, the necessary basic site information of studies, is often incomplete or missing in scientific publications (Hillebrand & Gurevitch, 2013). In this chapter we therefore describe which key site, study system, and study design variables and information should be collected, and how this information is best reported. We first discuss how to design and set up an experiment or observational study that may serve multiple uses beyond the needs of the particular project. Then we describe basic geographical location and basic site description (e.g. coordinates, elevation, land-use history, vegetation), physical (e.g. soil horizon, pH), chemical (e.g. nutrient availability), and meteorological variables, and finally how to report your data. Although some of this information may not directly relate to the particular research question or hypotheses, reporting all relevant site information is essential as it puts the study in a larger context and is the key to making data and results useful beyond the particular research for which they were designed.

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- Haddaway, N. R., & Verhoeven, J. T. A. (2015). Poor methodological detail precludes experimental repeatability and hampers synthesis in ecology. *Ecology and Evolution*, *5*(19), 4451-4454.
- Hillebrand, H., & Gurevitch, J. (2013). Reporting standards in experimental studies. *Ecology Letters*, *16*(12), 1419-1420.

How to cite a protocol:

E.g. To measure soil organic matter (SOM) we used the method described in protocol 1.3.2 Soil nutrients in the Supporting Information S1 Site characteristics and data management in Halbritter et al. (2020).

Halbritter et al. (2020) The handbook for standardised field and laboratory measurements in terrestrial climate-change experiments and observational studies (ClimEx). *Methods in Ecology and Evolution*, *11*(1) 22-37.

How to cite an updated protocol version:

E.g. To measure soil organic matter (SOM) we used the method described in protocol 1.3.2 Soil nutrients in the Supporting Information S1 Site characteristics and data management in Halbritter et al. (2020), using the updated protocol version, Date, available in the online version: www.climexhandbook.uib.no.

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1.1 Optimising the study design

1.1.1 What to measure and report and why?

A basic site description for an experimental or observational site includes the location where the study was performed and under what biotic and abiotic conditions. The documentation of the basic characteristics of the system and correct reporting of the basic data facilitates and improves comparisons with other studies and downstream usage of the data in meta-analyses and other data syntheses (Hillebrand & Gurevitch, 2013; Haddaway & Verhoeven, 2015; Gerstner et al., 2017). For any study it is thus important to report and measure the following information for each site: i) geographical location and basic site description, ii) abiotic and biotic properties of the ecosystem, iii) basic climate and weather data, and iv) experimental set-up, analysis, and results. More specific for climate-change experiments *sensu stricto*, variables that may be influenced by the manipulation need to be measured in all treatments. These include variables such as soil moisture, temperature, and nutrient availability, along with various biotic responses described in detail in other protocols (see Supporting Information S2 Carbon and nutrient cycling, S3 Water cycling, S4 Species and interactions and S5 Stress physiology). For *in situ* experiments (e.g. space and time), it is important to measure variables that may be influenced by the spatial or temporal component of the experiment.

The **geographical location and basic site description** should describe the study location i.e. coordinates, vegetation type, and climate (Hillebrand & Gurevitch, 2013; Morueta-Holme et al., 2018). This effort enables and facilitates further upscaling and modelling of the experiments beyond the observational boundary as well as increasing the potential for the dataset to be used in meta-analysis.

The pre-treatment measurements of the **abiotic and biotic properties** of the ecosystem report the conditions of the soil–plant–atmosphere system before the experimental manipulation (i.e. baseline ecosystem measurements) and enables between-experimental site comparisons in a global context. It is crucial to measure these key variables before the manipulations start in order to evaluate changes imposed by ambient, non-manipulated environmental conditions. Similarly, for natural experiments and long-term monitoring, baseline data (e.g. pre- and/or post-observational measurements) are important. The key variables that should be reported are of a physical, chemical, and biological nature, and also concern ecosystem services (e.g. water quality, carbon and nutrient cycling, biodiversity; Costanza et al., 1997). They are also important for process modelling and data usage in a meta-analysis. In addition to the before-after treatment measurements, it is advisable to have plots in the experimental design that test control vs. impact (Christie et al. 2019).

When conducting a climate-change study, it is crucial to measure **environmental conditions** during the study and if applicable in every treatment as these potential drivers of ecosystem functioning may deviate substantially from ambient conditions, and treatments may have effects on other drivers, such as warming leading to changes in the water balance (Damgaard et al., 2018). The most important climate variables include air temperature, relative humidity, and precipitation (which may be in the form of irrigation or precipitation removal), as well as soil temperature and moisture.

Finally, for the further use of the data from a climate-change studies, i.e. for a meta-analysis, it is crucial to correctly follow open science practise and report the study design, analysis, and results (Hillebrand & Gurevitch, 2013; Haddaway & Verhoeven, 2015; Gerstner et al., 2017).

1.1.2 Setting up a sustainable climate-change study

Once a decision on the important matters in the research are made, such as the research question, the experimental treatments and study design, the study system, site selection, and other important issues related to the research (which is not the purpose of this protocol and will not be discussed here), a number of more practical issues have to be decided. Climate-change studies are expensive and resource demanding and the goal should be to make the most out of them. One of the most important decisions is that of sustainability: how long will the study last, how many people and how much activity will be involved, and is there any chance that there will be new projects and measurements coming in? As a general rule of thumb, there is little to lose by erring on the side of optimism (as in expecting a lot of activity), although financial and spatial constraints may, of course, limit the options. Some general advice to guide decision-making is offered in Table 1.1 and Figure 1.1.

1.1.3 At which spatial scale should you measure?

The scale at which a variable should be measured has to be considered carefully. Measurements can be quick and simple or labour-intensive and expensive. Most importantly, the scale of the measurements should depend on the research question and the study system, but here are some general rules that are widely applicable, unless there are strong reasons to do differently related to the research questions or study design:

- Generally, all variables that are stable across the site and that are not affected by the experiment within the timeframe of the study should be measured at the site scale.
- Conversely, variables that are influenced by the experimental treatments should be measured at the plot scale.
- Variables that vary across the site should preferably be measured at the block or plot scale.

In practice, many of these decisions will be affected by economic and data analysis considerations, and there is likely to be a choice between expensive installations/optimal measurements with low replication (i.e. in some but not all treated plots) v. using cheaper/simpler measurements and technology which may allow measuring it across all replicates in the experiments. However, cheap technology and smaller and better loggers are developing rapidly, offering new opportunities. In heterogeneous habitats (horizontal and vertical), it is advisable to measure variables in several places.

1.1.4 What is the ideal sampling interval?

Similar considerations should be made for the sampling intervals. The frequency of the sampling will mainly depend on the temporal resolution of interest (annual variation, seasonal patterns, daily fluctuations) and also on whether manual sampling or automated loggers are used. If the data are used for modelling, it is important to consider the required temporal resolution of the model, which is often on a fine scale. For example, modelling of ecosystem gas exchange will require half-hourly to hourly climate data input. The calculation of some long-term variables such as seasonal temperature sums or daily maxima also requires relatively high-resolution data. Also here, rapid improvements in technology open up opportunities.

 Table 1.1 Checklist of questions to ask and guidance when setting up a sustainable and multi-purpose climate-change study from site to sub-plot level.

| Scale | Question | Guidance |
|---------------------------------|--|--|
| Site (selection) Figure 1.1A | What does the system represent? Which environment, habitat, ecosystem, former and present land use? | Sites should be placed within the limits of the relevant system, avoiding off-site effects when possible. Please note if former land use only affects part of the study area. |
| | Are there any specific factors like off-site effects (run-off, shade, nutrient inputs etc.) to consider? | Blocks (or other study design features) should be chosen to capture within-site heterogeneity due to environmental or biotic variability, or off-site effects. |
| | Single-site or multi-site study? Is the interest in the treatment effects per se, or across site comparisons of these effects? | Single-site studies are easier and cheaper to maintain, allowing resources to be spent on experimental factors and response variables. |
| | Are there other reasons for replicating across sites such as generality of the response, relevance across several systems etc.? | Multiple-site studies allow across site comparisons, but it is then important to consider your "outer design", i.e. should sites be along gradients or replicates in similar environmental settings, how many replicates are needed (e.g. to fit a regression at site level), etc. |
| | | Multi-site studies increase the costs and effort, but these also depend on the study and location of the different sites. |
| | Are any permits necessary and contacts to ensure site sustainability? | Obtain permits from the government and landowners well in advance. |
| | | Ideally get permits beyond the funding period for potential extension of the study. |
| | Is any site-scale maintenance needed? Fencing, grazing, mowing? | Building a fence and moving might need the agreement of the landowner. Calculate enough time and money for maintenance. |
| | Any control needed for that? | If the site maintenance affects your responses, you might need (yet) another set of controls. |
| | What site-scale data are needed? How many measurements are needed? | Report all the information needed to situate your study in space, time, and in the relevant ecological context. If you have a heterogeneous system, you likely need more than one measuring point in space (horizontal and vertical) and time. |
| | | Any variables affected by the experimental |

| | | treatments need monitoring on a finer scale (see below) Draw detailed maps of your study site with all blocks, plots, and installations clearly indicated. Take pictures; they are essential to document study set-up and design, for data collection (e.g. relocating samples), and outreach, and can be useful for data quality checks. |
|--|--|--|
| Within-site study design (blocks) Figure 1.1B | How to ensure sustainable site set-up ? | The area should be large enough to accommodate the study, treatments and the necessary unmanipulated controls / sampling areas for site-level factors. The site set-up should provide space (buffer area) to avoid contamination of for example treatments, and between blocks to carry out the measurements. In sensitive systems or when plots are small, consider installing boardwalks, etc. to minimise disturbance. A central pole supporting a ladder can be used in small plots to reach every point without touching the ground. The potential observer effects should always be considered and can be reduced by minimising disturbance, i.e. sampling intensely but not at the expense of statistical power (De Boeck et al., 2008). |
| | What block-scale data is needed? | Any response, predictor or co-variable that will be used in statistical analyses should be measured at this scale, if at all possible. This maximises the statistical power of the analyses. |
| | Is there space for additional experiments and/or measurements ? | Set aside areas for add-on measurements and project extensions - preferably within blocks, as that maximises opportunities for project integration. |
| Within-block study design (plots) Figure 1.1C | How to ensure sustainability of studies in plot set-up ? Where are data measured permanently and where destructively? | Plots should be large enough to encompass the responses of interest, and thus have to match the study aim and study system. The plot size should also take into account the complexity and heterogeneity of the study system (De Boeck et al., 2015). Plots should be large enough for sensor installation and sampling. It is recommended to divide the plots into destructive and non-destructive sampling |

| r | [| |
|--|--|---|
| | | areas. |
| | | Permanently mark and map all plots and sub-areas within plots individually, and include back-up marking (e.g. buried metal nails, or similar) in case primary marking is lost. A standardised (systematic) marking and within-plot design regime is easier to relocate if lost. |
| | What plot-scale data are needed? | All variables that are directly affected by a treatments should be measured at the plot scale. |
| | | It is advisable to have different control plots that control for before-after treatment effects and control vs. impact during the experiment (Christie et al. 2019). |
| | | Pictures of the plots at each sampling date are useful as they document the data collection and can also provide various kinds of additional data. |
| Within-plot study design (sub-plot, sample) | tudy designand where destructively?sub-plot,ample)What sub-plot data are needed? | Sub-plots can be used for dividing plots into zones, for destructive, non-destructive sampling. |
| Figure 1.1C | | Sub-plots can also be used directly for sampling purposes, e.g. frequency data or mapping. |
| | | Sensors and non-intrusive measurements can be installed in and under the permanent sub-plots, e.g. lysimeters, mini-rhizotron. |
| | Sub-plot data can be used directly in data analyses, and they are very useful for data checking, i.e. when resampling the same sub-plot over time, data from different time steps can be compared. | |
| | | In destructive sampling: plug soil-sampling holes with soil from within the block to avoid drainage issues and mark them to avoid resampling. |



Figure 1.1 Schematic figure of an example of a nested climate-change study design with four different levels: A) site, B) within-site (block), and C) within-block and plot level (plot and sub-plot).

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1.2 Geographical location and basic site description

1.2.1 History

Ecosystem history, which includes historical soil and land management (i.e. disturbance, grazing, harvests or harvest regime, nutrient input and contamination, species introductions or extinctions) gives crucial information as any such changes may have a knock-on effect on a range of responses (Sala et al., 2000; Kepfer-Rojas et al., 2015). Providing accurate details of the ecosystem history (e.g. the number and degree of prescribed burning, fertilisation schemes, timber volume removed or the quantity of grazing animals) can be useful when assessing the impacts of land-use history and comparing different sites. These historical factors may also affect the responses of ecosystems to future environmental manipulations, such as climate, nutrient, and land use (Luo & Chen, 2013; Domec et al., 2015; Kröel-Dulay et al., 2015; De Keersmaecker et al., 2016). Finally, ecosystem history can be of value if the ratio of C_3 to C_4 plants has changed, for example through crop rotations, as this allows for analyses of ecosystem processes that are based on stable isotopes without additional cost.

1.2.2 Location

Latitude and longitude coordinates should be given (e.g. via GPS), in addition to the resolution of the position (e.g. ±3 m). Coordinates can help with finding additional information about a study site, such as weather data from a nearby station, or remote sensing products. It is also common to give the name of the location of the study site, the region, and country or continent. Multiple locations can be shown on a map.

1.2.3 Elevation (metres above sea level), slope (degrees), and aspect (degrees)

Atmospheric pressure, temperature, and radiation (including UV-B) change consistently with increasing elevation, while many other factors such as precipitation and wind vary regionally along elevational gradients (Körner, 2007). Slope is helpful in explaining observations related to soil hydraulic properties as well as the radiative conditions. Both slope and aspect influence surface temperature and are useful to report on a hill and in mountains, where topography changes over short distances.

Elevation can be extracted from a GPS, and slope and aspect can easily be measured using a clinometer and compass, respectively. A number of free apps for these measurements are now available for smartphones. For slope, a long rigid plank can be placed on the ground and the clinometer sat flush on the plank in order to measure the slope. If the small-scale heterogeneity is high, a measuring post set to eye level > 10m away from the plot provides a more accurate measurement of the average slope. Aspect is measured using a compass, facing downslope. Note that these parameters can be measured on different spatial scales; plot, block, or site. At coarser scales, these parameters can be determined from reference map sources.

1.2.4 Climate data

Long-term climate data from each study site should be reported to enhance reproducibility (Morueta-Holme et al., 2018). Common variables are mean annual temperature and precipitation,

seasonality, and length of the growing season. Mean annual temperature and precipitation may not be very informative whereas summer maximum, winter minimum temperature, and growing season length are more relevant. It is important to cite the source of the data, the time frame over which the data were collected, name of the location if the data were obtained by a weather station, and (if applicable) to explain any data processing (Morueta-Holme et al., 2018).

1.2.5 Vegetation and habitat type

The habitat type (e.g. forest, grassland, desert), dominant plant functional type (e.g. trees, graminoids, forbs, mosses), cover and height of the dominant vegetation layers (e.g. trees, shrubs, dwarf-shrubs, herbaceous, cryptogams), and vegetation type (using relevant national or international classification schemes) of the study site should be described. It is also common to list the dominant species, and some biodiversity or structural information such as species richness and/or evenness.

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1.3 Soil type and physical characteristics

Soils are physically composed of mineral and organic particles in varying sizes. The combined particles form the soil matrix that shapes the structure and pore spaces of soil. In turn, soil physical properties determine many key soil processes from soil water-holding capacity to cation exchange capacity that affect other life forms and ecosystem function. The key soil physical characteristics include soil structure, bulk density, and texture.

For the description of standard methods for soil characterisation we refer the reader to the Soil Science Society of America (SSSA) series on Methods of Soil Analysis; in particular, part 3 – Chemical Methods (Swift & Sparks, 2009) and part 4 – Physical Methods (Dane & Topp, 2002). Although the SSSA - Physical Methods (Dane & Topp, 2002) lists a complete set of methods, note that some of these traditional methods are being replaced by newer technology. In addition, the USDA Soil Survey Field and Laboratory Methods Manual (NRCS, 2014b) can be used as a new and simple guideline for methods in determining soil physical properties.

In this protocol, we focus on providing a basic guideline and starting point for determining soil physical properties in the context of climate-change studies as our main focus is on understanding ecosystem processes and plant behaviour.

Other variables that can be of relevance in some cases are described in Supporting Information S4. Water cycling (for example water potential and water repellency).

Soil samples

Several protocols in section 1.3 and 1.4 require sampling of soils. Here, we describe in general how to sample soil and important things that should be considered. Note that the sampling can vary considerably depending on the research question, method and individual needs (also check individual protocols for more details).

Before taking a soil sample the vegetation and litter (i.e. dead vegetation) are removed. Note that in some systems, litter can form a thick layer and be part of the organic horizon. Whether this layer is removed or not depends on the research question. The **number of samples** depends on many factors (e.g. research question, experimental design, finances, short-range variability in soil properties across the area of interest). A power analysis can give you a hint of the needed sample size. The soil is generally heterogenous and it is advisable to take a minimum of 3-5 soil samples per study unit (e.g. plot or block level). Samples from the same unit can also be pooled to represent the variability and reduce costs and time, but should be avoided if possible. Climate change experiments often have limited space for destructive sampling, and it is advisable to plan the soil sampling beforehand. If samples are taken from several plots with the same layout, it is advised to take samples in a fixed location within a plot and/or with similar aboveground vegetation. This helps the comparability between plots. The researcher should also consider if the variable of interest changes with time, which will determine if the sampling should be done once or several times.

For some measurements it can be necessary to use gloves and clean the equipment well between each soil sample to avoid contamination (e.g. genetic analysis).

The **depth** of the soil sampling depends on the method, research question and type of soil. It is advised to sample the whole soil profile (down to the bedrock) for pool size assessment, or at

maximum root depth if the roots or microbial activity are the focus. Separate soil cores are usually taken from the organic layer defined as accumulated organic material on top of the mineral soil and mineral layer, but a higher resolution can be useful in deep soils and soils with several layers (Maaroufi et al., 2015).

Temporal scale (one time several times): if the aim is to assess total pool size of an element, it is advisable to sample only once as these numbers do not vary considerable with season. When the aim is to sample more dynamic pools (inorganic nutrients or microbial pool sizes) it is advisable to sample several times.

Spatial scale: if the aim is to assess the pool size in an area, it is recommended to take several soil samples, because the soil is often heterogeneous.

The **size of the soil core** depends a lot on the method, but often the diameter of the soil core is 3-8 cm. In general, structural and physical methods for measuring soil structure require diameters that are larger than the sampled soil depth to avoid damaging the soil structure. For processes related to microbial activity, it is advisable to take many small soil samples e.g. 10 samples with a 2 cm corer.

The **amount of soil** used is very variable and depends on the property of interest. For some methods it is crucial to know the volume of the soil sample (e.g. bulk density) to be able to scale up to an area basis, while for others a small amount of soil is needed (e.g. when concentrations or activity and not pool sizes are in focus).

Transport and storage - Each soil sample should be properly labelled including location, plot ID, profile number layer, depth, and date. The samples should not be exposed to the air or sun, as water will evaporate from the sample and warming can accelerate and activate unwanted biological processes. Soil samples are usually transported in plastic bags or boxes and kept cool during transport (ca. 4°C). For determination of very sensitive pools or processes, it may be recommended to place samples in a cooler with dry ice (e.g. for extraction of soil enzymes). Ideally, the soil is analysed immediately after sampling, and some methods require quicker processing than others. Often quick processing is however not possible (e.g. due to continuous field work) and then the soils should be stored in the proper way. There is no general rule for how to store soil before the analysis and it depends on the origin of the soil and the purpose and method. For example, tropical soils should not be stored in a fridge, since the low temperature can kill microbes and soil lysis will lead to underestimation of the microbial biomass. However if soil is sampled frozen in cold environments (e.g. arctic), then the samples should be kept frozen until the analysis. For some methods and soils the fridge or freezer is recommended for storage, while for others air or oven drying is the best practice.

For some analyses, the **roots and stones (>2 mm)** are removed from the soil samples, using a sieve of 2 mm mesh width. If the total mass of the samples needs to be known, the removed parts are weighed (e.g. for bulk density). This is especially important for stony soils. For measurements that require undisturbed soil (e.g. many physical measurements), the stones are not removed.

The soil samples are often used fresh, but for some analysis the soil needs to be **dried**. However, this depends largely on what the samples are used for. Soils can be dried at room temperature, low oven temperature or higher temperature. Table 1.3.1 shows how to process soil samples for the most commonly used methods (for more details check individual protocols).

Table 1.3.1 Temperature for processing soil samples for common methods.

| Temperature for drying | Method | Protocol |
|--|---------------------------------|----------|
| Fresh soil kept in fridge (4°C) | Soil microbial biomass | 2.2.1 |
| | Soil enzymatic activity | |
| | PLFA | |
| | Soil activity assays | |
| | Bulk density | 1.3.4 |
| | рН | 1.4.1 |
| Air dried, room temperature (25°C) | Soil water repellency | 3.6 |
| Oven dried, low temperature (max. 55°C) | Soil carbon and nutrient stocks | 2.2.4 |
| Oven dried, high temperature (70° - 105°C) | SOM (loss on ignition) | 1.4.2 |

Where to start

The International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (Cools & De Vos, 2016) and the Countryside Survey (Emmett et al., 2010) give a good overview over how to sample soils.

1.3.1 Soil types and horizons (layers)

Unlike sediments that are deposited over time, soils are developed as horizons from the parent material under the influence of local climate, topography, and biota. There are many soil types based on the composition of the parent material, texture, and organic matter. A soil horizon is a parallel layer to the soil surface with distinctly different physical, chemical, and biological characteristics from the layers above or beneath. A vertical layout of a soil (based on a soil pit) illustrates the different horizons in a soil profile (Figure 1.3.1). Determining soil horizons within a soil profile will provide important information about the life history of the soil as well as different characteristics of soil properties.

The soil profile should be described according to a soil classification that is compatible with the World Reference Base (WRB) system. Soil type should be reported according to the World Reference Base for



Figure 1.3.1 Soil profile showing the different soil horizons from the organic layers to the bedrock.

Soil Resources system (ISRIC, 1998; IUSS Working Group WRB, 2006, 2014). This is the international standard taxonomic soil classification system, which is endorsed by the International Union of Soil Sciences (IUSS) and replaces the FAO soil classification. Reporting the depth of soil layers or horizons should be included in any description as this is important for comparison and modelling. Most classification systems recognise six master soil horizons which are designated using the capital letters O, A, E, B, C, and R (Figure 1.3.1). Reporting O to B is usually sufficient for climate-change studies. O = Organic matter, characterised by high levels of organic material: dark in colour; A = Mineral topsoil, often organically stained: contains highly weathered parent material (rocks) and is lighter in colour than the O horizon; E = Eluviated - used to label a horizon that has been highly leached of minerals, clays, and sesquioxides: usually determined as a pale layer below the A horizon and only exists in older, well-developed soils; B = Subsoil, an illuviated layer that accumulates iron, clay, aluminium, and organic compounds: lighter in colour and often may be reddish due to the iron content; C = Broken-down bedrock, also known as substratum; R = Bedrock.

What and how to measure?

Traditionally, determining soil horizons involves excavating a pit exposing a clean vertical surface of approximately 1 metre depth and identifying the depths of different horizons based on colour and physical properties. This can be simplified by using a soil auger. First, identify the depth of soil until the bedrock or parent material layer. Using a soil auger, sample the continuous soil profile until bedrock or parent material is reached, and identify horizons based on different characteristics. Samples are often collected and taken back to the laboratory for analysis. A wide number of texts are available that describe soil analysis: those more widely used include NRCS (2014a) and Dane & Topp (2002).

1.3.2 Plant rooting depth and distribution by depth

The plant rooting depth is important to measure the distribution of roots throughout the soil profile as this may change when the plants are subjected to climatic treatments. If the site is heterogeneous (e.g. slope or changes in vegetation), rooting depth should be determined in more than one place. Plant rooting depth is measured by digging a soil profile (see 1.3.1 Soil horizon) and can be measured when the soil layers are determined.

1.3.3 Stone content (%)

Stone content defines the volume and mass in the soil containing stones > 2 mm. Stones do not contribute to plant nutrient supply or water-holding capacity in the soil and the weight and volume of stones are most often subtracted when > 5% of the soil volume (ICP, 2016). To calculate the stone content, an air-dried soil sample of known mass is sieved so that stones larger than 2 mm are removed. The stones are weighed and the % of stones above 2 mm can be determined and reported as a % of the total mass of soil.

1.3.4 Bulk density (g cm⁻³)

Bulk density is a measure of the amount of soil per unit volume of oven dried soil and gives information on the physical status of the soil. The soil organic matter content, soil texture, the minerals in the soil and degree of compaction define the bulk density. Bulk density varies substantially among soils. Mineral soils have a bulk density of around 1–1.6 g cm⁻³, while in organic soils and friable clay it is well below 1 g cm⁻³. Bulk density values are essential when determining soil carbon and nutrient stocks, as they allow a conversion from concentrations to mass per area (see also protocol 2.2.4 Soil carbon and nutrient stocks).

What and how to measure?

A range of equipment is available for the determination of bulk density and general guidance can be found in Grossman & Reinsch (2002). A volumetric core (the volume must be known), which should be at least 75 mm in diameter, with the depth no greater than the diameter, should be taken down the soil profile, ideally at 5 cm increments. Although dry bulk density can be determined on the entire soil sample (with stones), it is usually reported for soil sieved through 2 mm mesh, the fine-earth fraction, which is suitable for subsequent calculations of carbon and nutrient stocks. The stones (> 2mm) are removed and weighed separately. The soil samples are dried at 105°C and then weighed.

Dry bulk density is calculated using the following equation:

$$dry \ bulk \ density \ (g \ cm^{-1}) = \frac{(weight \ core \ (105 \ ^{\circ}C) \ (g) \ - \ stone \ weight \ (g))}{(core \ volume \ (cm^{-3}) \ - \ stone \ volume \ (cm^{-3}))}$$

The stone volume can be determined through the water displacement method using this equation:

stone density
$$(g \ cm^{-1}) = \frac{\text{stone mass } (g)}{\text{stone volume } (cm^{-3})}$$

The stone density is usually assumed to be $\sim 2.65 \text{ g cm}^{-3}$.

Resampling of bulk density is normally not required. However, especially severe drought may change the bulk density and resampling of bulk density is recommended after 3–5 years.

1.3.5 Soil texture

Soil texture is the particle size distribution of soil determined by a percent combination of sand, silt, and clay that presents coarseness of a soil. Soil texture partly determines soil water-holding capacity and permeability, which provides important characteristic information about soil physical properties. The percentage clay in particular is also highly relevant for nutrient availability, as the clay colloids serve as exchange places for cations such as NH_4^+ , K^+ , Ca^{2+} and Mg^{2+} , similarly to humus particles.

What and how to measure?

Particle size analysis (PSA) is used in soil science to determine soil texture (sand, silt, and clay content), and often used in soil physics to determine soil hydraulic properties. PSA is often reported for the fine earth fraction of soils < 2 mm in diameter. Soil samples returning to the laboratory are

usually sieved to remove particles > 2mm and root material. PSA can be determined using sedimentation with either the pipette method or the hydrometer method (Gee & Or, 2002). The texture of soil is expressed using the soil textural triangle from the composition of different particles (NRCS, 2014b). Texture in organic soils is not measured.

1.3.6 Water table depth (m)

The water table is the upper extent of the phreatic or groundwater zone, in which all soil pores and fractures are completely filled with water. The water table marks the end of the vadose zone. The phreatic zone and therefore the water table depth may vary between seasons and with dry or wet periods.

In areas with shallow water tables, for example in deltaic areas (van der Ploeg et al., 2012) or wetlands (Oosterwoud et al., 2017), or in soils with a perched water table owing to less permeable soil layers, the water table influences the amount of water available for vegetation and soil evaporation. For vegetation, the water table influences water availability as roots may grow towards the water table or via capillary rise of groundwater. Capillary rise is a physical phenomenon where water will rise in a hollow tube with a small diameter. The smaller the tube the higher the water will rise. Soil pores form a network of such hollow tubes, and the height of capillary rise depends on the soil texture and structure (Brutsaert, 2005, section 9.6), leading to a range from 0.2–0.5 m capillary rise for coarse textured soil (e.g. sand) to 0.8–several m for fine textured soil (e.g. clay). For soil evaporation, water is transported from the underlying soil layers by liquid and vapour transport, which can be influenced by groundwater through capillary rise. If the water table is expected to influence the climate-change studies it is recommended to monitor the water table fluctuations.

What and how to measure

The water table can be measured with a piezometer, which is a tube with a perforated, screened part for groundwater to enter the tube while soil material is kept out (Reeve, 1986). It can be installed by drilling a hole manually with a soil auger or with drilling equipment. Before installing in a site with perched water tables, ensure that the site's less permeable layers are not drained by making holes through them, thus altering the soil hydraulics. Once the piezometer is installed, measurements can be done manually with a measurement tape with a float or automatically with a pressure transducer (e.g. Oosterwoud et al., 2017).

1.3.7 References

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1.4 Soil chemistry and nutrient availability

The focus in terrestrial ecology is typically on carbon (C) and nitrogen (N), and to a lesser extent on phosphorus (P). But other nutrients such as potassium (K), magnesium (Mg), calcium (Ca), and zinc (Zn) can limit plant growth and influence ecosystem behaviour when in short supply (see e.g. Sardans & Peñuelas, 2015). However, the availability of individual nutrients can be difficult to assess, as, for example, no perfect method exists to determine availability of N or P to plants (Binkley & Hart, 1989; Holford, 1997; Neyroud & Lischer, 2003). Commonly used proxies are N mineralisation (protocol 2.2.5 Nutrient mineralisation), soil NO₃ and NH₄ concentrations (Keeney & Nelson, 1982), soil C:N ratio (protocol 2.2.4 Soil carbon and nutrient stocks), Olsen P and Bray P (Bray & Kurtz, 1945; Olsen, 1954), and/or ion exchange resin membranes like Plant Root Simulator (PRS) probes (1.4.2 Soil nutrient), but these all have their drawbacks (Binkley & Hart, 1989; Holford, 1997; Neyroud & Lischer, 2003).

Although no perfect method exists to determine nutrient availability, some soil properties are very indicative of the nutrient status of a soil (Vicca et al., 2018). These include bedrock, texture (1.3.5 Soil texture), pH (1.4.1 Soil pH), bulk density (1.3.4 Bulk density), cation exchange capacity, and soil organic matter (SOM). These key soil physical properties are relatively easy to measure and in combination are very indicative of the nutrient status (see e.g. Van Sundert et al., 2018; Vicca et al., 2018). These data on soil properties and nutrients allow disentangling the role of nutrient availability as well as classifying a study site as nutrient-poor, nutrient-rich, or moderately fertile (*sensu* Vicca et al., 2012; Terrer et al., 2016).

Plant N and P uptake can be calculated from plant N and P concentrations and plant growth:

N and P concentrations are important to determine the nutrient status of a study and thus important information for modelling and meta-analysis (see Table 2 in the main paper). Other variables that can be of relevance in some cases are described in stress physiology (Supporting Information S5) and carbon and nutrient cycling (Supporting Information S2).

Below, we present a brief description of complementary measurements that are relatively easy to conduct routinely at any site or study and can provide a robust characterisation of nutrient availability across sites. This list largely corresponds to the measurements recently suggested in Vicca et al. (2018), which we refer to for further reading on the interpretation and relevance of the different measurements.

1.4.1 Soil pH (unitless)

pH is a measure of acidity in the soil and affects many chemical processes, such as plant nutrient availability. Most soil methods are conducted on air-dry soil: however, given that understanding the chemical environment that plants experience is important in ecosystem studies, we propose the use of field-moist soil in preference to dried soil for pH measurements. Soil pH is then carried out on a suspension of fresh field-moist soil in deionised water (DIW), or 0.01 M KCl or CaCl₂. Often both DIW and a saline suspensions are measured because both values provide different information. Soils in their natural condition can vary widely in the salt content, also within the same soil the concentration of salts vary with the variation in soil water content, these variations in salinity have

an effect in the measurements of pH. The impact of these variations on pH is minimised when measured in 0.01 M of a saline solution and allows valid comparisons of soil pH between seasons and years.

What and how to measure

We focus on measurements in water. The soil:water ratio depends on the amount of organic matter in the soil with a ratio of soil to water of 1:2.5 to 1:5 by weight for mineral soil. The method described here is based upon that employed by the Soil Survey of England and Wales (Avery & Bascomb, 1974) and by the Countryside Survey (Emmett et al., 2010), but measuring soil pH in deionised water using a 1:1 mixture is reported in the NRCS (2014) handbook. Organic soils, however, require a much higher ratio of soil to water of 1:10 or 1:20 by weight

Calibrate the pH meter in buffer solutions. Check pH 4 & 7 buffer calibrations regularly within a sample batch, for example every 10 samples. If either buffer calibration is more than 0.02 of a pH unit from the correct value, repeat calibration. Weigh 10 g of fresh field-moist soil into a 50 ml plastic pH beaker. Add 25 ml of deionised water and stir the suspension thoroughly. Allow it to stand for 30 minutes, stirring occasionally. Measure soil pH electrometrically using the calibrated pH meter.

Include a suitable number of duplicate samples, i.e. carry out the pH measurement twice on approximately one-tenth of the samples. Thoroughly rinse the pH probe between samples with a stream of water from a deionised water wash bottle. Ensure the glass bulb of the pH probe is cleared of soil and be particularly thorough after probes have been immersed in pH buffers. If duplicated samples are not in agreement, repeat the measurements on a small set of samples; from this set of information, determine whether outliers skewed the measurements (remove the outliers if there is good reason, e.g. instrument failure), or whether soil pH was highly variable (report average and standard deviation).

New probes for field measurements of pH are available for instant and fast pH measurements, which enables high-resolution measurement of pH in space and time (e.g. Nielsen et al., 2017).

1.4.2 Soil nutrients

Integrated assessment of soil cation and nutrient availability for plants

Resin membranes like Plant Root Simulator (PRS) probes (Western AG, Saskatchewan, Canada) absorb anions or cations (depending on the type of probe) that are in the soil solution. They thus provide an indication of the nutrient availability as experienced by the biota during the time of burial and are particularly useful for assessing relative differences among treatments and studies. The probes are inserted into the soil for a short period (e.g. 7 days) and are subsequently analysed in the lab for the nutrients of interest (e.g. NO₃, NH₄, P, K, Ca, Mg, Mn, Fe, Zn). The results indicate the flux of each of these nutrients over the time of burial. Caution is needed to avoid saturation of the probes (i.e. burial time should not be too long) and the absorption of ions is sensitive to soil moisture, which may complicate interpretation in studies where soil moisture differs between the treatments. More information is available from the website of the commercially available PRS probes (<u>https://www.westernag.ca/innov</u>). Instead of buying the commercial product, it is also possible to produce the probes for low cost (see protocol 2.2.5. Nutrient mineralisation).

Cation exchange capacity, exchangeable base cations, and soil electrical conductivity

One of the most important properties of soil colloids (clay and organic matter particles < 0.001 mm diameter) is their ability to adsorb, hold, and release ions. Colloids are generally negatively charged and thus attract primarily positively charged ions, i.e. cations. The more negative charges, the higher the capacity of the soil to bind cations, and thus the higher its **cation exchange capacity** (CEC, typically expressed as the amount of positive charges that can be exchanged per mass of soil).

For soil fertility, the **total exchangeable base cations** (Mg^{2+} , Ca^{2+} , and K^+ in particular) are especially relevant. These are the base cations bound to the negatively charged colloids. They can be taken up relatively easily by plant roots through exchange for H^+ . The fraction of CEC that is occupied by exchangeable base cations is termed base saturation. This fraction can be small, especially in acidic and leached soils where many of the negative charges are occupied by (acidic) cations, such as H^+ , Al^{3+} , or Fe^{3+} .

What and how to measure

Cation exchange capacity and total exchangeable base cations can be determined using the most common method of Brown (1943), for which 1 M buffer ammonium acetate solution (NH_4Ac) at pH 7 serves as the extractant. Soil samples are collected and sieved (< 2mm) and air dried.

Soil electrical conductivity (EC; mS m⁻¹)

This is the ability of soil to conduct an electrical current and is commonly expressed in units of milliSiemens per metre (mS m⁻¹). EC estimates the concentration of ions in the soil, namely the anions Cl⁻, SO₄²⁻, and HCO₃⁻ and the cations Na⁺, Ca²⁺, K⁺, and Mg²⁺ (Friedman, 2005; He et al., 2012). Although the relationship between conductivity and salt concentration varies somewhat depending on ionic composition, EC provides a simple and reasonably accurate estimate of solute concentration (Carter & Gregorich, 2006). In addition, as soil EC is affected by several soil properties, its measurement can also be used as a proxy for estimating directly or indirectly the variations in these properties, including soil texture, bulk density, soil water content, water-holding capacity, cation exchange capacity, organic matter, and subsoil characteristics (Corwin & Lesch, 2005a; Grisso et al., 2005). For this reason, over the years, EC has been largely used in agriculture to estimate soil salinity, nutrient availability and loss, soil texture, and available water capacity, being considered a reliable and cost-effective measurement (NRCS, 2014). As an example, high EC values often reflect poor plant growth conditions and the potential for salinity problems (Karlen et al., 2008).

As this variable has been shown to be closely related with distinct soil properties, its measurement assumes special importance under the context of climate change where some properties are expected to alter with consequences for soil quality and its functioning.

What and how to measure

The first measurements of soil EC were made on soil samples, but it was found to be more consistent to measure EC in soil extracts. Hence, the standard laboratory method for determining the EC of a

soil is by using an aqueous paste extract of soil and to measure the electrical conductivity of the solution using a conductivity meter (Richard, 1954; Carter & Gregorich, 2006). The determination is carried out to obtain an indication of the content of water-soluble electrolytes in a soil. Because the saturated paste extract method requires time and skill, a fixed soil:water ratio (e.g., 1:1 to 1:5) has been generalised when measuring soil EC and solute concentrations (ISO 11265, 1994). Knowing that EC in soil is dependent on several properties and therefore is highly variable, several samples should be taken from multiple locations.

Besides the methods based on an aqueous paste extract of soil, the apparent EC (EC_a, bulk soil electrical conductivity) has become one of the most frequently used measurements to characterise the spatial distribution of soil salinity at field scales. Nowadays, EC_a is considered an invaluable tool for the establishment of spatial variation and for identifying the soil properties influencing crop production in precision agriculture (Corwin & Lesch, 2005a, 2005b). EC_a has also been used to identify homogeneous areas within a field to implement experiments. Field methods used to measure EC_a include the Wenner array or four-electrode, time domain reflectometry (TDR) and electromagnetic (EM) induction (Carter & Gregorich, 2006). The EM method, by using a non-contact sensor, is the most commonly used because measurements can be taken quickly over large areas, the large volume of soil measured reduces local scale variability, and measurements are possible on relatively dry or stony soils because contact is not required between soil and sensor (Hendrickx et al., 1990).

Carbon and nutrient stocks

The soil carbon and nutrient stock is the amount of C, N, P, K, and other nutrients stored in the soil. These stocks are coupled with net primary production and decomposition of above- and belowground material and highly related to climate. For further details and how to measure see protocol 2.2.4 Soil carbon and nutrient stocks.

Soil organic matter (SOM, %)

It is important to determine the soil organic matter (SOM), as changes in environmental factors such as temperature or water inputs may alter the SOM content (directly and indirectly by influencing organic matter inputs), and variation among studies may also be explained by differences in SOM. Soil organic matter can be determined using the Walkley Black method or the loss-on-ignition (LOI) procedure described in Nelson & Sommers (2009). LOI is generally preferred over the Walkley Black method because is less time consuming. One of the aspects to consider when measuring LOI is the choice of combustion temperature in the furnace and the duration of time used for combustion.

What and how to measure

We propose the method of Ball (1964) which is determined by subtracting the weight of a soil after 16 h drying at 105 °C from a soil after placing in a furnace overnight at 375 °C. The amount of soil used to determine SOM is adjustable but should not be lower than 10 g fresh soil to ensure a representative sample. For more details also see Countryside Survey (Emmett et al., 2010).

Soil inorganic carbon (mass fraction: g C g soil⁻¹)

Some soils contain significant amounts of carbonates, especially soils in dry climates. Changes to warming or the precipitation regime may alter the carbonate content. Reporting carbonates is not routine, but may be important if a total carbon balance is required. We refer the reader to the methods described in Nelson & Sommers (2009).

1.4.3 Soil trace metals

Trace metals are a group of metals and metalloids (e.g. arsenic (As) and selenium (Se); hereinafter called "metal") found in low concentrations (< 100 mg kg⁻¹), in mass fractions of ppm or less, in some specified source, for example, soil, water, plant, or tissue (Duffus, 2002; Hooda, 2010). The most common trace metals are beryllium (Be), aluminium (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), As, Se, molybdenium (Mo), silver (Ag), cadmium (Cd), antimony (Sb), mercury (Hg), thallium (TI), and lead (Pb). Trace metals are important elements in the biogeochemistry of terrestrial ecosystems (Driscoll et al., 1994). The concentration of trace metals in soil directly relates to the growth and development of vegetation and reflects the supply of mineral nutrition to plants by soil (Zhanbin et al., 2013). Depending on the dose, trace metals can become potentially toxic for life (Kabata-Pendias & Mukherjee, 2007; Kabata-Pendias, 2010). Although trace metals are naturally present in soils (Kabata-Pendias & Mukherjee, 2007; Kabata-Pendias, 2010), their concentrations in soils are significantly influenced by anthropogenic activities, which greatly alter the biogeochemical cycles of trace metals and their bioavailability (Driscoll et al., 1994). The observed changes in soil properties could affect soil functioning through their impacts on the composition and activity of microbial communities, which can provoke toxic responses in soil microorganisms, including reducing microbial biomass and decreasing carbon mineralisation and disturbing enzymatic activities. Furthermore, metal stress has been found to change the structure and diversity of microbial communities (Certini, 2005; Hart et al., 2005; Hartmann et al., 2005; Frey et al., 2006). This eventually affects the biogeochemical system's functions driven by these organisms, since soil microorganisms are important agents in nutrient cycling and energy flow. Assessing the levels of trace metals in soils is crucial to determining the environmental impacts of climate change on soil quality, structure, and functioning (Curran-Cournane et al., 2015).

What and how to measure?

Total concentration of trace metals in soils: sample digestion is often a necessary step before determining total element mass concentration in soils. Various digestion methods are used to determine the mass concentration of trace metals in soils, including different combinations of concentrated acids (Gaudino et al., 2007). The dissolution of soil samples can be obtained by rigorous digestion using the standardised aqua regia extraction protocol which consists of treating a soil sample in a heated 3:1 mixture of hydrochloric (HCl) and nitric (HNO₃) acids (ISO 11466, 1995; USEPA 3050B, 1996). This is a partial digestion of the soil solid phase consisting of a very strong acid digestion that dissolves almost all elements that become "environmentally available" (McLean & Bledsoe, 1992; USEPA 3050B, 1996; USEPA 3051A, 2007). Although the aqua regia digestion method is internationally accepted to measure concentrations in soil, fractions of elements extracted by this

method are not available for biological uptake (Gaudino et al., 2007). If a total trace metals concentration is required, the soil samples are treated with a mixture of $HNO_3 + HCI + HF$ (hydrofluoric acid) using microwave heating with a suitable laboratory microwave system (USEPA 3052, 1996; EN 13656, 2002). After the extraction procedures (ISO 11466, 1995; USEPA 3050B, 1996; USEPA 3052, 1996; EN 13656, 2002; USEPA 3051A, 2007), the extract is filtered through 0.45µm nitrocellulose membrane filters, diluted, and analysed by atomic absorption spectrometry (flame: FAAS or graphite furnace: GFAS) or inductively coupled plasma spectrometry (optical emission: ICP-OES or mass: ICP-MS).

Another commonly used procedure to measure the "total" concentration of trace metals is the digestion with hot HNO_3 and hydrogen peroxide (H_2O_2) procedure also outlined in USEPA 3050B (1996). This method adds H_2O_2 in order to enhance the destruction of the organic matter in soil.

Sequential extractions of trace metals in soils: chemical extraction is employed to operationally define trace metal fractions, which can be related to chemical species, as well as to potential mobile, bioavailable, or ecotoxicological phases of a sample. Fractionation is usually performed by a sequence of selective chemical extraction techniques, including the successive removal, or dissolution, of these phases and their associated metals (Hlavay et al., 2004). The extraction procedures consist of reacting a soil sample with increasing strengths of chemical solutions. Numerous extraction procedures have been developed for trace metals (Sposito et al., 1982; McLean & Bledsoe, 1992; Singh et al., 1998; Wenzel et al., 2001; Imperato et al., 2003; Hlavay et al., 2004; Hooda, 2010). Supernatants from each fraction will be analysed by FAAS, GFAA, ICP-OES or ICP-MS.

For the Sequential extraction there are numerous methods using different extractants at different concentrations. For example, extraction with specific extracting agents, especially containing chelating agents, allows examination of the distribution of soluble exchangeable forms. Ethylenediaminetetraacetic acid (EDTA), used as extracting agent for many trace elements, has been widely applied in soil science and environmental chemistry (Kocialkowski et al., 1999; Michaud et al., 2007; Komárek et al., 2008).

1.4.4 References

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1.5 Meteorological measurements

Weather includes abiotic factors that impact the functioning of an ecosystem. Meteorological databases can provide fundamental information for climatological and climate-change studies. These observations can be taken manually (weather observer), in automated mode (data-logging system applications or weather station data), or in a hybrid scheme where weather observer efforts are supplemented by automated weather measurements. The most commonly measured meteorological parameters relevant to studies of land–plant–atmosphere interactions are those relating to energy and water fluxes (De Boeck et al., 2017), which in turn affect ecosystem carbon dynamics. Precipitation inputs (e.g. rain and/or snow) and rates of evapotranspiration (commonly estimated on the basis of air temperature, relative humidity, and wind speed values) are needed for water budget assessments. The meteorological systems, both manual and automated, require permanent supervision due to the complex and often harsh ambient conditions. The World Meteorological Organization (WMO) has prepared an extensive reference work on meteorological observations that can be found online (WMO, 2012).

1.5.1 Weather station and nearby weather station data

A climate-change studies should be equipped with a weather station for measuring climate drivers. A wide variety of weather stations are available but a high quality and reliable automated weather station (hereafter AWS) that is calibrated is desirable. An AWS consists of a weather-proof enclosure containing data logger, telemeter (optional), and meteorological sensors. The whole system should be mounted on a mast and it is powered and backed-up with a battery that is charged with a solar panel, wind turbine, or regular power line if available. The weather station should be mounted above the studied plant canopy. Microclimate often varies considerably across the ground-atmosphere interface (Graae et al., 2012). If possible, multiple measurements should be taken, and in such a manner as to make them relevant for the biotic question at hand (for example, within the soil, on the surface, and/or in the vegetation canopy, inside/outside experimental structures such as open-top chambers (OTC) or rainout shelters) while at the same time allowing calibration with climate station data (which are typically taken 2 m above ground). The specific configuration may vary based on the purpose of the system and local conditions, thus the measurements must be defined before the study. Meteorological measurements that are influenced by an experimental treatment (e.g. air and soil temperature, soil moisture in OTC) should be repeated at each experimental treatment preferably with replicates, and variables that vary across the site should preferably be measured at the block or plot scale (Lamentowicz et al., 2016). These plot-level temperature and rainfall measurements, combined with the site-level AWS data, provide the minimum data needed to monitor and assess whether the planned experimental modifications of climate drivers are being achieved. For all the instruments we recommend using the manufacturer's instructions regarding set-up and use.

The system may report in real-time using telemetry, which is generally more energy demanding, or record data for collection later. The real-time measurements allow early detection of measuring process disruption, for example logger or sensor failure, and hence might minimise climate data loss, whereas a non-telemetric scheme of the system operation requires visits to the site providing opportunities to inspect the study and enable eventual repairs of the system. Whether to use

telemetry or not might be determined by the accessibility of the site, the type of power source, and additional routine manual observation requirements.

Separate sensors connected to the AWS can monitor air temperature, relative humidity, barometric pressure and tendency (change in pressure), wind speed and direction, total, net and photosynthetically active shortwave and longwave radiation fluxes, rainfall and water equivalent snowfall, and snow depth (if relevant). Additionally, soil moisture, soil temperature, and soil matric potential may be observed. Many of the soil moisture sensors measure temperature alongside. This reduces the number of applied sensors and consequent soil disruption. The installation of sensors will depend on the type and configuration of the instrument, but some general approaches to installation can be identified.

New technological advancement coming up with small climate stations that can be placed inside the plots of most climate change experiments and measure multiple microclimate variables for long time periods. One newly developed mini climate station is the tomst TMS-4 data logger (<u>https://tomst.com/web/en/systems/tms/tms-4/</u>), that measures air and soil temperature and soil moisture and has proved to produce reliable data for a range of different habitats (Wild et al., 2019).

Soil sensors are often used to monitor seasonal changes of the soil environment parameters. Daily changes occur, but can often be considered noise against the slower seasonal signal. Rather than measuring at fixed depths, it is of interest to know the moisture and temperature – and matric potential if measured – near the soil surface, e.g. within 5–15 cm. In shallow soils such as in alpine areas, 3–5 cm is recommended (Körner & Hiltbrunner, 2018) at a point corresponding to the



maximum root density (Figure 1.5.1). In the best of all worlds, a second set of sensors would be placed at greater depth, perhaps near the bottom of the root system. Such positioning would capture the rare drought or snowmelt events that deplete or refill the whole soil profile moisture. In boreal forests, these deeper sensors are often placed at 50 cm below the surface. As much as we would like to standardise these depths, the variation in diurnal/seasonal cycles and in root water depletion depths prevents convergence on a single recommendation.

Figure 1.5.1 Soil profile: location of sensors in the soil should be located in relevant areas such as the topsoil just below the soil surface, where maximum root growth occurs and in different soil layers. Sensor locations depend on vegetation (i.e. rooting patterns) and horizonation of the soil. It is advised to locate sensors in similar positions in each plot.

When installing sensors in a plot it is generally best to install them horizontally (to measure temperature at the desired depth), which can be achieved by excavating a small trench from outside the plot into the plot (Figure 1.5.1). This prevents preferential flow of water along the cables.

Generally, in areas with rodents, it can be useful to protect the wire of a sensor with PVC tubes to prevent damage. In alpine areas, where there is a lot of snow in spring, it can be advisable to protect the wire higher up, because rodents can climb up on the snow.

For each of the sensors, the optimal **sampling interval** is every minute and should be reported in the form of half-hourly to hourly averages. For example, modelling of ecosystem gas exchange will require half-hourly (e.g. Papale et al., 2006) to hourly data input.

Data management: high-resolution AWS data need to be quality checked due to the possibility of malfunctioning sensors, lack of power supply or system failures etc. The automated quality control procedures are used for selection (flagging) of uncertain data and the final data quality assessment must be performed by professional personnel that are familiar with local conditions. This visual inspection can be done with basic graphic tools that plot the data and trends for all measurements. Although this method seems less accurate than setting prescribed limits that data must fit within, it does allow for human interpretation and recall of conditions at the site – for example, an automated limit in a script will fail to remember if it was a particularly cold week.

Reporting climate data: when reporting climate data it is important to specify the timeframe in which the data were collected, name of the location if the data were obtained from a weather station, and (if applicable) explain any data processing (e.g. summer temperature, daily mean, cumulative temperature; Morueta-Holme et al., 2018).

Meteorological measurements

A site-based AWS should measure air, soil, and canopy temperature, relative humidity, photosynthetic photon flux density (PPFD), soil moisture, rainfall, and, in windy regions, wind speed (and, if relevant, direction). These measurements will help put plot-level data into context as, for example, air temperature and photosynthetically active radiation affect the level of photosynthetic uptake of atmospheric CO₂ by plants. They are also necessary for gap-filling eddy covariance gas flux measurements (protocol 2.3.1 Ecosystem CO₂ and trace gas fluxes), used to quantify the greenhouse gas balance of multiple ecosystem types (Kang et al., 2018).

1.5.2 Air temperature (°C)

Among other variables, air temperature affects tissue, canopy, and soil temperature and is thus relevant for plant growth, water cycling, microbial activity, and phenological response, and therefore the level of photosynthetic uptake of atmospheric CO_2 by plants.

Air temperature should be measured at 2 m above the ground surface, as this is a standard height to compare with weather station data; however, measuring air temperature in the canopy and at ground level is biologically more relevant for the plants. Standard heights for measuring canopy temperature are 20 cm and ground level in low vegetation, and above the canopy in high vegetation, such as forests (Barr et al., 2007; Reichstein et al., 2007). For experiments that affect temperature it

is important to measure the treatment effects. For example, an OTC will not affect the air temperature at 2 m.

Tissue temperature, which is relevant for metabolic rates and the water cycle, can be measured directly using infrared thermometers (protocol 5.5 Leaf temperature).

Air temperature should be measured every 1–10 minutes, from which daily minimum, maximum, and cumulative temperature sum can be calculated. The daily minimum and maximum air temperature can be used for a rough estimate of potential evapotranspiration using the Hargreaves equation (Jensen et al., 1997).

1.5.3 Soil temperature (°C)

Soil temperature is a primary driver of biogeochemical reactions, impacting responses that climatechange studies often quantify, such as soil respiration (carbon efflux) and nitrogen mineralisation (Knoepp & Swank, 2002; Curtis et al., 2005, also see protocol 3.5 Soil temperature).

Sensors should be installed horizontally and installation depths vary depending on the study objective. When associated with soil respiration or decomposition, sensors should, at minimum, be installed 5 cm beneath the soil surface (Wangdi et al., 2017). For leaf litter decomposition, an additional sensor should be placed at a depth of 2 cm. Soil microbial studies may require temperature profiles layered deeper in the soil (Angle et al., 2017; Che et al., 2018). At least one sensor at each depth should be installed in every plot, more in environmentally heterogeneous plots, such as those with varying topography. Sensors should be connected to a data logger and take measurements every 1–15 minutes. In the absence of a meteorological tower, for example when portable chamber-based gas flux measurements are applied, soil temperature can be measured with handheld thermometer probes or thermocouples installed within the chamber (Collier et al., 2014).

SOILTEMP is an initiative to build a global soil temperature database to provide more relevant temperature data for species (<u>https://soiltemp.weebly.com/</u>).

1.5.4 Photosynthetic photon flux density PPFD (μ mol m⁻ 2 s⁻¹)

Photosynthetically active radiation (PAR) is defined as the spectral range of solar radiation (0.4–0.7 μ m) that is used by plants within the process of photosynthesis. The density of the flux of these light molecules is called photosynthetic photon flux density (PPFD) and is a quantitative measure of the energy that reaches the plant canopy. PPFD impacts the rate at which plants photosynthesise, affecting growth and carbon storage. PPFD data are used to calculate scattered light conditions (cloudiness), sunshine hours, and light-use efficiency (LUE), as well as in gap-filling eddy covariance



Figure 1.5.2 Photosynthetic photon flux density (PPFD) sensors should be arranged above the canopy (n = 1), between the canopy and sub-canopy (n varies depending on plot size/heterogeneity), and on the ground surface (n varies depending on plot size and heterogeneity; tree clip-art made available by ian.umces.edu/imagelibrary).

gas flux measurements. In forest ecosystems, multiple sensors can be used to partition LUE of the canopy and sub-canopy, which is important when determining the recovery response (Reed et al., 2014; Stuart-Haëntjens et al., 2015).

One sensor should be mounted above the vegetation, on top of the meteorological platform or tower, positioned to avoid shading by other instrumentation. Due to cloud variation, PPFD should not be retrieved from nearby weather stations. Light environments below a forest canopy will likely change following climate manipulation experiments, so when a sub-canopy is present, additional sensors should be placed between the sub-canopy and canopy, and on the ground surface (Figure 1.5.2). Multiple sensors installed below the canopy capture the heterogeneous light conditions. Sensors installed between the canopy and sub-canopy can be mounted and levelled lower on the meteorological tower, on small tripods, or on hand-made PVC poles.

1.5.5 Relative humidity (%)

Relative humidity in combination with air temperature is used to calculate vapour pressure deficit, which can impact plant mortality, stomatal conductance, and, consequently, greenhouse gas fluxes (Breshears et al., 2009; Will et al., 2013; Yuan et al., 2016). This metric is growing more important as temperature increases due to climate change also raise atmospheric moisture demand, unless relative humidity increases (Breshears et al., 2013).

Relative humidity sensors can be installed on a meteorological station or tower, and in forests, should be placed above the canopy as well as below the canopy to assess sub-canopy growth dynamics.

1.5.6 Precipitation (mm)

Precipitation is often measured in climate-change studies, because it indicates the amount of water entering a system. It should, however, be stressed that precipitation is not the amount of water available for plants. Depending on the soil and prior meteorological conditions (e.g. a drought period), a considerable amount is intercepted by plant canopy, runs off, or drains into deeper layers of the soil. As such, soil moisture is a better measure of water availability for plants.

A ground-level storage rain gauge collects rain accumulated over a given period of time. Accumulated rainfall is measured in mL of water and converted to mm or rain where Rain [mm] = Rain [mL] / πr^2_{Funnel} [m²] of the rain gauge. The quantity of rainfall accumulated should never exceed the storage capacity of the gauge. Therefore, the frequency of emptying the rain gauge depends on the precipitation in a given area. Data from ground-level rain gauges are robust and far less vulnerable to issues such as logger downtime or loss of power, etc. In tall vegetation (e.g. forest), readings are often biased by turbulence, and usually precipitation data must be adopted from a nearby location.

The **tipping bucket rain gauge** consists of a plastic collector that is balanced over a pivot and collects the precipitation. A pre-set amount of precipitation tips the collector and actuates a switch which is then electronically recorded or transmitted to a remote collection station. The tipping bucket can be less accurate, i.e. if the rain stops before the lever has tipped, which is then added to the next rainfall event. Also, heavy rainfall and snow events are often underestimated with tipping buckets (WMO,

2012). In places with high vegetation, falling leaves and needles can plug the rain gauge and should therefore often be checked and cleaned.

Plot treatments such as drought or any rain-interfering structures such as scaffolding, will alter the plot-level rainfall input, therefore measuring direct water inputs to the plots is important. Often a simple manual rain gauge is sufficient on treatment plots.

1.5.7 Soil moisture

Soil moisture is the amount of water in the soil (Robinson et al., 2008; Vereecken et al., 2008). It provides the biological moisture pool for microbial activity and plant transpiration supporting terrestrial life. Soil moisture dynamics are likely to respond in different ways to climate change, depending on whether it leads to drought, warming, or excess rainfall (Seneviratne et al., 2010). This will have a direct effect on the biologically available moisture pool, and oxygen levels in the case of wet soils. Moreover, because soil moisture controls microbial activity, carbon and nutrient cycling will be affected, as will greenhouse gas fluxes such as CO₂, CH₄, and N₂O.

For how to measure soil moisture see protocol 3.1 Soil moisture.

1.5.8 Rain throughfall

In manipulation experiments where the experimental structures may reduce the amount of rainwater that enters the experimental plots, rain throughfall may be measured. Throughfall is measured easiest with a funnel-storage bottle construction which is placed into the plant canopy. The volume of throughfall is measured at the same time as rainfall accumulated by the rain gauge. In cold regions, bottles need to be exchanged and then thawed in the laboratory for the rain volume to be recorded. The rain throughfall volume on a plot basis is then converted to mm rainfall using the funnel diameter (see 1.5.6 Precipitation). Throughfall and rain gauge values are used to calculate a percentage reduction in rainfall per plot.

1.5.9 Wind speed (m s⁻¹) and direction (degrees)

Wind speed and direction can help to interpret temperature measurements, snowmelt date and photosynthetic activity, and is most often associated with ecosystem scale gas flux measurements obtained by eddy-covariance flux towers. Sonic anemometers should be installed at 1.5–2 times vegetation height. Calculations of wind speed and direction may need to be adjusted in hilly terrain (Zitouna-Chebbi et al., 2015).

1.5.10 References

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1.6 Open science practice, reproducible workflow, and data management

Many ecological questions nowadays are related to complex drivers and mechanisms on large spatial and temporal scales which increasingly demands collaborations (i.e. research networks), handling of large datasets, and data sharing. For this, the study design, data analysis, and results need to be correctly and comprehensively reported, which are surprisingly often not the case (Hillebrand & Gurevitch, 2013; Haddaway & Verhoeven, 2015), frustrating researchers aiming to synthesise and upscale research developments (Halbritter et al., 2018; Morueta-Holme et al., 2018). Open science practice, reproducible workflow, and data management have recently received much attention in ecology and in science and when successfully applied these practises ensure high-quality data, which is available to others and in the future (Lind 2013). Funding bodies and publishers have recognised this and now often ask for a data management plan and open science practice (British Ecological Society, 2018). "Prereproducible" practise – a holistic approach of providing sufficient information about data and workflow – is becoming more common (Stark, 2018).

Data management is the planning of the "data cycle" in a research project, including how to create, process, document, share, store, and re-use the data (British Ecological Society, 2018). It can be applied to small and large projects and should be planned well ahead of the start of a project.

Alongside the planning of the study design and research questions, the workflow from collecting raw data, to the final results, should be planned, i.e. data curation, transformations, quality check, visual examination and analysis, data storage, and data availability beyond the project (create, process and store data). The raw data should always be retained and the workflow should follow a well-documented and script-based approach. This allows the script to be revised and rerun at any time and thus ensures transparency, reproducibility, and a robust workflow (British Ecological Society, 2017). Version control such as Git combined with a host (e.g. GitHub, Bitbucket) ensures transparency and reproducibility of the workflow. The data should always be stored in non-proprietary software formats to ensure long-term availability beyond the project. A common practice in medical and social sciences to enhance good research practice, though rarely applied in ecology, is to preregister the planned data analysis (Nosek et al., 2015). This ensures a thorough thinking about what data and analysis are needed and reduces problematic research practice (e.g. clarifies projects aims vs. hypothesis-testing or hypothesis-generating research, reduces risk of cherry picking; Fraser et al., 2018).

Thorough data documentation and metadata ensures that the data are available in the long term. Data documentation should be started early, done consistently, and updated regularly to ensure an overview of the methodology, data, data manipulation, and analysis. Complete data documentation and metadata is important for inter-study comparisons (see above) and enables data sharing and re-use.

Here we provide a list for how to correctly report study design, data analysis, and results from climate-change studies to make research reproducible and for synthesis (Table 1.6.1). This table was compiled from Hillbrand & Gurevitch (2013), Haddaway & Verhoeven (2015), and Gerstner et al. (2017).

 Table 1.6.1 Issues and guidance for how to correctly report study design, data analysis, and results from climate-change studies. Adapted from Hillbrand & Gurevitch (2013), Haddaway & Verhoeven (2015), and Gerstner et al. (2017).

| | Issue | Guidance |
|---|--|--|
| General | Methodology | Each study and dataset should be described in detail in a readme file, including a data dictionary and annotated dataset (Table 1.6.2; British Ecological Society, 2018). |
| | Necessary meta-data provided | Correctly report site characteristics: i.e. geographic location, elevation, vegetation type, soil physical and chemical properties, meteorological data (see protocols 1.2 - 1.5), and author information. |
| | | Results (including master theses, internal reports, etc.) should be publicly available. |
| | Data and study should be easy to find, and be accessible. | Data should be publicly available in a data repository. Funding bodies and journals are increasingly requiring this. |
| | | Publications should have useful keywords and titles to enable them to be easily found. |
| Study design | Study design is reported in sufficient detail | The description of the study design should be thorough; parts of it can be reported in the appendix if there is limited space. Correctly report: start, end date, and duration of the study treatment factors, levels, and interactions, design structure, e.g. factorial, nested, hierarchical level of replication: number of sites, blocks, plots, and sub-plots; including selection and randomisation process at each level spatial scale: size of the study unit, distance between sites, populations type of data sampled (predictors and covariates), and sampling precision for each (including any within-replicate sampling or pseudoreplication) sampling schedule: timing, frequency, including study design aspects such as treatment-control, before-after-control-impact, etc. (also see Table 1.1) description of the manipulated organism, population, or community should follow accepted taxonomic literature, e.g. The Plant List (TPL; http://www.theplantlist.org/) and the Taxonomic Name Resolution Service (TNRS; Boyle et al., 2013) and national or international classification schemes. |
| Response variables, predictors, and covariates | Measurements should be relevant, reproducible, and convertible | Follow established protocols, and guidance on which and how to measure predictors, response variables, and covariates. |
| | | Report which protocols are used, and describe any |

| r | | |
|----------------------------|--|---|
| | | adjustments that are made. |
| | | Describe all variables fully and report in readme files, data dictionaries, and datasets. |
| | | Measure useful covariates for synthesis and upscaling (see Table 2 in the main paper). |
| Data handling and analysis | Data manipulation is described in sufficient detail | Each step of data manipulation should be described and explained and be repeatable and reproducible (British Ecological Society, 2017). |
| | Comprehensive description of data analysis | Type of statistical tests used, response variables, covariates (explanatory factors) tested, posthoc or planned comparisons carried out, definition of statistical metrics if different from commonly accepted terms should be described. |
| | | Statistical software, packages, and versions used need to be reported. |
| | Reproducible workflow | The workflow from data manipulation, coding, analysing, and results output should be repeatable and reproducible (Lind 2013, British Ecological Society, 2017). |
| Results | Units need to be reported | Units for each variable should be reported. |
| | Raw data should be provided | Raw data or summary statistics with mean (or median), variation around the mean and sample size should be reported. |
| | Negative results should be reported | Report negative results. |

Table 1.6.2 Content for a readme file for a research project and a data dictionary.

a) Readme file

- 1. Project information
 - a. Project summary
 - b. Funding information
 - c. Primary contact information
 - d. Project partners, students, collaborators
 - e. Research sites and basic site information
 - f. Information on data repositories
 - g. Naming conventions for data files, datasets, co-variables (taxa, studies/experiments, treatments, sites), response variables
 - h. Data access, authorship rights, data policy and acknowledgements
- 2. Studies and/or experiments
- 3. Publications
 - a. Publications

b. Master/doctoral theses, reports etc.

4. References

b) Data dictionary

- 1. Content of the dataset
- 2. Data collection methods
- 3. Dataset authors and collaborators
- 4. Location of data collection
- 5. Time of data collection
- 6. Study design
- 7. Data development and curation
- 8. Other relevant datasets within the project (e.g. predictors)
- 9. Data usage publications
- 10. Data dictionary (variable name, type, range, factor level, measurement type and unit/format)

Where to start?

Gerstner et al. (2017) give guidance on how to make the reach of your research broader and longer lasting; Haddaway & Verhoeven (2015) explain how to correctly describe methodology in ecology to make the research repeatable; Hillebrand and Gurevitch (2013) provide a checklist for reporting study details in manuscripts. The British Ecological Society (2017) have produced a useful guide for Reproducible Code in Ecology and Evolution. Lind (2013) presents lessons learned about data management in NutNet.

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