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Handbook of standardized protocols for collecting plant modularity traits

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ABSTRACT

Plant modularity traits relevant to functions of on-spot persistence, space occupancy, resprouting after disturbance, as well as resource storage, sharing, and foraging have been underrepresented in functional ecology so far. This knowledge gap exists for multiple reasons. First, these functions and related traits have been considered less important than others (e.g., resource economics, organ-based traits). Second, collecting data for modularity traits can be difficult. Third, as a consequence of the previous points, there is a lack of standardized collection protocols. We now feel the time is ripe to provide a solid conceptual and terminological framework together with comparable protocols for plant modularity traits that can be applicable across species, regions and biomes. We identify a suite of 14 key traits, which are assembled into five groups. We discuss the functional relevance of each trait, supplying effective guidelines to assist in the use and selection of the most suitable traits in relation to specific research tasks. Finally, we are convinced that the systematic study and widespread assessment of plant modularity traits could bridge this knowledge gap. As a result, previously overlooked key functions could be incorporated into the functional ecology research-agenda, thus providing a more comprehensive understanding of plant and ecosystem functioning.

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1. Introduction

Plant functional ecology started as a discipline describing plant life history strategies in small geographical regions (e.g., Grime, 1979) and developed to broad-scale, comparative syntheses (Wright et al., 2004; Chave et al., 2009). This has been facilitated by using trait-based approaches that allowed the identification of i) important axes of plant differentiation, such as size and economics spectra reflective of allocation trade-offs (e.g., Westoby, 1998; Wright et al., 2004), and ii) drivers of community assembly (Götzenberger et al., 2012). At the same time, functional ecology is falling short with regards to some crucial plant functions, such as belowground nutrient acquisition, clonal multiplication, and vegetative regeneration (Laliberté, 2017; Ottaviani et al., 2017). This gap has been partly caused by focusing on a set of functional traits that are mainly associated with resource acquisition and sexual reproduction (Westoby, 1998; Wright et al., 2004; Chave et al., 2009). These traits are, in most cases, relatively easy to collect and measurable on aboveground organs, namely leaves, stems, and flowers/fruits, for which standardized procedures were developed (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013).

So far, most trait-based studies and approaches rarely considered that plant organs are assembled into modules – defined as the basic architectural building blocks of a plant which are accumulated during growth (Fig. 1). The position, size and persistence of the modules i) generate the intra- and interspecific variability of plant shapes, and ii) most importantly, affect plant functioning. Understanding plant modularity has been a major challenge for comparative plant ecologists for quite some time (Raunkiaer, 1934; Hallé et al., 1978; White, 1979). We now feel the time is ripe to consistently incorporate plant modularity into functional ecology.

Plant modularity traits are ecologically relevant, as they can effectively capture functions of on-spot persistence, space occupancy, resprouting after disturbance, resource storage, sharing, and foraging (for c overviews, see Ottaviani et al., 2017 and Klimešová et al., 2018). Yet, they remain largely understudied compared to e.g., traits related to resource acquisition. We therefore consider it essential to provide standardized protocols for fully including plant modularity traits into functional ecology analyses. Data comparability is indeed crucial for syntheses, and the publication of the previous trait-collection handbooks triggered widespread trait-based studies (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013). These handbooks (while very useful for aboveground, organ-based traits) contain only a limited number of modularity traits and do not provide the necessary framework and level of detail required for their appropriate sampling and assessment. The primary aim of our initiative is to fill this gap, namely to present standardized collection protocols for 14 key modularity traits (Table 1) potentially applicable across species and biomes. We do so by offering i) a robust conceptual and terminological framework, ii) synthetic description of functional relevance of each trait, and iii) detailed protocols for trait assessment including field- and lab-procedures.

1.1. Aims and structure of the handbook

We identified 14 plant modularity traits arranged into five major trait-groups: Anatomical features, Bud bank, Carbohydrate storage, Clonality, and Longevity and growth (Table 1). Based on our expertise working in different biomes and focused on different growth forms, we selected what we consider to be the most relevant plant traits that are informative on functions of on-spot persistence, space occupancy, resprouting after disturbance, resource storage, sharing and foraging (Weiher et al., 1999; Ottaviani et al., 2017; Klimešová et al., 2018). While some of these traits are fairly well-known (e.g., clonal and bud bank traits) for a few biomes, community types or growth forms, others (e.g., anatomical features, carbohydrate storage, longevity) have rarely been collected for large sets of species in a comparable, meaningful way for functional ecology.

To better explain and guide readers through plant modularity, we present four sections that are not specific to any one trait. The first section on "*Plant modularity*" provides a general framework and vocabulary for trait description and assessment. The second part reports the "*Field sampling protocol*" where essential information and guidance related to plant collection are described (see also Appendix I "*Standardized site description and sample labelling*"). The third section on "*Experimental assessment of resprouting after disturbance*" (included as Appendix II) illustrates methods and ideas for testing functionality of the proposed bud bank traits. The fourth part is the "*Glossary*" (included as Appendix III) explaining specialized terminology. Plant ecologists should be aware that the assessment of plant modularity traits is time-consuming and requires destructive sampling of plants. We are convinced that combining plant modularity traits with other more widely studied traits is necessary to achieve a more realistic and comprehensive understanding of plant and ecosystem functioning.

2. Plant modularity: a conceptual framework

A major challenge for assessing plant modularity is the absence of a standard practice of how to look at the plant body as a whole. For developing comparable, standardized collection procedures for

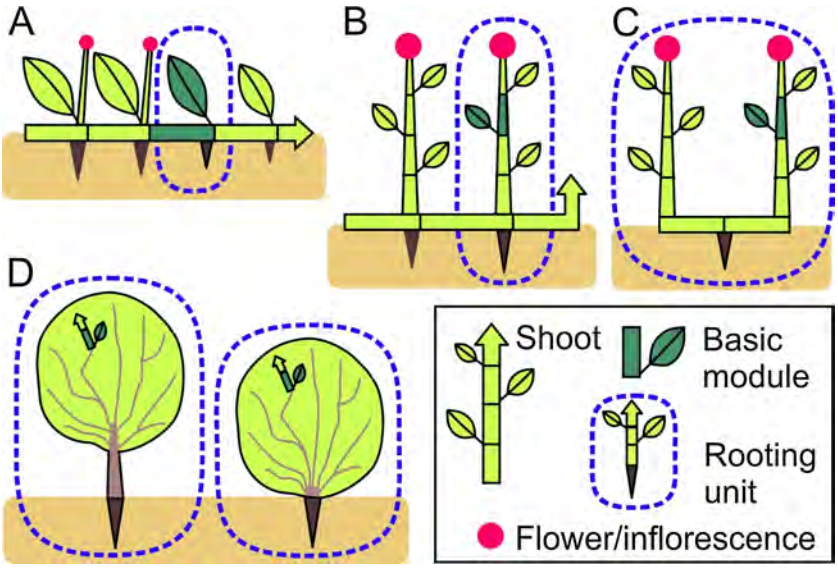

Fig. 1. Hierarchical nature of plant modular structure. A – rooting unit corresponding to one basic module (creeping herb); B – rooting unit (shoot) composed of several basic modules (clonal herb); C – rooting unit composed of several shoots (nonclonal herb); D – rooting unit formed of a branched shoot system (nonclonal woody plant, e.g., tree and shrub).

Table 1
Overview of plant modularity traits (variable type, units, definition) with trait-groups ordered alphabetically as presented in this handbook, with related functions and effects on ecosystems.

Trait-group	Specific trait [Variable type; Units]	Definition	Major functions	Effects on ecosystem
Anatomical features	*1. Conduit diameter* [Continuous; μm]	Stem base cross-sectional conduit diameter	Transport capacity; Growth potential; Resistance to frost and drought; Recovery after damage	Tissue decomposability; Productivity; Carbon sequestration; Water uplift
	2. Tissue type ratio [Continuous; Dimensionless]	Proportion (percentage) of tissue for storage, transport and support in the oldest stem parts		
Bud bank	*3. Bud bank size* [Continuous; Numerical]	Total number of buds stored in bud bearing organs per rooting unit	Recovery after damage; Space occupancy; On-spot persistence; Competitive ability; Protection of vital tissues	Productivity; Carbon sequestration; Soil fixation
	4. Bud bank fluctuation [Continuous; Dimensionless]	Maximum to minimum number of buds in bud bearing organs per rooting unit within a growing season		
	5. Bud preformation [Discrete; Categories]	Degree of shoot development in a bud before sprouting from bud bank		
	6. Bud protection [Discrete; Categories]	Bud bank coverage by a plant organ (leaf), tissue (wood, bark) or soil		
Carbohydrate storage	*7. Carbohydrate type* [Discrete; Nominal]	Composition of individual sugars forming total non-structural carbohydrates in storage organs	Resource storage; Recovery after damage; On-spot persistence; Competitive ability; Osmotic protection	Carbon sequestration; Productivity; Biogeochemical cycles; Tissue decomposability
	8. Carbohydrate concentration [Continuous; mg g^{-1}]	Concentration of total non-structural carbohydrates in storage organs		
Clonality	*9. Type of clonal growth organ [CGO]* and *bud-bearing organ [BBO]* [Discrete; Nominal]	Type of organ(s) enabling plants to regenerate and/or reproduce vegetatively	Space occupancy; Resource storage, foraging and sharing; Recovery after damage; Competitive ability	Carbon sequestration; Productivity; Counterbalancing environmental heterogeneity; Soil fixation
	10. Lateral spread [Continuous; m $year^{-1}$]	Distance between offspring rooting units and parental rooting unit		
	11. Multiplication rate [Continuous; Dimensionless]	Number of offspring rooting units produced by a parental rooting unit per year		
	12. Persistence of connection [Discrete; Categories]	Period of connection between offspring rooting units and parental rooting unit		
Longevity and growth	*13. Age* [Continuous; Years]	Potential maximum plant age	On-spot persistence; Competitive ability	Carbon sequestration; Biogeochemical cycles; Plant-soil feedback
	14. Radial growth [Continuous; mm]	Average annual plant increment in plants with secondary thickening		

modularity traits, we base our approach on the hierarchical structure of plant bodies. We do so by combining previous knowledge, approaches, and methods (Hallé et al., 1978; Givnish, 1979; Serebryakova, 1981; Groff and Kaplan, 1988).

2.1. Modular growth

All plants grow through the addition of modules (White, 1979), which are construction units produced by apical meristems (Godin and Caraglio, 1998), and consist of an internode, a node, a leaf and an axillary bud. The repetitive production of a series of similar basic modules (modular growth) results in the development of a complex plant body. One of the advantages of modularity is the possibility of losing some modules (for example, in the case of stress or disturbances) without jeopardizing overall plant survival, which is an important characteristic of plants.

A single basic module could be either i) a potentially self-standing individual with its own roots (rooting unit), as in a creeping herb (Fig. 1A), ii) several basic modules together forming a shoot, and the shoot is a self-standing rooting unit (Fig. 1B), iii) several basic modules assembled together into a vertical shoot and several of these shoots having a common root forming a rooting unit (Fig. 1C), and iv) a basic module being a tiny piece of a large hierarchical body (shoot system), as in a tree or shrub (Fig. 1D).

2.2. Architectural models

The diversity of modular constructions can be described as architectural models (Fig. 2) using the following parameters: number of rooting units, shoot types, branching, shoot longevity, aboveground woodiness and Raunkiaer's life forms. We will refer to these models and parameters in each trait description.

Number of rooting units. A rooting unit is a potentially physically independent individual with a single connection between aboveground (shoot or shoot-system) and belowground plant organs (root or root-system). Some plants are formed with one rooting unit for their whole life (genet; Fig. 2A–C), whereas others produce more rooting units during their lifespan (a genet with many ramets; Fig. 2D–G). Aboveground shoots may also generate new rooting units by i) producing them on aboveground horizontal stems (stolons), or ii) shedding vegetative propagules (bulbils or plantlets) originating from aboveground plant parts capable of generating adventitious roots once they reach the soil. Shoots freely floating in water may produce independent ramets even without root production, i.e., only by fragmentation of shoots.

Shoot type. The initial shoot of a plant is called the primary shoot. Some plants only consist of a single shoot throughout their entire life (Fig. 2A). If a plant body is composed of multiple shoots or by a single shoot that replaces a previous one, these shoots may have one of two origins: they may derive from a preceding shoot (axillary shoot; Fig. 2D–E) or from root sprouting (adventitious shoots; Fig. 2F–G). To recognize whether a shoot derives from another shoot or from a root, one can use morphological and anatomical markers (Fig. 3).

Some plants are capable of producing shoots and, eventually, rooting units from leaves, for example, some species of *Saintpaulia*, *Begonia*, *Kalanchoe*, and *Asplenium*. In most of these species, sprouting from leaves is not necessary for completing the plant life cycle, but it is an additional shoot-production mechanism. Some plants have their bodies so reduced that shoots cannot be distinguished (e.g., *Rafflesia* spp., *Streptocarpus* spp., Podostemaceae; Fig. 2H).

Branching. Stem branching is described as either monopodial or sympodial. In monopodial branching, a shoot keeps growing vegetatively for a potentially unlimited period without its apical meristem ever transitioning to flowering (vegetative shoot), but its branches may flower (flowering shoots; Fig. 2D). Sympodial branching means that branches are replacing or overtopping each other and all of them are potentially able to produce flowers (Fig. 2B, C, E).

Shoot longevity (Cyclicity). Although the whole plant may be polycarpic (i.e., iteroparous), individual shoots when flowering are always

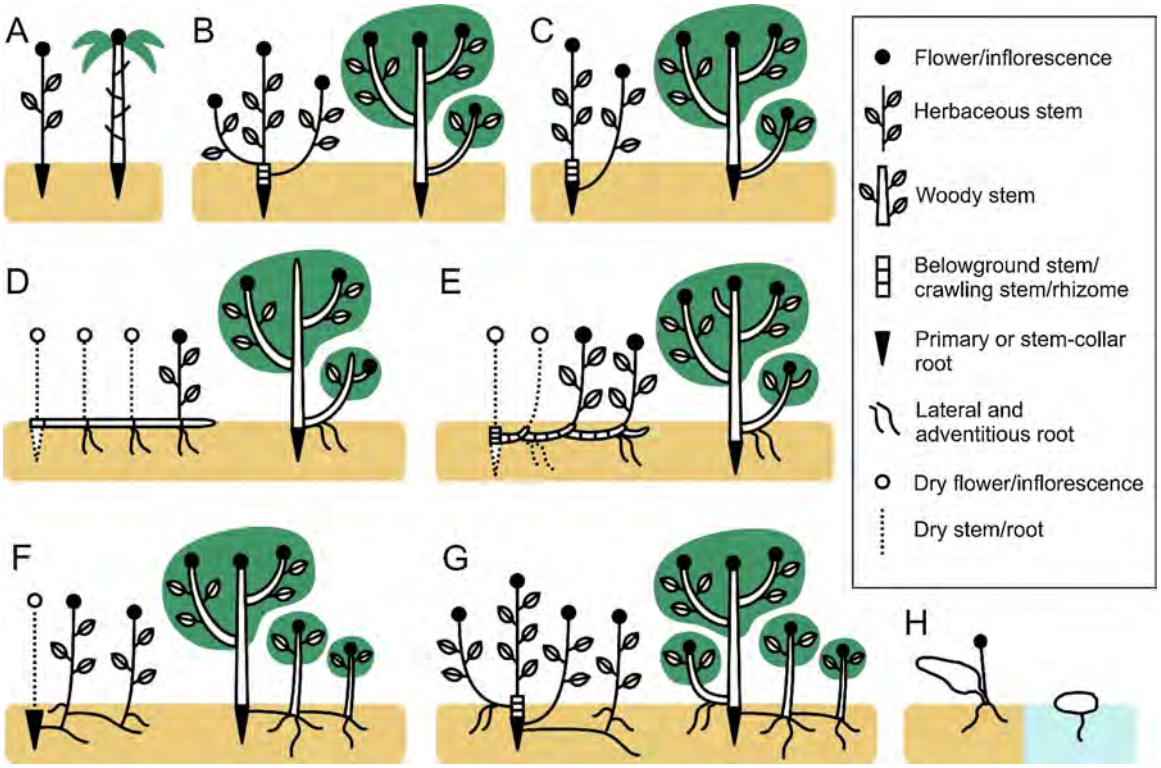

Fig. 2. Architectural models for herbaceous and woody plants. One rooting unit: A – only a primary shoot; B – nonclonal perennial; C – root sprouting nonclonal perennial; Several rooting units: D – monopodial clonal; E – sympodial clonal; F – only root sprouting; G – root sprouting and axillary branching; H – special cases with reduced plant body. Cyclicity and life form may be assessed in each model (except for type H).

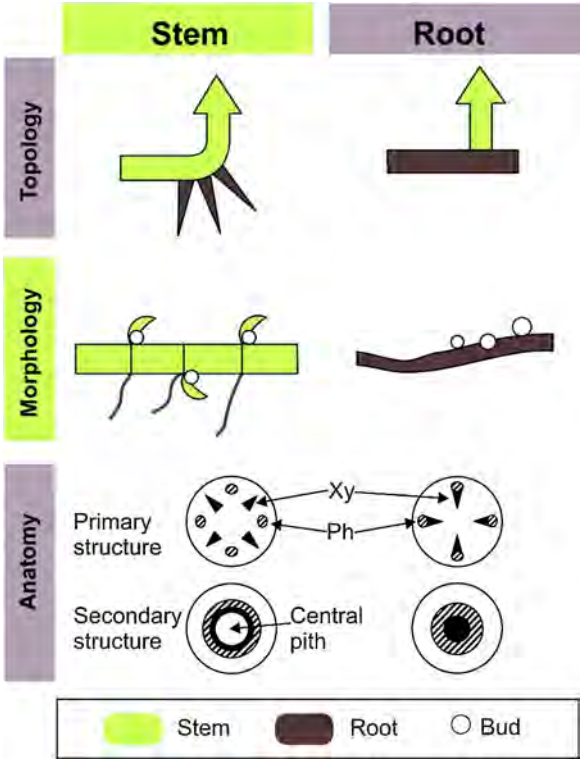

Fig. 3. Recognition of shoot origin – topological, morphological and anatomical markers. A stem with axillary buds is characterized by the presence of nodes, leaf-scales or their scars and centrifugal maturation of xylem in the centre of the organ (primary structure) or by the presence of a central pith in the organ (secondary structure). A root with adventitious buds is characterized by the lack of nodes, leaf-scales or their scars and centripetal maturation of xylem in the centre of the organ (primary structure) or by the absence of a central pith in the organ (secondary structure). Xy: primary xylem; Ph: primary phloem.

monocarpic (i.e., semelparous) and hence they cease growing after flowering and fruiting. The number of years (or seasons) occurring between bud-sprouting and shoot-flowering determines shoot longevity (cyclicity, Klimešová et al., 2016a). Cyclicity of a shoot may be recognized according to the remains of old leaves or leaf scars. Monocyclic shoots develop in a single growing season and no remains of old leaves are visible at the stem base. Polycyclic shoots live for more than one growing season and their development in a population is not synchronized: current-year shoots could be vegetative, while shoots from preceding years may be flowering. These shoots have remnants of last-year's leaves at their base, and the development of successive shoot generations may overlap.

Aboveground woodiness and Raunkiaer's life forms. In seasonal climates, herbs lose aboveground biomass during adverse dry or cold seasons and then sprout from buds located belowground (geophytes, Raunkiaer, 1934) or close to the soil surface (hemicryptophytes). Woody plants (shrubs and trees) in seasonal climates persist aboveground and continue to grow after rest from buds located aboveground (chamaephytes and phanaerophytes). This difference is connected to the anatomical structure of aboveground stems. Woody plants have long-lived aboveground stems that contain a large amount of lignified tissue, while herbs have generally softer aboveground stems that are short-lived. Intermediate growth forms do occur, namely as sub-shrubs with woody stem bases (close to the ground) and herbaceous upper parts.

3. Field sampling

Basic information about sampling sites and species should be collected according to the standardized protocol proposed in Appendix I ("*Standardized site description and sample labelling*"). Always sample 3–5 mature, healthy, well-developed individuals per species. To be able to assess modularity traits, a plant sample must include its belowground organ(s). In woody plants problems arise due to plant size, whereas herbs have more issues related to the fragility of belowground organs, particularly in dense vegetation where individuals are tightly intermingled with each other. Here, we provide common procedures applicable across species, growth forms and regions, with special cases described for each individual trait.

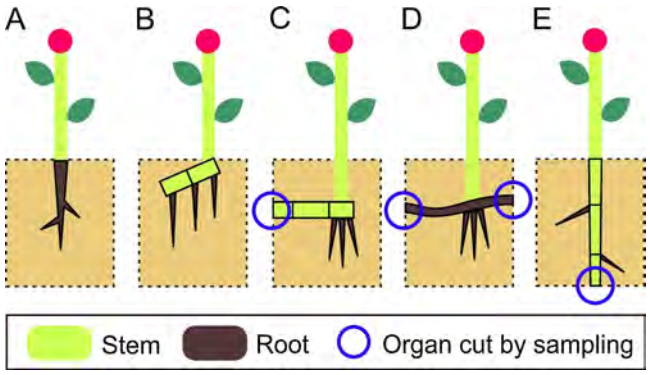

Fig. 4. Possible scenarios of relative position among different organs during plant sampling. A-B – examples of successful sampling where all important organs for a trait evaluation were sampled; C-E – examples of unsuccessful sampling, where crucial organs for assessing modularity traits were not collected.

Sampling herbaceous plants. First, locate an upright flowering shoot and excavate it including the soil from the approximate area of the projected foliar coverage at ground level (Fig. 4). Sampling depth should be: i) about 10 cm in shallow skeletal soils, ii) about 20 cm in dry soils, and iii) 30 cm or more in wetlands. For large herbs (e.g., more than 1 m tall), sampling should go deeper. After obtaining the sample, separate the target shoot, its belowground parts (cleaning them from soil) and dead parts (e.g., leaves and shoots). Then, visually inspect the sample to determine whether the whole structure of the perennial belowground organs (excluding fine roots) was extracted (Fig. 4A, B). If there are signs indicating that major organs were involuntarily broken and left behind during excavation (Fig. 4C–E), the whole procedure should be repeated with special attention to depth and growth direction of the organs. In this case, start sampling a new individual going deeper and/or wider than previously done. If the plant is not upright but creeping on the soil surface or has a visible aboveground horizontal stem, follow it carefully and excavate all rooted parts. Because one plant may combine aboveground and belowground horizontal stems, caution at this stage is recommended. Caution should also be exercised when a shoot is emerging laterally from a thin root (Fig. 4D) because roots can be easily broken and overlooked. Missing this thin connection of a shoot from a thin root could prevent the possibility of recognizing the rooting unit and, therefore, correctly assessing clonal growth.

Sampling woody plants. If equipment to sample whole plants is not available, try to find small saplings and proceed as described for herbs. Examine whether saplings have a primary root system (Fig. 4A) or have shoots derived from roots or belowground stems (Fig. 4B–E). The formation of a right angle between the trunk of the plant and its major belowground axis is an indication that the sapling was derived from a pre-existing plant (which may or may not be present at the time of sampling). If sampling large, mature shrubs or trees, excavate a trench crossing approximately the diameter of the projected crown at soil level. Proceed carefully, leaving belowground organs in place and only removing soil to the depth of the coarse roots.

For each of the five trait-groups (Table 1), we propose specific parts of rooting units to be sampled in order to effectively assess the trait(s)

of interest – see each trait description for specific guidelines. When fresh samples are necessary (i.e., clonal and bud bank traits), ensure that sampled material will not decay or continue to grow before trait assessment. In temperate climates, maintain the collected samples under wet and cool conditions (below 20 °C in plastic bags) until the samples are transported to the laboratory for subsequent analyses. This plant material can be stored in the lab for one week at 4 °C. If sampling in remote areas experiencing hot climates (e.g., mediterranean, tropical), cooling is necessary to prevent tissue degradation. Samples should be wrapped in moist paper or cotton gauze embedded with 70% ethanol. The wrapped samples should be put into a plastic bag inside a thermal container. If a refrigerator is available, put ice gel inside the bag and replace gel each day. These samples can be stored for 7–10 days until they are brought to the laboratory where preservation in 70% ethanol is recommended. For carbohydrate trait assessment, plant material should be processed immediately after field collection (for details, refer to the trait description below).

4. Traits

4.1. Anatomical features

Plant growth and allocation to different tissues are constrained by plant size, evolutionary history and ecological variables, producing tradeoffs within a plant body (Schweingruber et al., 2014). Connection between aboveground photosynthetic tissues and belowground absorptive and anchorage organs is vital for any plant. Stems are employed in the key roles of storing and transporting resources and supporting aboveground biomass. Belowground, roots acquire nutrients to be transported aboveground.

Anatomically, three main tissues (cells sharing a common function) provide specific functions: (1) parenchymatic cells serve primarily for carbohydrate storage (potentially affecting recovery after damage); (2) fibers and lignified cells support the plant body against gravity; and (3) conducting cells (vessels or tracheids) are responsible for transport capacity and growth potential as well as resistance to embolism caused by frost and drought (e.g., Petrucco et al., 2017; Olson et al., 2018; Fig. 5). We propose analysing cross sections of the stem base to gather information on *Conduit diameter*, and the oldest part of stem for *Tissue type ratio* (proportion of different tissues) – see below.

4.1.1. Conduit diameter

Embedded in a matrix of parenchymatic cells and fibers, a network of vessels or tracheids carries out the key function of transporting ascending water and nutrients in vascular plants. Plants optimize transport efficiency by widening the size of the water conduits from the plant top downwards (Anfodillo et al., 2013; Rosell et al., 2017; Olson et al., 2018). The benefits of narrower conduits for avoiding embolism are well established: they reduce the water conductive efficiency (hydraulic conductivity) of xylem but provide more security under drought or freeze-thaw cycles (embolism less likely; Mayr et al., 2006, but see Gleason et al., 2016). Large comparative studies of woody plants, showed that smaller conduit diameter is typical for short stature species inhabiting regions with freezing temperatures while conduit diameter is very variable in the tropics (Hacke et al., 2016). Although we have only limited data for herbs, they show the same pattern, and conduit diameter in the stem of graminoids tends to decrease with altitude in cold deserts (Doležal et al., 2019).

Trait definition: Stem base cross-sectional conduit lumen diameter.

Type of variable and units: Continuous; μm.

Sampling procedure: Collect plant samples from the stem base (the lowest part of aboveground shoot close to rooting point), taking note of plant height (length in plants growing horizontally). In herbs, cut small discs (ca. 2 to 6 cm long). Place samples in plastic bags containing 40% ethanol to prevent plant tissues from drying out and degrading (Schweingruber and Poschlod, 2005; Gärtner and Schweingruber, 2013). In woody species, extract wood cores preferably along the slope contour to avoid reaction wood. In trees and large shrubs, extract a wood core at breast height (1.3 m aboveground) using a stem increment borer. In smaller shrubs, cut small discs at the base, avoiding possible basal enlargements or burls.

Trait assessment: Cross section wood cores and discs with a microtome, stain them with Astrablue/Safranin and preserve them in Canada Balsam (for details see Gärtner and Nievergelt, 2010; Gärtner and Schweingruber, 2013). All lignified cell-walls accept Safranin and are therefore red-stain. Non-lignified walls accept Astrablue and are blue-stained. Vessel lumen areas remain white. Quantify the cross-sectional conduit lumen diameter using image analysis (e.g., ImageJ; Schneider et al., 2012, or ROXAS; von Arx and Carrer, 2014) by measuring the 5–50 largest conduits in each cross section. For woody plants the same cross section may be used for *Tissue type ratio, Age* and *Growth* traits (see related sections).

Special cases and problems: None.

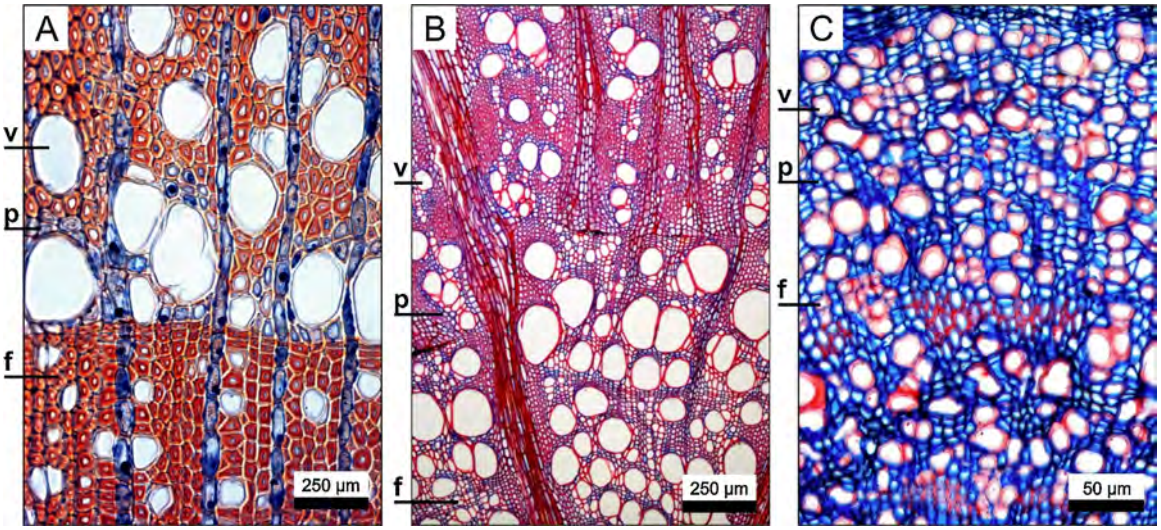

Fig. 5. Examples of different cells and tissue arrangements in stems. A – *Syringa vulgaris*; B – *Clematis vitalba*; C – *Silene galatea*. v: vessels (conduits; empty white, transport); p: parenchymatic cells (blue cells, storage); f: fibers (red cells, support).

Recommended literature: Schweingruber and Poschlod (2005); Gärtner and Nievergelt (2010); Anfodillo et al. (2013); von Arx and Carrer (2014).

4.1.2. Tissue type ratio

Parenchymatic (storage), water conductive (transport), and mechanical tissues (support) provide key functions for plants. These tissues are constrained and controlled by genetic and environmental factors (Plomion et al., 2001), and interspecific differences are substantial, especially in herbaceous plants (Doležal et al., 2018; Fig. 5). *Tissue type ratio* has rarely been used in the comparative ecology of herbs, while this trait is more frequently studied in trees. We know from large comparative studies that the amount of parenchyma in secondary xylem varies interspecifically along climatic gradients and growth forms (Morris et al., 2015). Studies also indicate that anatomical structure constrains carbohydrate storage as plant organs differ in their amount of parenchyma (e.g., Plavcová et al., 2016).

Trait definition: Proportion (percentage) of tissue for storage, transport and support in the oldest stem parts.

Type of variable and units: Continuous; % of different tissues (dimensionless).

Sampling procedure: Collect plant samples from the oldest part of a stem, taking note of plant height (length in plants growing horizontally). In nonclonal herbs cut small discs (ca. 2 to 6 cm long) from the oldest part of the plant (i.e., transition between root and shoot, root collar; Fig. 6A, B). In clonal herbs with stem-derived clonality, sample the oldest well-preserved living rhizome (ca. 2 to 6 cm long; Fig. 6C, D). In clonal herbs with root-derived clonality, sample the stem base close to its attachment to the root (Fig. 6E, F). Place samples in plastic bags containing 40% ethanol to prevent plant tissues from drying out and degrading (Schweingruber and Poschlod, 2005; Gärtner and Schweingruber, 2013). In woody species, refer to *Conduit diameter*.

Trait assessment: Follow the same procedure for preparing cross sections as described in *Conduit diameter*. Under the microscope, characterize a zone of the cross section between two rays (more than one ray can be included in the selected zone). If the wood is rayless or it lacks secondary thickening, select ¼ of the stem cross-sectional area. Next, estimate the total area (cell lumina plus cell walls) of the zone excluding one of the rays on either side. Then, estimate area of all conduits, fiber area, ray area (if present) and axial parenchyma area (blue-stained) by drawing multiple polygons that enclose each tissue type (see details in Crivellaro et al., 2012). Any image analysis software (e.g., ImageJ, ROXAS) can measure this trait.

Recommended literature: Crivellaro et al. (2012); von Arx and Carrer (2014).

4.2. Bud bank

Plants accumulate buds on their bodies by producing new modules as they grow (see "*Plant modularity*"). These buds may be used for branching, flowering, or seasonal regrowth or may remain dormant for future use (e.g., to recover after damage) (Klimešová and Klimeš, 2007; Vesk and Westoby, 2004; Pausas et al., 2018). Dormant buds waiting for resprouting opportunities are usually protected against damage caused by disturbance(s) and are collectively recognized as the bud bank (BB) of a plant. In this handbook, we consider BBs composed of only buds located belowground (buds protected by soil; Vesk and Westoby, 2004) or aboveground buds covered by bark (Pausas, 2015), sunken inside the woody tissue (Burrows et al., 2010; Charles-Dominique et al., 2015), protected by leaf bases (apical buds, e.g., palms) or originating as a specialized leaf structure (e.g., colleters; da Silva et al., 2012). Plants with a bud bank generally store carbohydrates which provide energy for resprouting (Pausas et al., 2018).

The vital organ for plant growth and sprouting is the stem, often containing most of the buds. However, stems need to employ growth modifications in order to be located belowground and store resources

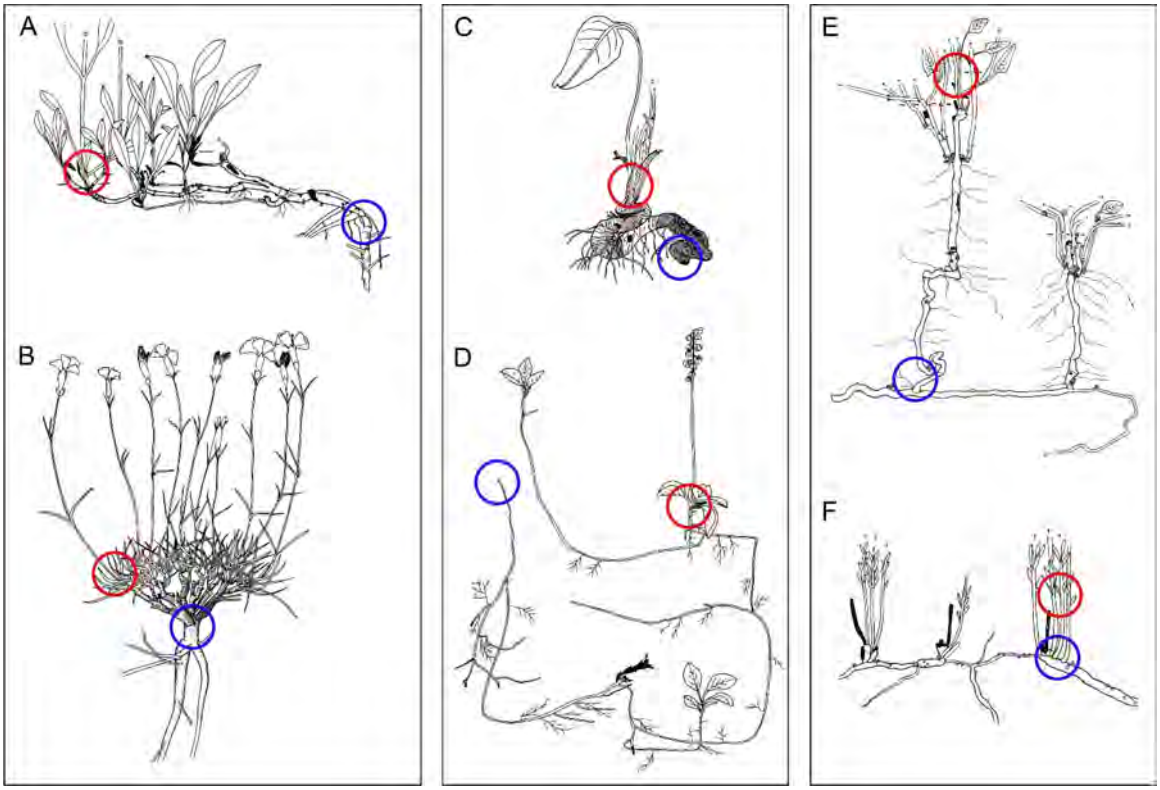

Fig. 6. Plant parts in herbs to be collected for anatomical traits and age are marked by circles. Red circles – *Conduit diameter*, A-F – the base of aboveground shoot; blue circles – *Tissue type ratio*, Age and *Radial growth*. A-B – root collar in nonclonal herbs (*Silene nutans*, *Dianthus sylvestris*); C-D – oldest part of rhizome in herbs with stem-derived clonality (*Polygonum bistorta*, *Pyrola minor*); E-F – oldest part of the stem in herbs with root-derived clonality (*Convolvulus arvensis*, *Euphorbia cyparissias*).

where buds for resprouting are sheltered from disturbance(s). One common type of modified stem is the rhizome, which is located belowground and supplies buds and carbohydrates (Klimešová and de Bello, 2009). Buds may also be formed on roots that are usually located deeper belowground than stem-derived rhizomes (Klimešová et al., 2018) and are therefore better protected from disturbances that reach upper soil horizons (e.g., ploughing). The formation of these bud bearing organs (BBOs – see "Clonality" for details), either on stems or on roots, is often at the expense of vertical growth, especially in woody plants (Midgley, 1996).

Possessing a bud bank is considered an important strategy for plants under various disturbance regimes (Vesk and Westoby, 2004). Traits related to the bud bank include *Bud bank size*, *Bud bank fluctuation* (temporal variation of bud bank size), *Bud preformation* and *Bud protection*. *Bud bank size* and *Bud protection* are traits relevant to disturbance frequency and severity respectively, whereas *Bud bank fluctuation* and *Bud preformation* are relevant to the timing of the disturbance event. Another important bud bank trait (described in the section of "Clonality" with clonal growth organs (CGOs), but applicable also for nonclonal plants) is the type of bud bearing organ (BBO). BBOs are traditionally categorized based on morphology and generally constrained by evolution, i.e., certain types of BBOs tend to be shared among related species (Pausas et al., 2018).

4.2.1. Bud bank size

According to the model by Bellingham and Sparrow (2000), bud bank size should be small or even absent at very low or high frequencies of severe disturbance but should peak at intermediate frequencies. This model is supported by plant communities in Central Europe and Brazilian subtropical grasslands where bud bank size was found to reach a maximum at intermediate disturbance frequencies across species (Fidelis et al., 2014; Herben et al., 2018). Bud bank size also varies intraspecifically in relation to changing environmental conditions such as disturbance regimes (e.g., Paula and Ojeda, 2006) and drought (e.g., VanderWeide and Hartnett, 2015).

Trait definition: Total number of buds stored in bud bearing organs per rooting unit (Fig. 7).

Type of variable and units: Continuous; Numerical.

Sampling procedure: In herbs, sample bud bearing organs at the time of flowering. In woody plants, buds may not be visible if covered by thick bark and wood (especially in fire-prone areas), and therefore we recommend sampling woody stems of several individuals (3–5 cross-section samples) between the soil surface and 1.3 m (breast height). Some woody plants (e.g., in palms) lack axillary buds and depend on protection of the stem apical meristem by leaf bases.

Trait assessment: Use a magnifying lens or stereomicroscope (10–40x) to examine bud bearing organs. First, identify whether a bud is dead or alive: a dead bud has a soft or mealy brown interior that crumbles easily, and a living bud is moist and fleshy with a white or yellow color. Then, count living buds per rooting unit (Fig. 7). When several rooting units are connected (forming a clonal fragment), count all buds of the clonal fragment and divide the total number of buds by the number of rooting units. As bud density may vary along the stem of woody plants, to facilitate comparison, stem samples (sections) where buds are counted should be standardized according to plant size, and other characteristics (for detailed anatomical procedures, see Burrows, 2002; Burrows et al., 2010).

Special cases and problems: Adventitious buds on roots and lignotubers are sometimes formed only after injury so that this type of bud bank cannot be reliably quantified. In this case, experimental evaluation of resprouting ability is recommended (see Appendix II).

Recommended literature: Klimešová and Klimeš (2007); Vesk and Westoby (2004); Burrows et al. (2010); Pausas et al. (2018); Ott et al. (2019).

4.2.2. Bud bank fluctuation

Bud bank size is not a static feature of plants. As plants grow, new buds are produced (bud natality), and older buds sprout to become new shoots (e.g., resprouting after disturbance) or die (bud mortality; Ott and Hartnett, 2012, 2015). *Bud bank size* can fluctuate due to

Fig. 7. Rhizome of grass species with buds highlighted by circles. This sympodial growth form shows three generations of stems with their buds. The parent shoot (far left-only the belowground stem base remains) developed two rhizomes whose apical meristems developed into terminal offspring shoots. These offspring shoots also produced rhizomes with terminal shoots. Therefore, three generations of buds are present in this sympodial rhizomatous grass.

synchronized natality and/or mortality, and may lead to bud limitation within a season, thus affecting sprouting ability (e.g., Russell et al., 2017). Bud bank size is expected to fluctuate less in long-lived BBOs (e.g., trees and herbs with long-lived rhizomes) than in short-lived BBOs (e.g., bulbs in herbs; Suzuki and Hutchings, 1997).

Trait definition: Maximum to minimum number of buds in bud bearing organs per rooting unit within a growing season.

Type of variable and units: Continuous; Ratio of maximum to minimum number of buds (dimensionless).

Sampling procedure: Refer to *Bud bank size*, and repeat this procedure at the end of the growing season (in seasonal climates) or after half of year in aseasonal climates.

Trait assessment: Refer to the procedure for measuring *Bud bank size*. Repeat this procedure twice: once at the time of flowering (expected minimum number of buds) and once at the end of the growing season (expected maximum number of buds).

Special cases and problems: Refer to the procedure for measuring *Bud bank size*. In temperate seasonal grassland systems, the minimum bud bank size occurs after seasonal sprouting before new shoots produce new buds to replenish the bud bank supply, and the maximum bud bank size coincides with flowering and the end of the growing season (Ott and Hartnett, 2012, 2015). In aseasonal climates, assessment of this trait has never been carried out, and would deserve research attention.

Recommended literature: Ott and Hartnett (2012, 2015); Ott et al. (2019).

4.2.3. Bud preformation

In a bud bank, not all dormant buds have equal chances of sprouting or are equally prepared for sprouting. In some cases, the shoot is preformed in a bud before sprouting, while in other cases a bud remains approximately the same size over the season as at the time of its formation. In seasonal climates, bud preformation happens during the season preceding sprouting (Körner, 2003). Bud preformation is frequently found in early-flowering plants, and plants with this trait tend to prevail in regions experiencing a short growing season (e.g., Rawat and Gaur, 2004). In aseasonal climates, bud preformation can be unrelated to environmental conditions and can lead to periodic growth in trees (Hallé et al., 1978). Bud preformation may constrain a species' response to changing environments (e.g., Geber et al., 1997).

Trait definition: Degree of shoot development in a bud before sprouting from bud bank.

Type of variable and units: Discrete; Categories (1 = whole generative, 2 = partial generative, 3 = only vegetative).

Sampling procedure: In herbs, sample bud bearing organs and focus on the largest or terminal bud at the end of the growing season. In woody species, inspect buds on twigs of last year before the sprouting of new shoots in a seasonal climate. In an aseasonal climate, search the branching pattern and examine buds with the highest probability to produce a new stem increment.

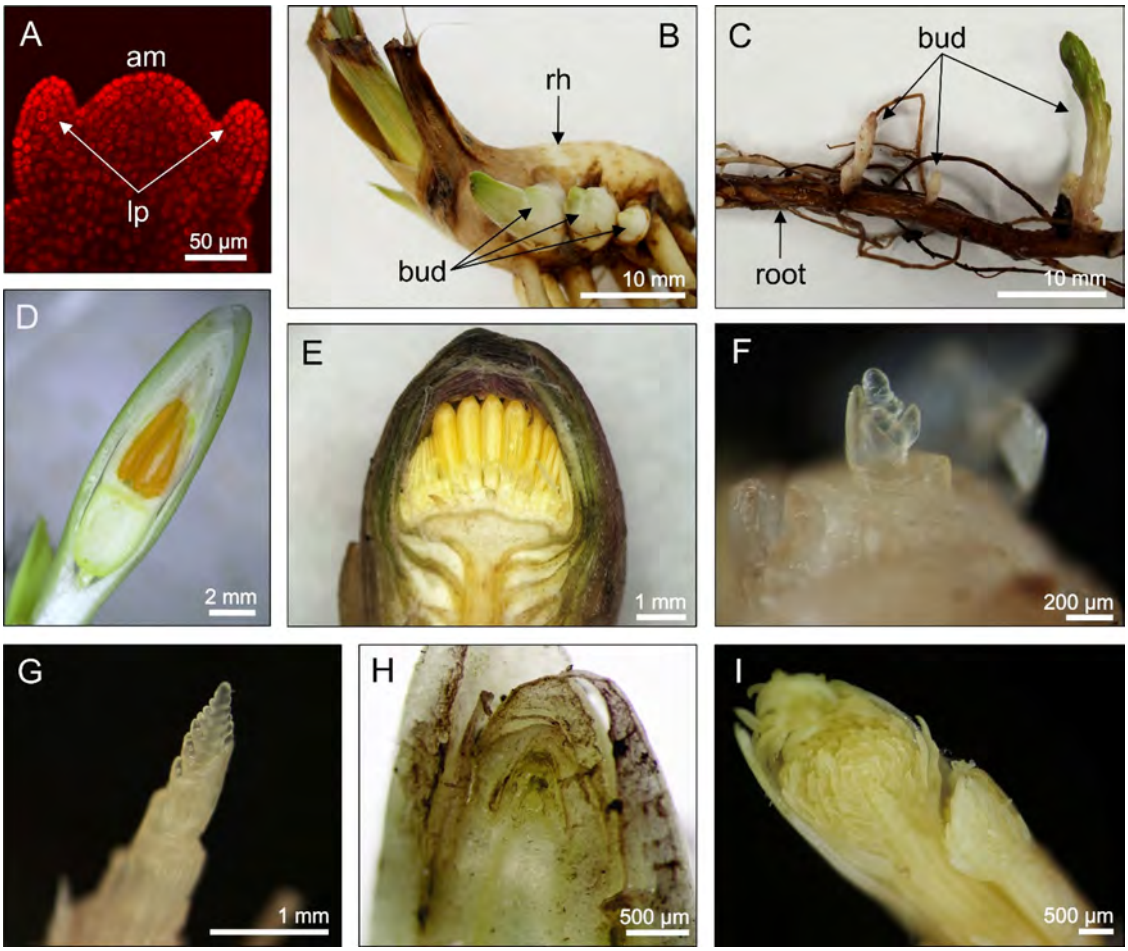

Fig. 8. Different type of bud preformation. A – Shoot apical meristem of *Salvia nemorosa*. Nuclei are red-stained. B-C – Buds on rhizome of *Iris aphylla* (B) and on root of *Euphorbia cyparissias* (C). D-E – Preformation of the whole inflorescence including anthers and an ovary in *Galanthus nivalis* (D) and *Tussilago farfara* (E). F-G – Partial preformation of the developing inflorescence meristem in *Carex remota* (F) and *Festuca rupicola* (G). H-I – Preformation of vegetative parts in *Epilobium hirsutum* (H) and *Adonis vernalis* (I). Picture A is stained by propidium iodide as seen from a confocal microscope (excitation 514 nm). Pictures B to I are from a stereomicroscope. am: apical meristem; lp: leaf primordia; rh: rhizome.

Trait assessment: Use a magnifying lens or stereomicroscope (10-40x) to examine bud bearing organs by searching for the largest buds (Fig. 8B, C), as these are the most likely ones to develop into a shoot. Dissect the bud to examine its apical meristem (Fig. 8A). For woody plants, buds need to be preserved in 70% ethanol for two weeks before dissection to soften resins and outer scales. We distinguish three categories of bud preformation: 1) whole preformation, the shoot with all its leaves and flower(s) (for the next growing season or future growth in an aseasonal climate) is fully formed inside the bud (Fig. 8D, E); 2) partial preformation, leaves and flower(s) of the shoot are not entirely preformed inside the bud (Fig. 8F, G); 3) preformation of vegetative parts, only leaves but no flowers are preformed prior to sprouting (Fig. 8H, I).

Recommended literature: Hallé et al. (1978); Geber et al. (1997).

4.2.4. Bud protection

Buds can be protected by plant structures like xylem, bark and leaf-derived structures (Burrows et al., 2010; Charles-Dominique et al., 2015; Pausas, 2015; Fig. 9). However, a very effective bud protection is conferred by the soil, which prevent buds from experiencing the damaging effects of disturbance such as fire, heat, frost, trampling, herbivory and ploughing; consequently, many species store buds belowground (Klimešová and Klimeš, 2007; Pausas et al., 2018). For example, the position of bud banks in the soil is important in arable fields where buds of successful perennial weeds are commonly located deep in the soil and therefore are safe from ploughing (Leakey, 1981).

Trait definition: Bud bank coverage by a plant organ (leaf), tissue (wood, bark) or soil.

Type of variable and units: Discrete; Categories: 1 = protected aboveground buds (specify if protected by bark, wood or leaf-derived structures), 2 = buds at soil surface, 3 = buds located up to 5 cm belowground, 4 = buds located deeper than 5 cm belowground.

Sampling procedure: Note the position of belowground bud bearing organs in relation to the soil surface and use their position to measure bud bank depth. Then, refer to *Bud bank size*.

Trait assessment: Identify living buds following the procedure for measuring *Bud bank size*, and check whether and how buds are protected. For woody plants, section aboveground stems to assess the occurrence of buds under/in the bark or embedded in the wood (buds emerging from the stem surface are considered unprotected; Charles-Dominique et al., 2015; Fig. 9).

Recommended literature: Burrows et al. (2010); Charles-Dominique et al. (2015); Pausas (2015); Pausas et al. (2018).

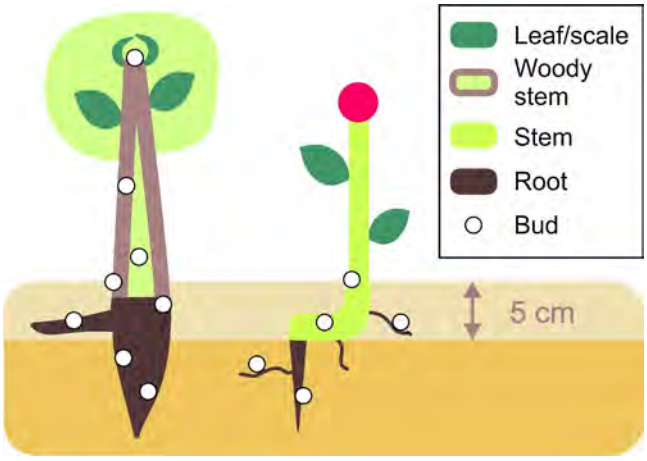

Fig. 9. Vertical distribution and protection of buds in a tree (left) and herb (right). According to our definition, aboveground buds are part of a bud bank only when they are protected by plant parts (bark, wood or leaf-derived organs). Belowground buds are protected by soil and we distinguish whether they are located in the upper soil layer (up to 5 cm belowground) or deeper belowground.

4.3. Carbohydrate storage

Carbohydrates are the main source of energy and carbon for plants, sustaining metabolism and growth (Patrick et al., 2013; Hartmann and Trumbore, 2016). They are produced by CO_2 fixation during photosynthesis, and the products not directly used for growth and maintenance form the main reserve of resources accumulated in roots or in specialized storage organs, such as rhizomes and tubers (Chapin et al., 1990; Janeček and Klimešová, 2014). These stored resources could be employed by plants to exert different key functions e.g., recovery after damage (e.g., Moreira et al., 2012). The carbohydrates directly used to build the plant body are named structural carbohydrates (e.g., cellulose), while those used for metabolism and storage are total non-structural carbohydrates (TNCs, such as starch), and are the focus of this handbook.

Carbohydrate storage, in conjunction with the presence of buds, affects seasonal sprouting and post-disturbance resprouting. Additionally, soluble sugars (e.g., oligosaccharides) contribute to plant protection against environmental adversities, such as drought, salinity or cold (Bartels and Sunkar, 2005; Peshev et al., 2013). Sugars are also recognized as signalling molecules that can modulate plant responses to changing environmental conditions (Rolland et al., 2006). Here, we consider traits related to carbohydrate storage, which are, at least potentially, relevant to (re)sprouting. We propose two traits: *Carbohydrate type* and *concentration* (Table 2; Fig. 10).

4.3.1. Carbohydrate type

Carbohydrate composition indicates the main storage compounds that are accumulated by plants in dedicated organs. Types of carbohydrates stored can be clade-specific, i.e., varying among plant families or even genera. Different types of carbohydrates differ in their functions. Generally small sugars such as mono-, di- and oligosaccharides are used for carbon transport and osmotic protection, while polysaccharides such as fructans and starch are used for carbon storage (Patrick et al., 2013; Van den Ende, 2013). Intraspecifically, this trait can also be affected by abiotic factors such as temperature and moisture (Wilson et al., 2001). Next, we describe the major types of carbohydrates accumulated by plants.

Mono- and disaccharides: The most common and simple sugars are the monosaccharides glucose and fructose and the disaccharide sucrose. They are the main compounds of more complex oligo- and polysaccharides. Sucrose is the main saccharide transported from sources to sinks in phloem.

Raffinose family oligosaccharides (RFOs): These are sucrose-based oligosaccharides with α-galactosyl extensions (Dos Santos et al., 2013). RFOs are prominent reserve compounds in Lamiales (Bachmann et al., 1994), and are also important transport and storage compounds in other plant orders, such as Cucurbitales (Hendrix, 1982) and Celastrales (Turgeon et al., 2001). The trisaccharide raffinose is also widespread in various vegetative tissues and seeds of angiosperms.

Fructans: These are oligo- and polysaccharides of polymerized fructosyl units, occurring in nearly 15% of angiosperms (Hendry, 1993). Fructans are vacuolar and water-soluble carbohydrates, and, depending on plant species and their phenological phase, occur in a series of molecules with several degrees of polymerization. A good example is offered by different Asteraceae species: for instance, *Smallanthus sonchifolius* accumulates mostly small fructans, *Chrysolaena obovata* stores medium-sized fructans, and *Aldama discolor* stocks bigger fructans (Carvalho et al., 1997; Itaya et al., 2007).

Starch: It is the main carbohydrate stored in the majority of plants. This polysaccharide is composed of glucose units creating two types of polymers, amylose and amylopectin. These molecules then jointly create starch granules, which define their properties, e.g., enzymatic digestibility (Zobel, 1988; Gallant et al., 1992).

Table 2
Recommended analytical procedures for extraction and detection of the main carbohydrate types stored in plants.

Carbohydrate type	Extraction method	Chemical or biochemical treatment	Detection analysis
Mono- & disaccharides			
Glucose, Fructose, Sucrose	80% ethanol in 80 °C		HPLC, HPAEC enzymes (glucose oxidase and peroxidase) + spectrophotometry
Oligo- and polysaccharides			
RFOs	80% ethanol in 80 °C	enzymatic hydrolysis	HPLC, HPAEC
Fructans	80% ethanol in 80 °C, water in 60 °C	enzymatic hydrolysis	spectrophotometry HPAEC
Starch	Using sediment free from soluble sugars	gelatinization, enzymatic hydrolysis	enzymes (glucose oxidase and peroxidase) + spectrophotometry

Trait definition: Composition of individual sugars forming total non-structural carbohydrates in storage organs.

Type of variable and units: Discrete; Nominal.

Sampling procedure: In herbs, ensure that each sample is collected from a different genetic individual because connected ramets may exchange and share carbohydrates. Note that genets of some clonal species can occupy large areas, so sampled individuals should at least be 10 m apart. Excavate belowground organs, remove their dead parts and wash fresh organs to remove soil. Note that soil particles, which can occur mainly in root fissures, can greatly affect dry weight and, consequently, carbohydrate concentration. It is important to make sure that organs are sampled at similar developmental stages as this can influence the levels of non-structural carbohydrates. In woody plants, sample aboveground stems protected by thick bark or leaf-derived organs by taking discs or wood cores using an increment borer (for details, refer to Quentin et al., 2015; Landhäusser et al., 2018). In belowground organs of both woody and herbaceous plants, carbohydrates may be located in specialized storage organs without buds and/or in bud bearing organs. Note that individual tissues (e.g., bark, outer and inner wood) and organs (e.g., root and rhizome) can differ in their concentration of non-structural carbohydrates (Zhang et al., 2014; Rosell, 2016), and should be sampled separately and then combined to create a final sample.

Depending on the preservation method that will be applied, fresh weight of the sample may be assessed in the field (see methods below). From fresh material, carbohydrate type can be identified by laboratory assessment. However, in most cases immediate processing is not possible and plant material must be preserved before extraction. Sample preservation requires the inactivation of enzymes that can metabolize carbohydrates, thereby depleting and/or altering the composition of non-structural carbohydrates. We recommend two methods for preservation.

The first method is to use liquid nitrogen or dry ice (solid CO_2) to freeze samples immediately after collection. This is the most recommended method, but difficult to use in remote areas. After this step, samples can be lyophilized, weighed for dry mass after dehydration and stored until extraction. Note that storing lyophilized samples for a long time may decrease the carbohydrate content and change the carbohydrate composition due to residual enzyme activity (Perkins, 1961).

The second method is to weigh the fresh mass of whole samples in the field, and then take aliquots of the fresh samples for dry mass assessment. Samples can be preserved by boiling pieces of plant material in aqueous ethanol (80%) for 3–5 min (Pollock and Jones, 1979). Then, samples can be stored at ambient temperature (kept in sealed containers preventing evaporation) to preserve solid and liquid phases, as a portion of the carbohydrates will already be in the solution. Such

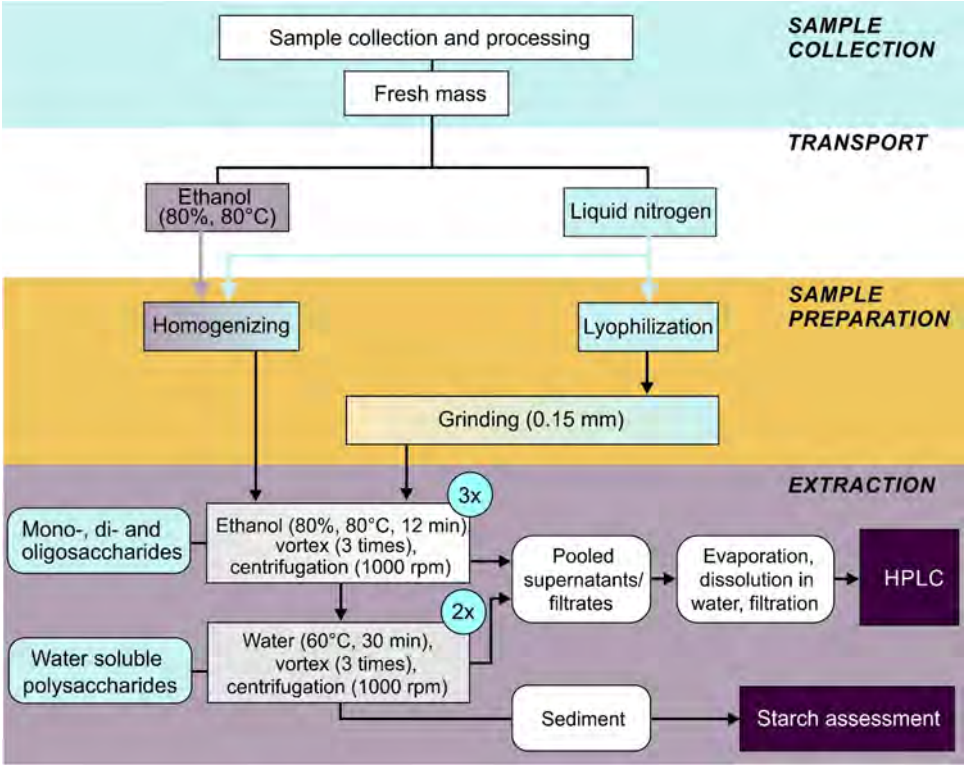

Fig. 10. Schematic guidance for sample collection, transport, preparation and extraction of non-structural carbohydrates.

treatments can be useful when the research is performed in remote regions (Chlumská et al., 2014). The aliquots (e.g., 1 g of fresh material) to be used for dry mass assessment of each replicate should be properly dried (through lyophilization as described above or by oven-drying) to estimate the proportion of dry mass for calculations of the carbohydrate storage traits.

Trait assessment: Prior to extraction, pay attention to the pulverization and homogenization of the sample, as TNCs are extracted from a diversified chemical matrix. Plant material (dry samples) has to be milled into small particles (< 0.15 mm; Quentin et al., 2015). If samples are not dry (fresh samples), they should be homogenized using a mortar and pestle. Before starting with further analyses, the type of carbohydrate(s) to be analyzed must be known. Some information can be found in the literature, but will need to be confirmed by carbohydrate screening testing for the presence of different carbohydrate types (Fig. 10).

Mono- and disaccharides: They can be extracted using aqueous alcoholic solvents even under low temperatures and short-time extraction conditions (i.e., 3–5 min. duration; Davis et al., 2007). The alcoholic extraction is composed of three fundamental steps: i) incubation in hot alcohol (hereinafter described as ethanol, but methanol may be used), ii) centrifugation, and iii) deposition of supernatant with dissolved carbohydrates. We recommend the following extraction protocol (Table 2): 1) put 100 mg of a dry, ground sample into a centrifuge tube, add 5 mL of 80% ethanol, incubate at 80 °C in a water bath for 12 min, and vortex three times during this procedure; 2) centrifuge for 15 min at 1000 rpm; 3) transfer the supernatant into a 30 mL glass bottle (the sediment in the centrifuge tube has to be free of ethanol extractable saccharides to be used for subsequent analyses, e.g., of starch and hemicellulose); 4) evaporate the collected supernatant, dissolve the pellet in 10 mL of distilled water, and deionize if necessary; 5) filter the solution for HPLC (High Performance Liquid Chromatography) analysis. Specific explanation of individual chromatographic methods – e.g., HPLC, gas chromatography, High Performance Anion Exchange Chromatography (HPAEC) – is out of the range of this protocol, and details can be found elsewhere (Chaplin and Kennedy, 1994). However, we strongly recommend using HPLC as it is the most commonly used technique (Table 2).

Raffinose family oligosaccharides: Similar to mono- and disaccharides, these oligosaccharides can be extracted and directly analyzed by HPLC (Gangola et al., 2014). If proper conditions for HPLC are unavailable, the enzymatic hydrolysis of RFOs using α-galactosidase can be used and the total amount of RFOs (relevant to the next trait, *Carbohydrate concentration*, but described here for simplicity) can be determined as the difference between soluble carbohydrates (galactose, glucose, fructose and sucrose) before and after enzyme addition to the extract (Janeček et al., 2011).

Fructans: Fructan extractions can be fractionated between oligosaccharides and polysaccharides due to their differential solubility in alcohol and water. Oligosaccharide fructans are mostly extracted in 80% aqueous ethanol (Table 2) similar to the extraction of mono- and disaccharides (Pollock and Jones, 1979). Polysaccharide fructans are insoluble in pure ethanol, but extractable in water. Hence the inclusion of water is necessary to extract these larger molecules. When evaporating extracts containing fructans, the pH needs to be monitored as fructans can undergo acid hydrolysis at high temperatures. To measure fructans, both HPLC and the enzymatic method can be used, but we recommend HPAEC/PAD (HPAEC with Pulsed Amperometric Detection; Benkeblia, 2013).

Starch: Starch content can be examined using the sediment created during mono- and disaccharide extraction (described above) but the sediment must be free from soluble sugars (Table 2; Fig. 11). The initial step for starch analysis is gelatinization – the process during which high temperatures in the presence of water disrupt the bonds among starch molecules and new bonds between starch molecules and water are created. This process increases the availability of starch for subsequent

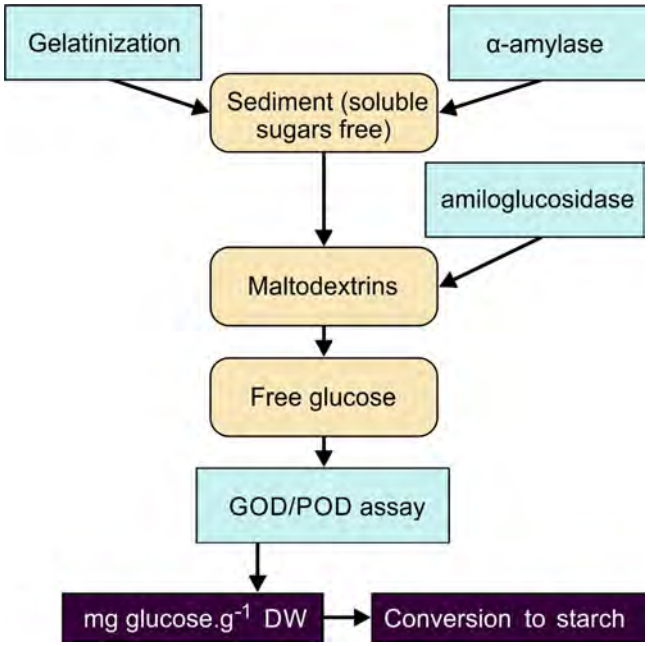

Fig. 11. Procedural steps for starch determination. GOD: glucose oxidase; POD: peroxidase; DW: dry weight.

enzymatic hydrolyses, and is commonly used when cooking starch-rich food. After gelatinization, the enzymatic hydrolysis of starch first produces maltodextrins and then free glucose (Batey, 1982), which can be detected through various methods (Galant et al., 2015). We recommend using commercially available assay kits with the enzymes glucose oxidase (GOD) and peroxidase (POD), whose reaction can be measured by spectrophotometry (Galant et al., 2015). Alternatively, if the HPLC instrumentation is available, glucose can be determined similarly to mono- and disaccharides from the extract. The HPLC approach is nevertheless usually more expensive and time-consuming.

Special cases and problems: Storage organs may also contain rarer carbohydrates. For example, hemicelluloses are important in building elements of cell walls, but in some cases they may represent an additional source of carbon and energy (Hoch, 2007; Schädel et al., 2009). The analysis of hemicelluloses is complex due to their chemical and structural heterogeneity. Hemicelluloses first need to be isolated from the sediment created during mono- and disaccharide extraction. Following their extraction using acid hydrolysis, the determination of hemicellulose-derived monosaccharides can be done using HPLC (e.g., Schädel et al., 2010).

Recommended literature: Chapin et al. (1990); Benkeblia (2013); Chlumská et al. (2014); Quentin et al. (2015).

4.3.2. *Carbohydrate concentration*

This trait is highly variable interspecifically, and higher values of TNCs are expected to be functionally advantageous under recurring severe disturbance (Canadell et al., 1991). Also, this trait varies intraspecifically, as concentrations of TNCs in storage organs usually decrease after disturbance (Moreira et al., 2012; de Moraes et al., 2016). In seasonal climates, such as in temperate regions, the concentration of carbohydrates in storage organs varies in relation to changing environmental conditions over the year and across phenological stages (Bartoš et al., 2011) being the lowest at the time of renewal of aboveground biomass after seasonal rest and the highest at the end of season. Carbohydrate storage, similarly to bud bank size, is expected to fluctuate less in long-lived storage organs (e.g., trees and herbs with long-lived rhizomes) than in short-lived ones (e.g., bulbs in herbs; Suzuki and Hutchings, 1997).

Trait definition: Concentration of total non-structural carbohydrates

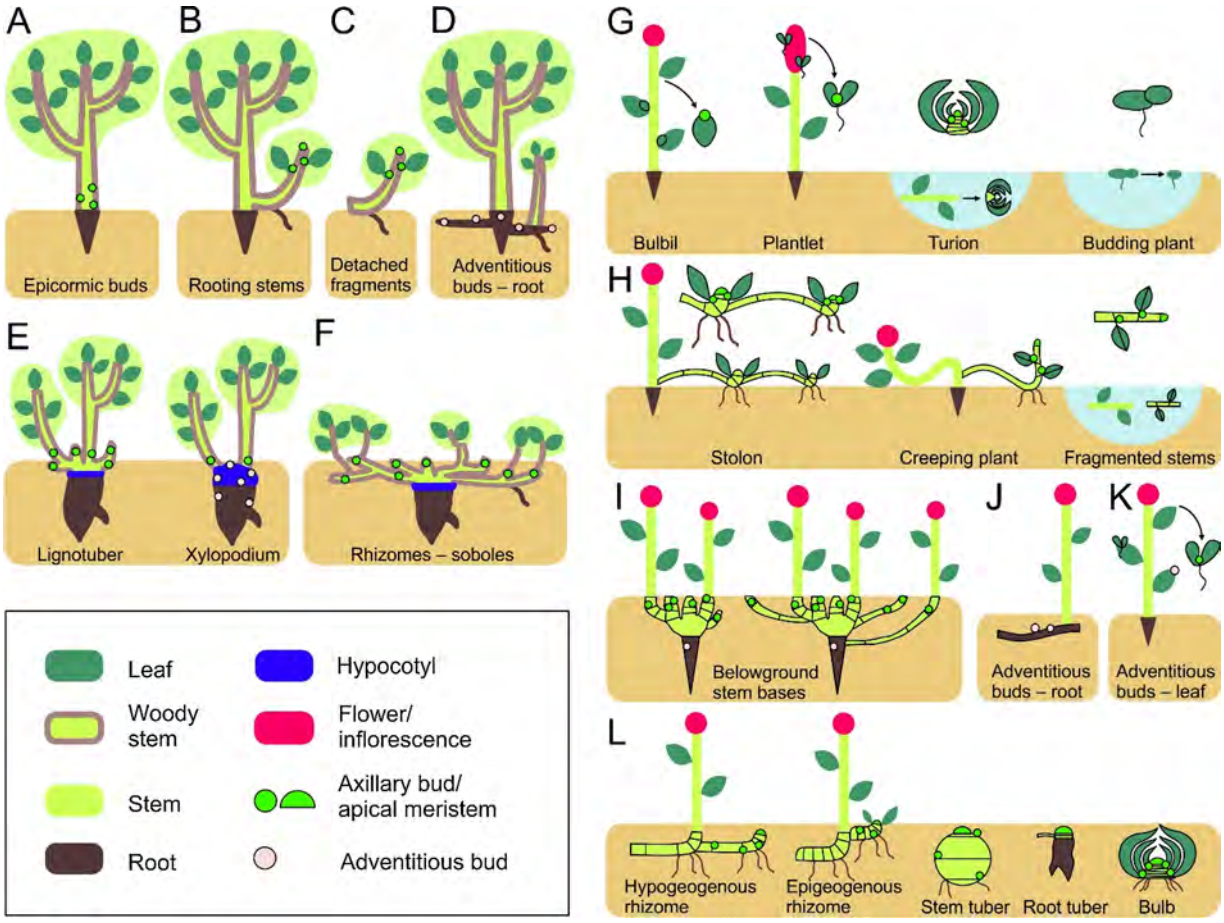

Fig. 12. Examples of clonal growth organs (CGOs) and bud bearing organ (BBOs). A–F: Woody plants. A – nonclonal plant with protected aboveground buds; B – aboveground rooting branches; C – rooting detached fragments; D – clonal plant; E – main root with bud bank on stem bases (left) and hypocotyl and root (right); F – belowground stem-derived clonality. G–L: Herbaceous plants. G – aboveground detachable buds; H – aboveground rooting stems; I – main root with belowground stem bases; J – root-derived clonality; K – leaf-derived clonality; L – belowground stem-derived clonality.

in storage organs.

Type of variable and units: Continuous; mg carbohydrates g^{-1} dry weight.

Sampling procedure: Refer to *Carbohydrate type.*

Trait assessment: Follow the extraction and quantification procedures as per *Carbohydrate type*. Measure the amount of each carbohydrate type, pool them (hence obtaining TNC), and divide their weight by the dry mass of the sample.

Special case and problems: Although it is practically impossible to find the best universal method for carbohydrate analysis for all types of plant materials, some common mistakes should be avoided to prevent wrong interpretations or results:

i) Before starting any analyses, review the literature for possible carbohydrate types in the target species and/or related taxa. For example, species belonging to Asteraceae mainly store fructans and species from Lamiales preferentially store RFOs (Van den Ende, 2013). Although this information is available in literature, it is sometimes neglected (e.g., Atkinson et al., 2012, 2014).

ii) Enzyme deactivation must be done as soon as possible after sample collection. Otherwise, the concentration of TNCs will decrease and carbohydrate composition will change over time (Smith, 1973; Chlumská et al., 2014). Therefore, we do not recommend using air-dried plant samples, such as herbarium specimens, because the enzymes will remain active during the slow-drying process and could change both the carbohydrate composition and concentration.

iii) Acid hydrolysis methods are not specific and can hydrolyse various carbohydrates, including those not relevant to storage (Sullivan, 1935; De Ruiter et al., 1992). Strong acids used in hydrolysis can partially damage monosaccharides (Raessler, 2011). Thus, acid hydrolysis may not give the accurate concentration of non-structural carbohydrates. Regardless of this problem, acid hydrolysis is still frequently used in ecological research. We strongly recommend avoiding the use of acid hydrolysis, except for hemicellulose analysis.

Recommended literature: Bartoš et al. (2011); Van den Ende (2013); Chlumská et al. (2014); Landhäusser et al. (2018).

4.4. Clonality

In addition to generative reproduction, many plants are capable of multiplying clonally, namely producing rooting units that potentially could become physically independent (i.e., clonal offspring, or ramets). This ability can be affected by internal and external factors. Internal factors are related to the modular structure of plants, such as the ability to form adventitious roots and/or shoots, and to lose old parts (i.e., splitting clones). External causes are associated with a diffuse distribution of resources (both aboveground and belowground) and adversities that can limit vertical growth (e.g., disturbances), but, at the same time, enable horizontal growth (Klimešová et al., 2018).

Clonal growth leads to multiplication affecting plant fitness in terms of population persistence and size. Clonal organs (e.g., rhizomes and

stolons) can provide numerous functions such as space occupancy, resource foraging and sharing (Yu et al., 2004; Klimešová et al., 2018). Connections between the parental plant and its offspring may be advantageous when competing intra- or interspecifically for resource capture. Additionally, clonal organs may carry buds and store carbohydrates that can boost resprouting after damage (Yu et al., 2008; Klimešová et al., 2018).

Clonal growth can be an additive (facultative) or a necessary (obligate) feature of plants (Klimešová and de Bello, 2009). The obligate nature implies all individuals of a species are able to reproduce vegetatively, and the facultative feature means that only some individuals and/or populations of a species are equipped with such a strategy. This variability is associated with ontogeny and environmental factors. Also, clonal plants are not uniformly distributed across regions and along environmental gradients. Most studies have been restricted to Central Europe, which is the only region where clonality has been studied in detail and data are available for an entire flora (CLO-PLA3 database; Klimešová and de Bello, 2009). Here, we describe how to identify *clonal growth organs* (CGOs) together with *bud bearing organs* (BBOs) as qualitative, categorical traits. We then report three quantitative clonal traits, that is, *Lateral spread, Multiplication rate*, and *Persistence of connection* among ramets. These quantitative traits have been described and identified specifically for herbaceous plants, whereas little is known about woody species.

4.4.1. Type of clonal growth organs and bud bearing organs

Organs that provide plants with clonal growth and/or enable vegetative regeneration after damage can be distinguished from one another morphologically (Leakey, 1981; Klimešová, 2018). CGOs and BBOs are considered together as there may be (but not necessarily) a large overlap between them (Fig. 12). For example, rhizomes are very common among grassland species serving as a source of buds and carbohydrates for regeneration after herbivory or fire and, at the same time, their growth results in vegetative multiplication and lateral spread (Klimešová and Klimeš, 2007). Conversely, lignotubers of woody plants that store carbohydrates and buds provide plants with the ability to regenerate after fire (Paula et al., 2016; Pausas et al., 2018), but do not lead to clonal multiplication.

Trait definition: Type of organ(s) enabling plants to regenerate and/or reproduce vegetatively.

Type of variable and units: Discrete; Nominal.

Sampling procedure: Focus on large individuals as some organs (e.g., rhizomes) are only entirely formed later in plant ontogeny. To assess clonality of aboveground organs, observe the plant repeatedly during the growing season as vegetative reproduction may be restricted to a short period. For example, turions of aquatic plants are formed at the end of the season, and detachable buds of some understory forest plants are produced during flowering.

Trait assessment: In the laboratory, wash the organs and use a magnifying lens or stereomicroscope (10-40x) to examine them. Follow this procedure: 1) determine whether buds are located on stem or root organs, 2) identify whether storage compounds are deposited in stems, roots or leaves, 3) note where the organs are located in relation to the soil surface, and 4) examine the organs for adventitious roots. For belowground organs, one assessment per season (at the time of flowering) may be sufficient as they are usually long-lived. For short-lived belowground organs (some tubers and bulbs), the end of season may be more appropriate for assessment. For aboveground organs, multiple visual inspections of growing plants along their seasonal development are necessary as they may be short-lived. The correct identification of CGOs and BBOs requires skilled morphologists and repeated observations and/or cultivations. We therefore introduce a simplified categorization which can be effectively applied across different biomes based on a limited set of characters (Table 3). For a more detailed classification, refer to specialized literature (Alonso and Machado, 2007; Appezzato-da-Glória et al., 2008; Pausas et al., 2018; Klimešová, 2018).

The following CGO/BBO types are identified and described (Fig. 12).

i) Nonclonal plants (dicotyledonous plants: long-lived primary root without adventitious roots; monocotyledonous plants: only adventitious roots)
   a Lack belowground CGOs and BBOs: some woody plants (e.g., non-resprouters) and annual herbs (Fig. 12A).
   b Belowground stem bases: belowground stem bases (storing carbohydrates and buds) connected to the perennial main root (Fig. 12E, F, I). Stem bases may be either short and vertical or long and horizontal. In woody plants, this organ when bulky, is called a xylopodium (when constituted by the junction of the primary root with the hypocotyl) or a lignotuber (when formed from stem base; Fig. 12E) – which is especially relevant in arid, fire-prone ecosystems.
ii) Clonal plants: stem-derived organs (short-lived primary root, replaced by adventitious roots, bud bank located on stem).
   a Stolons, rooting fragments and creeping plants: aboveground horizontally growing stems, aboveground vertically growing stems which bend and then root when touching soil surface, or rooting from aboveground branches and fragments. They form adventitious roots and bear buds. However, they usually do not contain prominent carbohydrate storage (Fig. 12B, H).
   b Dispersible aboveground buds: small buds in leaf axils (e.g., bulbils) or within inflorescences. They are shed early from parental plants, usually are not dormant, and do not form a bud bank in the soil. In some aquatic plants, turions can be formed to overwinter, and are specialized dormant buds similar to bulbils (Fig. 12C, G).
   c Rhizomes: belowground horizontally growing stems initiated either aboveground (epigeogenous rhizomes) and later pulled belowground (or buried by litter) or directly formed belowground (hypogeogenous rhizomes). Also, positive geotropism, producing belowground parts, may occur (rhizophores). In woody plants, the organ is called a sobole or woody rhizome, and its persistence is much longer than similar structures of herbaceous plants (Fig. 12F, L).
   d Tubers and bulbs: Belowground organs specialized for surviving adverse seasons, and multiplying. Carbohydrate storage may occur in the stems (stem-tuber), roots (root-tuber) or leaves (bulb). Tubers and bulbs usually produce offspring ramets of two types: 1) large offspring individuals replacing the parental ramet (usually 1–3), and 2) small offspring individuals that split early from the parental ramet (similar to seedlings, usually 1-many; Fig. 12L).
iii) Clonal plants: root-derived organs (both primary and adventitious roots may be involved)
   a Root with adventitious buds: roots capable of producing adventitious buds spontaneously or after injury (Fig. 12D, J).
iv) Clonal plants: leaf-derived organs (short-lived primary root, replaced by adventitious roots)
   a Leaf with adventitious buds: leaves able to produce adventitious buds spontaneously or after injury (Fig. 12K).

Special cases and problems: Some species may have more than one type of bud bearing organ, while in others the organ may vary among individuals or populations (Klimešová and de Bello, 2009). Clonality may depend on plant age as it may take several years before the CGO is formed. In some species, root-tubers only have the function of storing carbohydrates so that they are unable to regenerate when separated from the parental plants and cannot be considered a CGO or BBO. Regeneration ability of root tubers and roots (primary as well as adventitious ones) has to be examined experimentally by fragmentation (see *Experimental assessment of resprouting after disturbance*). Clonality

Table 3
Examples of CGO/BBO types in relation to Raunkiaer life forms, general morphological categories, and their functioning as bud bearing or clonal growth organ. NA = not applicable.

Growth form	General morphological categories	CGO	BBO	Examples of CGO/BBO types
Woody plants	Aboveground buds not protected	No	No	NA
Phanaerophytes, Chamaephytes	Aboveground buds protected	No	Yes	Epicormic stem buds
	Aboveground rooting branches	Yes	No	Aboveground stems
	Aboveground rooting detached fragments	Yes	No	Detached fragments
	Main root with belowground stem base	No	Yes	Lignotuber, Xylopodia
	Belowground stem-derived clonality	Yes	Yes	Rhizomes
	Root-derived clonality	Yes	Yes	Roots with adventitious buds
Herbaceous plants	No belowground storage of buds or carbohydrates	No	No	NA
Hemicryptophytes, Geophytes, Hydrophytes, Therophytes	Aboveground detachable buds	Yes	No	Bulbils, turions, plantlets, budding plants
	Aboveground rooting stems	Yes	No	Stolons, creeping stems, and fragmented stems of aquatic plants
	Main root with belowground stem bases	No	Yes	Belowground stem bases
	Belowground stem-derived clonality	Yes	Yes	Rhizomes, tubers, bulbs
	Root-derived clonality	Yes	Yes	Roots with adventitious buds
	Leaf-derived clonality	Yes	No	Leaves with adventitious buds

induced by fragmentation or plant injury has proved to be ecologically important in disturbed habitats (e.g., arable land; Martínková and Klimešová, 2016).

Recommended literature: Klimešová and Klimeš (2008); Klimešová et al. (2017); Pausas et al. (2018).

4.4.2. Lateral spread

Clonal spread allows plants to move horizontally, and enhances their competitive ability (Klimešová et al., 2018). Lateral spread has proven to be the most informative clonal trait. For instance, lateral spread can positively influence clonal reproduction and negatively affect seed reproduction (Herben et al., 2012). It is also positively correlated with the establishment success of plants in meadows during restoration using seed mixtures (Mudrák et al., 2018). Lateral spread through belowground structures (rhizomes, roots) is very successful in fire-prone ecosystems (Pausas et al., 2018). Clonal plants with extensive lateral spread tend to prefer high soil moisture (Klimešová et al., 2016b). Lateral spread may also vary intraspecifically (Sammul, 2011).

Trait definition: Distance between offspring rooting units and parental rooting unit.

Type of variable and units: Continuous; Distance per year (m $year^{-1}$).

Sampling procedure: Refer to *Type of CGO*. Note that the last two generations of rooting units are needed, that is, both parental and offspring rooting units.

Trait assessment: The first step is to identify the CGO type(s). If a plant possesses multiple CGOs, assess the trait for each CGO separately so that different values of the trait can be obtained – as in the case of species with short rhizomes and dispersible buds (bulbils). The evaluation procedure depends on CGO origin, branching and shoot cyclicity, so it can be described according to the following categories (Fig. 13).

A Sympodially branching stems (stolons, rhizomes, tubers and bulbs) with monocyclic or polycyclic shoots: Check whether the connection between an offspring shoot and the parental shoot is preserved. If the connection still exists, measure the distance between the parental shoot and the offspring shoots. If not, examine the plant repeatedly in one season. i) in monocyclic shoots, measure the distance between the current-year shoots and shoot remnants from last year; ii) in polycyclic shoots, measure the distance between the young (not yet flowering) shoots and the older (flowering) shoots.

B Monopodially branching stems (stolons, rhizomes, tubers and bulbs): Identify morphological marks which indicate annual increments on stems (e.g., traces produced by flowering shoots) and measure the distance, e.g., between flowering shoots of this year and last year (annual increment).

C Dispersible aboveground buds: Measure the dispersal distance due to natural dispersion or by experimental manipulation by detaching buds from parental plants.

D Root-sprouting, monocyclic or polycyclic shoots: Exact measurement is difficult as new shoots may resprout on older as well as younger parts of the root system. Therefore, proceed similarly as in category “A” but note that the measured values are only approximations.

Special cases and problems: In sympodially branching plants with polycyclic shoots and in monopodially branching plants flowering may not occur each year so that lateral spread cannot be measured. Branching from older parts of a rhizome may occur in some species, so searching for the youngest parts of the rhizome system is recommended. Long-distance dispersal of vegetative buds, especially in aquatic plants, is very difficult to observe. Dispersibility of aquatic propagules may be assessed indirectly by either studying populations along water bodies or by implementing molecular techniques. Long-distance dispersal of vegetative propagules is also possible in terrestrial systems due to human-related activities, both intentional (e.g., spread of ornamental plants multiplied by cuttings) or unintentional. In this case, plant molecular techniques may help to quantify the spreading distance. For woody plants, assessing lateral spread is challenging, and very limited data are available (e.g., Wiehle et al., 2009). Generally, offspring at the edge of the clone need to be dated as the distance between parental and offspring rooting units divided by offspring age is used to infer lateral spread per year.

Recommended literature: Klimešová and Klimeš (2008); Klimešová et al. (2016b); Sammul (2011).

4.4.3. Multiplication rate

The number of rooting units per plant can differ considerably among species. Some plants form extensive clones consisting of hundreds of interconnected ramets, while others form small clones consisting of a few ramets. In plants with multiple CGOs, multiplication rate may largely differ between CGOs. Multiplication rate is highly variable intraspecifically, and depends on growing conditions, particularly resource availability and competition (Klimešová et al., 2016b).

Trait definition: Number of offspring rooting units produced by a parental rooting unit per year.

Type of variable and units: Continuous; Dimensionless.

Sampling procedure: Refer to *Type of CGO*. Note that the last two generations of rooting units are needed, that is, both parental and offspring rooting units.

Trait assessment: The first step is to identify the CGO type(s). If a

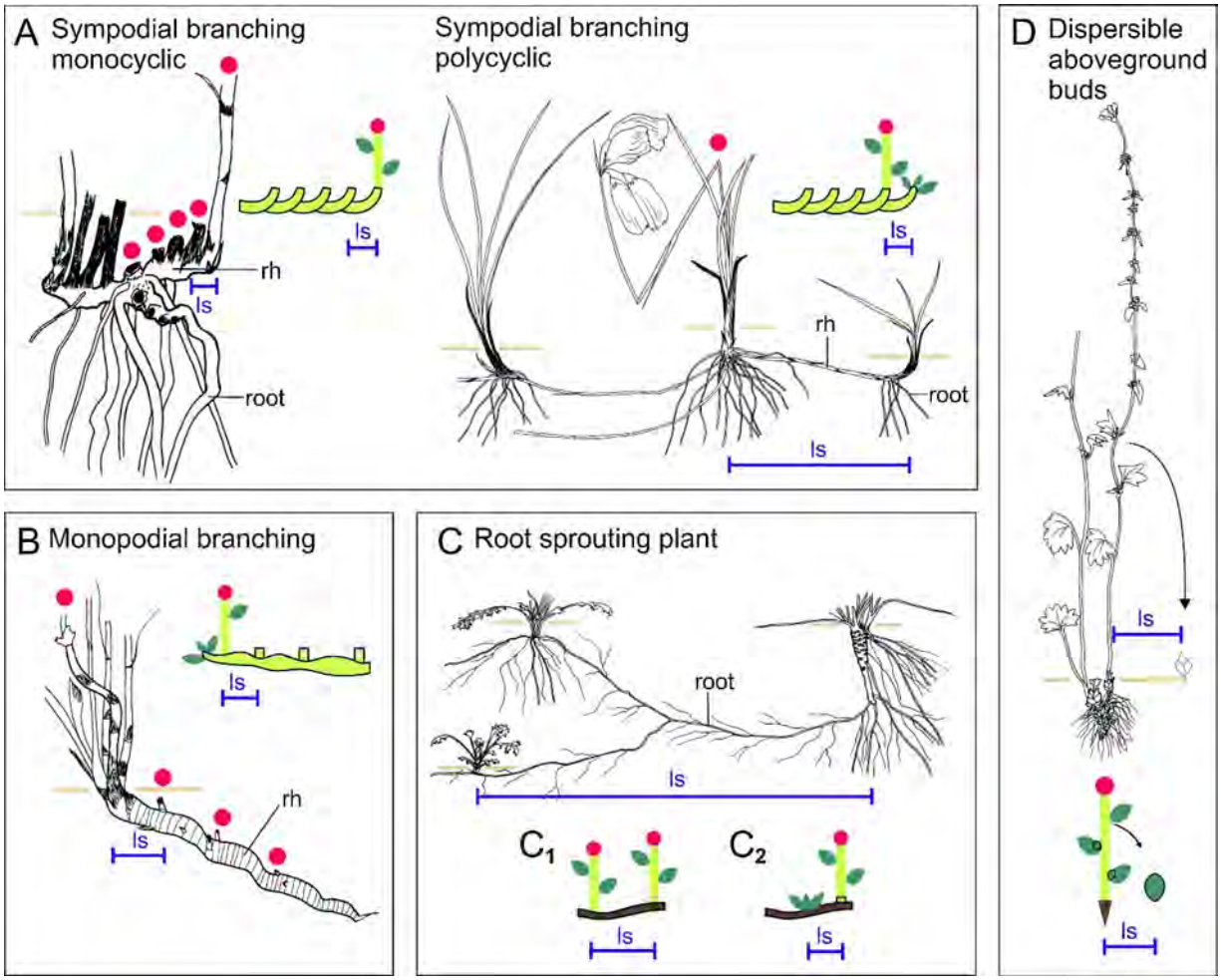

Fig. 13. Morphological categories for evaluation of quantitative clonal traits: A – sympodially branching stems with monocyclic (*Agrimonia eupatoria*) or polycyclic shoots (*Eriophorum angustifolium*); B – monopodially branching stems (*Alchemilla xanthochlora*, roots are not shown); C – root-sprouting, monocyclic (C_1) or polycyclic shoots (C_2) (*Ajuga genevensis*); D – dispersible aboveground buds (*Saxifraga cernua*). Red dots: flowering shoots from the current year and remnants of flowering shoots from previous years. ls: lateral spread; rh: rhizome.

plant possesses multiple CGOs, assess the trait for each CGO separately so that different values of the trait can be obtained – as in the case of species with short rhizomes and dispersible buds (bulbils). The evaluation procedure depends on CGO origin, branching and shoot cyclicity, so it can be described according to the following categories (Fig. 13).

A Sympodially branching stems (stolons, rhizomes, tubers and bulbs) with monocyclic or polycyclic shoots: In the case of monocyclic shoots that have preserved connections to the parental shoot, calculate how many current-year shoots belong to one previous-year shoot and this is equal to the multiplication rate. In polycyclic shoots, a parental shoot may produce offspring for several years so that only the youngest of current-year offspring should be counted when calculating the multiplication rate. If the connection to the parental shoot is not present, examine the plant repeatedly for offspring during one year (season).

B Monopodially branching stems (stolons, rhizomes, tubers and bulbs): Identify morphological marks which indicate annual increments on stems (e.g., traces produced by flowering shoots) and count the number of branches (i.e., offspring) per one annual increment.

C Dispersible aboveground buds: Inspect the plant when dispersible buds are formed (but not shed yet) and count them.

D Root-sprouting, monocyclic or polycyclic shoots: Exact measurement is difficult as new shoots may resprout on older as well as younger parts of the root system. We therefore propose the following approximation: i) for monocyclic shoots, count current-year shoots and previous year shoot remnants. Then calculate multiplication rate by dividing the number of current-year shoots by number of remnant shoots from last year; ii) for polycyclic shoots, count young (not yet flowering) shoots and older (flowering) shoots and calculate multiplication rate by dividing the number of young shoots by the number of older shoots.

Special cases and problems: In sympodially branching plants with polycyclic shoots and in monopodially branching plants, flowering may not occur each year, making multiplication difficult to assess. Branching from older parts of a rhizome may occur in some species, so searching for the youngest parts of the rhizome system is recommended. Multiplication may occur so rarely that you cannot observe any offspring. When the connection between offspring and parental rooting units is short-lived, it is difficult to determine the offspring-parent link, and we recommend repeated observations over a year (growing season). For woody plants, assessing multiplication rate is challenging, and only limited data are available (Wiehle et al., 2009). To calculate this trait, estimating age of the parental tree is necessary. Then, it is necessary to identify all offspring that can be attributed to one parental rooting unit, and determine the offspring age. Finally, multiplication rate can be calculated as number of offspring produced

by a parental rooting unit per year.

Recommended literature: Klimešová and Klimeš (2008); Klimešová et al. (2016b).

4.4.4. Persistence of connection

There is great interspecific variability in the persistence of connection among rooting units. Some clonal offspring split from the parental plant soon after they are formed while others remain connected to the parent for decades (Jónsdóttir and Watson, 1997). Intraspecific variability of this trait is largely unknown (but see Šťastná et al., 2010). Long persistence of connection is more common in nutrient-poor and dry environments than in nutrient-rich and wet conditions (Klimešová et al., 2016b). Connected ramets may have an advantage to take up more resources from larger areas and share them through their clonal network (Yu et al., 2008). Long-term connection can support young ramets in competitive environments while early detached dispersible buds resemble seedlings as they must rely on storage deposited inside themselves to establish.

Trait definition: Period of connection between offspring rooting units and parental rooting unit.

Type of variable and units: Discrete; Categories (< 1, 1–2, > 2 years).

Sampling procedure: Refer to *Type of CGO*. Note that the entire interconnected clonal fragment must be excavated.

Trait assessment: The first step is to identify the CGO type(s) of a plant. If the plant possesses multiple CGOs, assess the trait for each CGO separately so that different values of the trait can be obtained – as in the case of species with short rhizomes and dispersible buds (bulbils). The evaluation procedure depends on CGO origin, branching and shoot cyclicity, so it can be described according to the following categories (Fig. 13).

A Sympodially branching stems (stolons, rhizomes, tubers and bulbs) with monocyclic or polycyclic shoots: For monocyclic shoots, check if remnants of previous season shoots are preserved and if branching is regular towards the distal (older) part of the stem. If these criteria are met, then age of connection can be assessed. For polycyclic shoots, such accurate age determination may not be possible as flowering and branching may not occur each year. However, if the remnants of flowering shoots are still visible, then the persistence of connection category can be assigned with > 2 years.

B Monopodially branching stems (stolons, rhizomes, tubers and bulbs): Identify morphological marks which indicate the annual increment on stems (e.g., traces produced by flowering shoots) and count the number of annual increments. Such accurate age determination may not always be possible as flowering may not occur each year. However, if some parts of the stem are older than the flowering shoot remnants, the category of connection persistence can be assigned as > 2 years.

C Root-sprouting, monocyclic or polycyclic shoots: Exact measurement is difficult, so we propose to examine the remains of shoots. If the shoots have decayed to different degrees, then the persistence of connection category can be assigned as > 2 years. If the root has secondary thickening, count the number of rings (e.g., dicots; see “Age”) and assign the category of connection persistence based on growth rings in roots.

D Dispersible aboveground buds: Aboveground CGOs usually disintegrate during the season they are formed and should be assigned the persistence of connection category < 1.

Special cases and problems: Caution should be exercised as branching from older rhizome portions may occur in some species. For some plants, especially those found in productive, wet or aquatic habitats, their belowground organs are short-lived so that no remnants of previous shoots are visible. In this case, we recommend assigning < 1 year as the category of connection persistence. For woody plants, assessing persistence of connection is extremely challenging, and no data are available. This trait can be estimated by measuring the age of connection among rooting units if the oldest part of the connection can be found and connections show secondary thickening (thus counting tree-rings is possible; refer to *Age* below).

Recommended literature: Klimešová and Klimeš (2008); Klimešová et al. (2016b).

4.5. Longevity and growth

Plants are sessile organisms usually occupying the same spot for their whole lifespans and, in the case of most clonal plants, their close surroundings. This persistence has several consequences: i) pre-empting space and resources in a community (promoting competitive ability), ii) adjusting their phenotype to local conditions, iii) affecting soil biota (plant-soil feedbacks), and iv) influencing biogeochemical cycles following death. Persistence of established individuals is a key demographic process in population dynamics of perennial plants, and considered more important than seed production or seedling establishment (e.g., Salguero-Gómez et al., 2016). Interspecific differences in plant longevity also reflect species positioning along the fast–slow continuum, with short-lived plant species at one end and slow-growing, long-lived species at the other (Salguero-Gómez et al., 2016). This knowledge is, however, based on demographic data without direct measurements of plant age.

Plant persistence is characterized by *Age* and *Growth*. Traditionally, the age of a plant can be classified using life history categories, such as annual, biennial, and perennial. More detail and reliable data on plant age and growth are possible in species with secondary thickening, which form growth rings, especially in regions experiencing seasonal climates. For plants that lack secondary thickening or plants without distinct growth rings (e.g., monocots or plants from aseasonal biomes), other methods for age and growth determination need to be used (de Witte and Stöcklin, 2010). For woody species, dendrochronology is a well-established discipline allowing accurate age determination (e.g., Schweingruber, 1988), while for herbaceous species chronological techniques have been developed only recently (e.g., Schweingruber and Poschlod, 2005). These methodological advances enabled accurate estimates of longevity (maximum potential age of the species) for perennial herbs by counting growth rings.

Growth indicates the speed of net biomass accumulation of plants. Plants respond to changing environmental conditions – temperature, precipitation, and disturbances such as fire, grazing – and these abiotic variations are reflected in the ring formation of secondary thickening species. For example, a perennial plant exposed to drought should show reduced growth (i.e., narrower rings than plants of the same species not experiencing drought). However, by averaging ring increments over years, evidence suggests that ring formation is a species-specific plant trait allowing comparisons among-species (Doležal et al., 2018). Also, *Age* and *Radial growth* are expected to have strong tradeoffs, as shown by demographic studies (Salguero-Gómez et al., 2016).

4.5.1. Age

Information stored in perennial structures of plants with distinct annual growth rings is used to understand how long a plant can potentially live (e.g., Dietz and Fattorini, 2002; von Arx et al., 2006; Nobis and Schweingruber, 2013). From the first implementations of these techniques, we know that plant *Age* tends to be greater in harsh environments (e.g., cold; Nobis and Schweingruber, 2013; Doležal et al., 2016). The age of non-clonal herbs also exceeds the persistence of rhizomes in clonal herbs (Klimešová et al., 2015) indicating a close link between clonality and longevity (e.g., studies using demography, Salguero-Gómez, 2018).

Trait definition: Potential maximum plant age.

Type of variable and units: Continuous; Numerical (years).

Sampling procedure: Refer to *Tissue type ratio*. In this case, collection

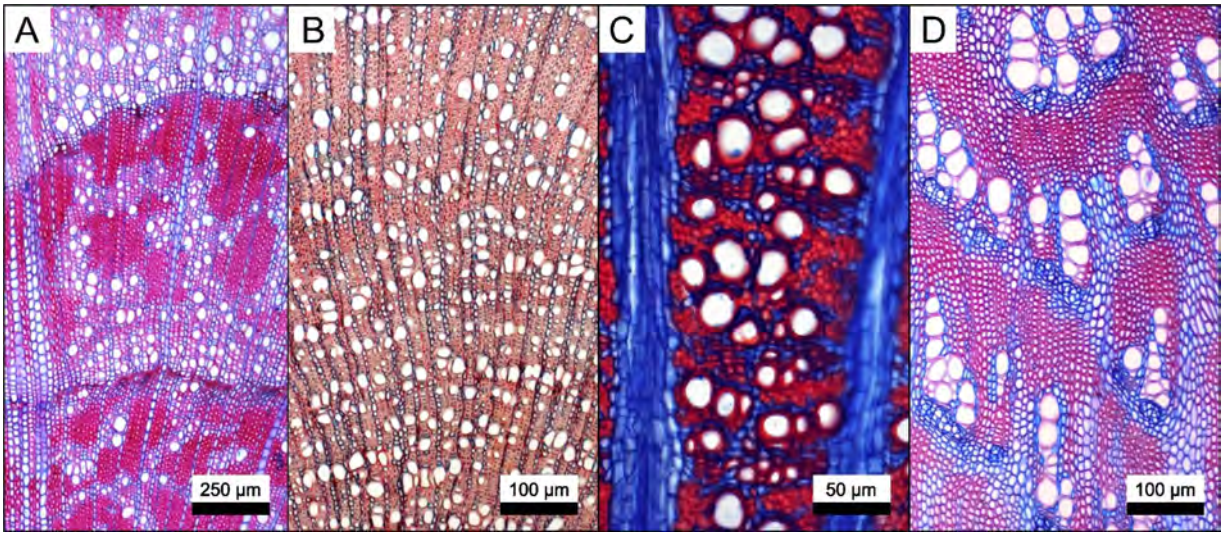

Fig. 14. Growth ring distinctiveness: A – Type a: all ring boundaries are clearly demarcated; B – Type b: clearly demarcated rings are only visible along some radii and some rings may be ill-defined due to tangential intra-annual bands or wedging rings. In such cases, it is important to examine the complete cross section; C – Type c: Growth zones may either look like annual rings, or be weakly expressed, or only visible in small areas of the cross section. Growth ring numbers indicate approximate plant age estimation; D – Type d: Growth rings are invisible or growth-ring formation is insignificant.

should be focused on the oldest individuals in a population. For woody plants with a basal resprouting capacity or for multi-stemmed shrubs, stem age does not reflect plant age and the same sampling technique as applied for herbs is recommended (i.e., sampling the oldest part of belowground stem, that is, root crown or distal part of a rhizome).

Trait assessment: Refer to *Tissue type ratio* for preparing cross sections. Count the number of growth rings along two radii from pith to bark: the maximum number of counted growth rings represents plant age. Observe the cross section from pith to bark direction, and look for anatomical changes that mark growth ring boundaries. Growth rings are not always clearly distinguishable, and Schweingruber and Poschlod (2005) proposed the following classification of growth ring distinctiveness:

Type a: All ring boundaries are clearly demarcated (Fig. 14A).

Type b: Clearly demarcated rings are only visible along some radii and some rings may be ill-defined due to tangential intra-annual bands or wedging rings. In such cases, it is important to examine the complete cross section (Fig. 14B).

Type c: Growth zones may either look like annual rings, or be weakly expressed, or only visible in small areas of the cross section. Growth ring numbers indicate a rough plant age estimation (Fig. 14C).

Type d: Growth rings are invisible or growth-ring formation is insignificant (Fig. 14D).

If annual growth rings are distinguishable (i.e., Type a, b), the following growth ring markers should be used: 1) thick-walled and radially flattened latewood fibres or tracheids versus thin-walled earlywood fibres or tracheids (Fig. 15A), 2) a marked difference in vessel diameter between latewood and earlywood of the following ring (Fig. 15B), and 3) the presence of marginal parenchyma at the growth ring boundary (Fig. 15C).

Special cases and problems: In clonal species, age can be determined only for the clonal fragment (e.g., ramet) (Fig. 6) and not for age of the whole genet. Growth rings may occasionally be missing, and a second or "false" ring may be deposited during a single year, such as after an insect defoliation or due to summer drought (Pausas, 1999).

Recommended literature: Schweingruber and Poschlod (2005); von

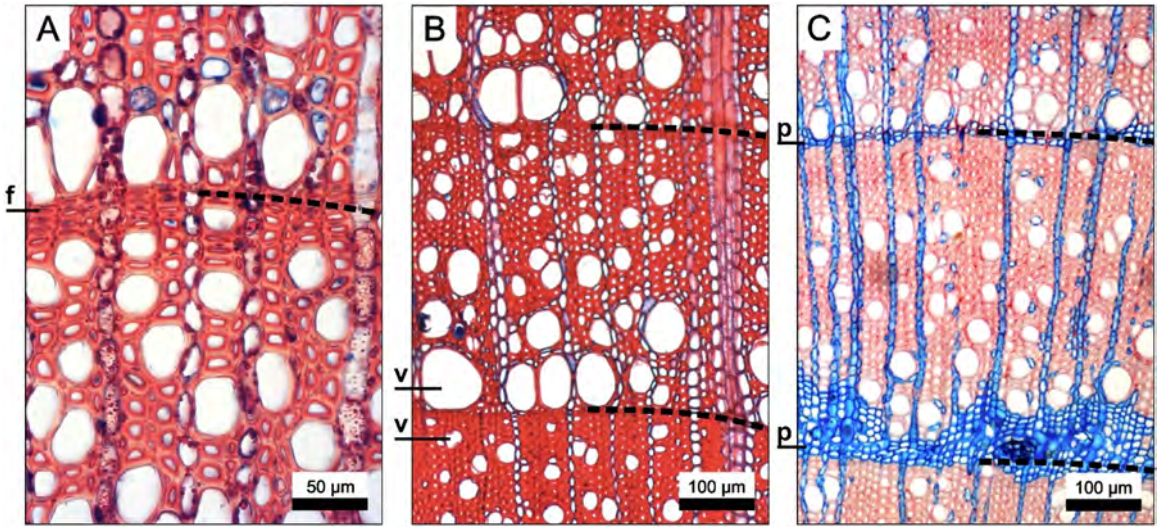

Fig. 15. Anatomical growth ring markers: A – demarcation identifiable by differences in cell wall thickness; B – demarcation identifiable by abrupt changes in conduit diameter; C – demarcation identifiable by presence of marginal parenchyma. f: fibers; v: vessels (conduits); p: marginal parenchyma. Dashed line: growth ring boundary.

Arx et al. (2006); Nobis and Schweingruber (2013); Doležal et al. (2018).

4.5.2. Radial growth

Despite the fact that plant growth responds to changing environmental conditions, their average annual growth increments (i.e., average of growth ring width) can be used as a reliable species-specific trait (Doležal et al., 2018). Functionally, growth rate contributes to shaping the fast-slow continuum in life history strategies (Salguero-Gómez et al., 2016).

Trait definition: Average annual plant increment in plants with secondary thickening.

Type of variable and units: Continuous; mm.

Sampling procedure: Refer to *Tissue type ratio*.

Trait assessment: Refer to *Age*. Measure all ring widths in a section (for Type a and b; see above), then average by the number of rings.

Special cases and problems: As per *Age*.

Recommended literature: von Arx et al. (2006); Doležal et al. (2018).

5. Conclusions

In this handbook we have provided operational guidelines for collecting 14 key plant modularity traits relevant to functions of on-spot persistence, space occupancy, resprouting after disturbance, resource storage, sharing, and foraging. Only by studying modularity traits in combination with other well-studied traits (e.g., leaf traits) can we achieve a more realistic and comprehensive understanding of plant and ecosystem functioning. We believe that, as happened after the publication of previous handbooks for trait assessment (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013), these protocols have the potential to trigger the systematic inclusion of plant modularity traits into the core toolbox of functional ecology.

Author contributions

JK conceived the original research idea; JK and GO led the writing; GO organized the research network and workflow; JK, JM, MGdM, JP, ŠJ, AC, JD, JPO, SP, RS contributed to specific topics; JGP, TH assisted in developing ideas and structure; All co-authors actively contributed to manuscript revisions.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ppees.2019.125485.

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