

## Appendix I: Standardized site description and sample labelling

Standardized sampling for trait assessment requires a basic description of the characteristics of plots (Table I) and samples (Table II). Such information will help to assess, e.g., whether the sampling effort was representative of the species distribution and whether the data are comparable. We recommend collecting the following information during field sampling.

*Locality of sampling* (Table I): Locality name; Country; Coordinates; Elevation, Aspect, Slope.

*Date of sampling*: day/month/year

*Habitat description*:

Habitat type: e.g., wet meadow, road verge

Vegetation structure: sparse herbaceous/woody (cover of vegetation lower than 50 %), dense herbaceous/woody (cover of vegetation more than 50 %)

Soil moisture: dry (dry and hard soils, plants partly dry); mesic (soft, rich soils, plants green); wet (soils soaked with water); aquatic (plants at least partly submerged)

Disturbance regime: report any information related to severity and frequency of disturbance; describe signs of disturbance, e.g., heavily grazed by livestock

Vegetation height: average height of the sampled community per vegetation layer, e.g., understory and overstory

*Plant description*:

Species name and authority

Family

Voucher specimen (y/n)

Replications (number, code) and sampling design (e.g., random, stratified)

Size of sampled individuals (plant height in cm) and phenological stage

Sampled organ(s)

Trait(s) to be measured

*Notes*: other features that may be important in the study site (e.g., soil depth).

Example of sample labelling is reported in Table II.

**Table I.** An example of sampling protocol reporting features at plot scale and describing sample information. Black text - headlines; blue text - data to be added during sampling.

Locality name	Date of sampling	Country	Coordinates	Elevation	Aspect	Slope
<i>Hill</i>	<i>4 May 2018</i>	<i>Czechia</i>	<i>49°00'42.3"N 14°47'06.5"E</i>	<i>434 m a.s.l.</i>	<i>NE (45°)</i>	<i>mild (10°)</i>
Habitat type	Vegetation structure	Soil moisture	Disturbance regime	Vegetation height		Notes
<i>meadow</i>	<i>dense herbaceous</i>	<i>moist</i>	<i>usually mown but not yet this year</i>	<i>40 cm</i>		
Species name	Family	Voucher specimen (y/n)	Replications (number, code), sampling design	Size of sampled individuals, phenological stage	Sampled organ(s)	Trait(s) to be measured
<i>Taraxacum officinale</i> L.	<i>Asteraceae</i>	<i>No</i>	<i>3, random</i>			
			<i>TO1/1</i>	<i>12, flowering</i>	<i>storage root</i>	<i>carbohydrate type</i>

			<i>TO1/2</i>	<i>13, flowering</i>	<i>storage root</i>	<i>carbohydrate type</i>
			<i>TO1/3</i>	<i>10, non-flowering</i>	<i>storage root</i>	<i>carbohydrate type</i>

**Table II.** An example of information to be appended to each sample collected in the field. Black text - headlines; blue text - data to be added during sampling.

Locality	Date of sampling	Plant name	Plant height in cm	Plant part	Code	Traits to be measured
<i>Hill</i>	<i>4 May 2018</i>	<i>Taraxacum officinale L.</i>	<i>12</i>	<i>storage root</i>	<i>TO1/1</i>	<i>anatomy, longevity</i>

## Appendix II: Experimental assessment of resprouting after disturbance

In pot or field experiments, different parameters related to disturbance regime, namely type, frequency, severity, and timing of the event, can be manipulated. Disturbance type is defined by the injuring agent causing biomass removal (e.g., fire, mowing, grazing); frequency is defined as how often the disturbance event occurs within a certain period of time; severity typically describes the proportion of biomass removed or the proportion of plant affected; timing refers to the period of the year or to the plant life cycle phase when disturbance happens (Miller et al., 2011). Resprouting ability can be assessed as survival (% of resprouting individuals), resprouting vigour (regenerated biomass), or any other fitness component (e.g., number of ramets, flowers, and seeds).

### Pot experiments

Experiments can vary from those where plants are grown in ideal conditions without competition to those mimicking more or less natural conditions, i.e., manipulating competition or resource availability. Note that plant responses to disturbance may be affected by disturbance history as well as current and previous biotic and abiotic conditions (Latzel et al., 2016). Be aware that bud bank and carbohydrate storage change during ontogeny, and adult plants tend to have larger buds and carbohydrate storage and thus higher probability to resprout than younger plants.

In nonclonal plants, as soon as seeds germinate on wet and sterilized sand in Petri dishes, transplant young seedlings to pots, with one seedling per pot. In clonal plants, ramets can be used instead of seedlings. Transplantation should be done early to protect plants from any root injury. If the study aims at gathering data on belowground biomass, pots should be large enough to reduce root distortions, and filled either by washed sand or garden substrate and sand mixture in e.g., 2:3 ratio. Pots with plants can be put into experimental gardens, greenhouses or growth chambers, and supplied with regular watering in combination with medium level of nutrient availability. We recommend liquid nutrition such as basic Rorison solution (Hunt et al., 1993). Experimental plants have to be protected against any unwanted disturbance before and after experimental injury. After plants are well established, treatments with different disturbance regimes can be applied (e.g., Kraaij and Ward, 2006). New resprouts from belowground or close to soil surface might start to appear already during the first week following the experimental injury. However, we recommend the resprouting activity to be traced for at least three weeks for herbs and several months for woody species.

### Field experiments

Experimental disturbance carried out in the field may be applied on whole communities or on selected plants (Table I). Evaluation of disturbance effects described here is done at species level. Before the disturbance event is implemented, select random replications of target species (10 or more replicates per species), map their positions or mark them to ensure that mortality during disturbance can be recorded. When a clonal plant is examined, ensure that the whole clonal fragment (i.e., all connected ramets) is subjected to the same treatment as interconnected ramets share resources hence their responses to disturbance may be affected. The recovery time depends on the study system and may last from several weeks for herbaceous plants to several years for woody plants.

**Table I.** List and details of different disturbance types according to their severity, method(s) to assess resprouting ability, and selected references.

Disturbance type	Disturbance severity	Method	References
Mowing, or grazing by large herbivores	Removal of nearly all the aboveground biomass	For mowing, remove all the aboveground biomass to 5 cm above soil. For grazing, apply height of cutting depending on	Vesk and Westoby (2004); Kraaij and Ward (2006)

the herbivore height of grazing.			
Grazing by small herbivores	Total defoliation; selective removal of leaf portions or leaf category (e.g., young leaves only)	Mimic damage by selected small herbivores.	Boege (2005); Lautent et al. (2017)
Landslide, ploughing	Fragmentation of belowground plant parts	Take belowground part out of soil, fragment it into pieces and replant, or apply disturbance directly by cutting plant in soil by sharp knife, spade or by ploughing.	Bímová et al. (2003)
Flooding	Total submergence of plants for a prolonged time period – for terrestrial plants	Depending on plant size, choose tanks of sufficient depth so that the whole plant is submerged. Some plants react to flooding by boosting their vertical shoot growth in order to reach above the water surface, avoiding anoxia, and this needs to be accounted for. After the treatment has been applied, do not remove any biomass, including dead parts. We recommend 7 days of flooding.	Striker et al. (2008)
Frost	Heavy and sudden (e.g., late-spring) frosts	Event has to be applied out of usual occurrence of frost season in the study area. Use a freezer or a chamber with temperature regulation and expose plants to -5°C for 5 hours (for temperate herbs).	Prozherina et al. (2003)
Drought	Heavy and long-lasting drought	Use experiments (e.g., plots with rain shelters) and apply drought exceeding usual dry period in the study area.	VanderWeide and Hartnett (2015)
Burial by sand or sediment	Partial or complete burial of plants in sand	Depending on plant size, choose tanks of sufficient depth so that the plant can be partially or completely buried in sand.	Maun and Lapierre (1984); Yu et al. (2004)
Erosion by wind or water	Partial or complete exposure of belowground plant parts to the air	Use pots or field experiments so that plants can be partially or completely exposed out of the substrate, mimicking wind or water erosion.	Yu et al. (2008)
Fire	Partial or total removal of aboveground biomass (e.g., crown fires) and heat	Experimental studies involve individual species or community-wide comparative survey before and after fire, or in similar habitats with or without experiencing fire.	Vesk et al. (2004); Kral et al. (2015)
Logging or wind	Partial or total removal of	Field studies involve individual species or community-wide	Cooper-Ellis et al. (1999); Moreira et

throw	aboveground biomass	comparative survey before and after logging or wind throw, or in similar habitats with or without experiencing logging or wind throw.	al. (2012)
Root fragmentation	Removal of all stem- and leaf-derived organs	Use root fragments from 5-10 plants. Cut off root fragments at comparable position with comparable size. Weight the fragment and put it in a pot covered by sand. Keep the fragment under moist conditions.	Martínková and Klimešová (2016)

#### Assessment of resprouting ability

For woody plants, resprouting ability is typically assessed in the next growing season or after approximately one year (Paula et al., 2009; Moreira et al., 2012). Plants could be monitored for several years to record changes in life-history mode (e.g., from monocarpic to polycarpic, from biennial to perennial) or lifespan. Depending on research questions, absolute or relative plant performances can be assessed (Table II).

**Table II:** Examples of research questions, and suggested experimental set-ups to quantify resprouting success.

Questions	Experimental approach
Is the plant able to cope with disturbance? If so, how?	Assess performance of disturbed vs undisturbed plants.
Is resprouting more advantageous than sexual regeneration under disturbed conditions in terms of fitness (e.g., survival, biomass recovery)?	Quantify performance of resprouting plants (e.g., regenerated from fragments) vs plants established from seeds at the time of disturbance.
What is the capacity of plants to compensate for biomass loss? How does disturbance severity affect plant performance? What is the role of plant size?	Compare performance of the same plants before vs after the treatment (e.g., removed vs regrown biomass).
What is the capacity of a population to recover after disturbance?	Estimate survival of disturbed plants.
How does repeated disturbance affect plant performance?	Assess performance of plants disturbed repeatedly vs those disturbed only once.

Special cases and problems: Note that axillary buds can be exhausted by repeated injury, whereas adventitious buds are potentially indefinite, and their availability may have large ecological implications for plants (e.g., affecting resprouting ability after fire, mowing, grazing). In the field, it may be highly difficult to distinguish between a new sprout and a seedling, especially long after disturbance. Therefore, either remove seedlings regularly when they are recognizable or inspect belowground parts in order to quantify the number of sprouts.

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### Appendix III: Glossary

**Amylopectin:** glucose polymer with  $\alpha(1-4)$  and  $\alpha(1-6)$  glycosidic bonds resulting in branched chains composing starch.

**Amylose:** Glucose polymer with  $\alpha(1-4)$  linkages between the glucose moieties. This polymer has mostly linear chains and is a component of starch.

**Clonal fragment:** physically independent part of a clone – formed either by one ramet or by several interconnected ramets.

**Cyclicity:** the number of years (or seasons) occurring between bud-sprouting and shoot-flowering which determines shoot longevity.

**Distal:** plant parts situated further away from the centre of the body or from the point of attachment (opposed to proximal).

**Disturbance frequency:** how often a disturbance event occurs within a certain period of time.

**Disturbance regime:** the combination of different disturbance parameters (e.g., type, frequency, intensity, severity, and timing).

**Disturbance severity:** the magnitude that a disturbance event affects the plant, which can be measured by, e.g., the proportion of plant biomass removed, the percentage of plant height affected, or the proportion of individuals killed by the disturbance event.

**Disturbance timing:** the period across ontogeny and/or growing season when disturbance happens, e.g., the time of the year when the disturbance usually occurs.

**Disturbance type:** the type of injuring agents causing biomass removal (e.g., fire, mowing, grazing, flooding, trampling, wind blast, frost, strong current, burial and wind erosion).

**Earlywood:** the part of a secondary-growth ring that is formed early in the growth season in plants with secondary thickening (in dicots and gymnosperms), usually characterized by a lower density and larger cells than the latewood.

**Genet:** the product of a zygote or a genetic individual, i.e., a rooting unit in nonclonal plants and a collection of all rooting units derived from a single zygote in clonal plants.

**Growth ring width:** xylem added by the cambium during a single growth period (in dicots and gymnosperms).

**HPAEC/PAD (High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection):** a technique similar to HPLC, but using both stationary and mobile phases in alkaline conditions, allowing anion exchange. Under this condition, carbohydrates tend to have electroactive groups interacting in a solid anode when positive and negative potential pulses are applied alternatively, allowing a more precise identification of individual carbohydrates.

**HPLC (High Performance Liquid Chromatography):** a technique for separation, identification and quantification of compounds based on the use of a mobile phase (liquid) which flows through a stationary phase (column). The column is prepared with smaller particles as compared to the particles used in regular column chromatography. This provides higher resolution during separation of compounds.

**Hypocotyl:** linkage between root and shoot systems evident in seedlings. In later ontogenetic stages, it becomes part of root collar in nonclonal species, while it is decaying in clonal plants.

**Latewood:** the part of a secondary-growth ring that is formed late in the growth season in plants with secondary thickening (in dicots and gymnosperms), denser and composed of smaller cells than the earlywood.

**Lyophilization:** a method for tissue dehydration, in which the frozen samples are placed in reduced pressure, under vacuum and temperature variations to allow conversion of solid water directly into the gaseous state.

**Maltodextrins:** oligosaccharides composed of  $\alpha(1-4)$  linked glucose units produced by partial hydrolysis of starch.

**Monopodial branching:** a shoot with potentially endless apical growth, and lateral shoots are derived from axillary meristems on that shoot.

**Plant functional trait:** defined by three key properties: i) measurable by standardized procedures at individual plant level, ii) interspecific differences higher than intraspecific variability, and iii) tightly associated with specific plant function(s).

**Proximal:** plant parts located closer to the centre of the body or the point of attachment (opposed to distal parts).

**Ramet:** potentially independent or fully independent part of a genet, i.e., a developing or fully developed rooting unit in clonal plants. Interconnected ramets form a clonal fragment.

**Resprouting:** emergence of shoots after disturbance.

**Rooting unit:** the smallest plant part capable of surviving independently, i.e., a ramet in clonal plants, and a genet in nonclonal plants.

**Root-system:** similarly to shoot-system, roots can be classified in three types: 1) primary root (growing from the embryo's root pole), 2) root branch (growing from the primary root), and 3) adventitious root (growing from a stem). Plants capable of producing adventitious roots can form more than one rooting unit during their lifespans and, therefore, grow clonally.

**Shoot:** a chain of modules, i.e., internode(s) plus node(s) with leaf and axillary bud(s), produced by an apical meristem. Each plant starts its growth by forming a primary shoot from the shoot pole of a seed embryo. New shoots developing from axillary meristems of the primary shoot may be added. Some plants can produce shoots from adventitious buds of roots or leaves (adventitious shoots). Therefore, three types of shoots can be distinguished based on origin: primary, axillary and adventitious.

**Shoot apical meristem:** the meristematic tissue producing shoot (stem with leaves) which may be long-lasting or turn into a generative structure (e.g., inflorescence) or another type of dead-end structure (e.g., thorn).

**Shoot-system:** in perennial herbs, the aboveground stem consists of shoots that are shed after senescence or after flowering, and the basal, belowground part of each shoot often remains active and functions as bud bank. Woody plants add new shoots to older ones by aboveground branching, and older shoots usually undergo secondary thickening and build up



perennial aboveground shoot systems. Therefore, the aboveground structure often consists of individual shoots (in herbs) or of a branched shoot-system (in woody plants).

**Sink:** plant organs or regions importing carbon compounds through phloem transport.

**Source:** plant organs or regions exporting carbon compounds through phloem transport.

**Spectrophotometry:** the quantitative measurement of light that is absorbed or reflected by a material having specific wavelengths. This method is often used to quantify compounds that, in reaction, produce coloured substances that can be detected in a spectrophotometer.

**Sprouting:** seasonal emergence of shoots, which occurs throughout the life cycle of a plant.

**Stem base** (= root collar = root crown): the connection between root- and shoot-system in nonclonal plants, representing the oldest part of these plants. The hypocotyl is part of this structure.

**Sympodial branching:** a shoot stopping its growth after developing into a dead-end structure (e.g., inflorescence, thorn) or simply due to growth cessation. This shoot can be replaced by at least one axillary shoot that overtops and continues growing.

**Tracheid:** a conductive cell that has no perforations, as contrasted with vessel elements.

**Vessel:** a tube-like series of conductive elements, with perforated cell walls (especially in dicots).

**$\alpha$ -galactosidase:** enzyme which hydrolyses terminal  $\alpha$ -galactosyl moieties from higher molecules, such as oligosaccharides from the raffinose family.