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Article type : Methods on vegetation science
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Tree death and damage: a standardized protocol for frequent surveys in tropical forests

Running title: Tree mortality and damage protocol

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Funding information: This work was supported by the US Department of Energy, Office of Science, Office of Biological and Environmental Research through the Next Generation Ecosystem Experiment-Tropics (https://ngee-tropics.lbl.gov/). ForestGEO workshops were supported by the US National Science Foundation [DEB-1046113 and DEB-1545761] to SJD.

Abstract

Tree mortality drives changes in forest structure and dynamics, community composition, and carbon and nutrient cycles. Since tropical forests store a large fraction of terrestrial biomass and tree diversity, improved understanding of changing tree mortality and biomass loss rates is critical. Tropical tree mortality rates have been challenging to estimate due to low background rates of tree death, and high spatial and temporal heterogeneity. Furthermore, the causes of mortality remain unclear because many factors may be involved in individual tree death, and the rapid decomposition of wood in the tropics obscures evidence of possible causes of tree mortality. We present a field protocol to assess tree mortality in tropical forests. The protocol focuses on the rapid, repeatable and inexpensive assessment of individual tree death and damage. The protocol has been successfully tested with annual assessments of >62,000 stems in total in several ForestGEO plots in Asia and the Neotropics. Standardized methods for the assessment of tree death and biomass loss will advance understanding of the underlying causes and consequences of tree mortality.

Key words: above-ground biomass, forest carbon, ForestGEO, tree mortality, tropical forests, tree damage

1. Introduction

Tree death alters tree population and community structure. It also increases light availability and changes soil properties (Franklin et al. 1987). In tropical forests, variation in carbon stocks depends on tree mortality more than growth (Johnson et al. 2016; Longo et al. 2019; Hubau et al. 2020; Pugh et al. 2020). Recent increases in mortality rates are therefore expected to alter forest structure and dynamics, community composition, and carbon and nutrient cycles (McDowell et al. 2020). Although key to predict forest response to global changes (Cavaleri et al. 2015; Smith et al. 2016; Hartmann et al. 2018; McDowell et al. 2018), much uncertainty remains on causes and consequences of tropical tree mortality.

Tree death involves interrelated drivers from global or regional (e.g., drought, increasing temperature) to local scales (e.g., biotic outbreaks, fire, wind). Linking tree death to drivers is particularly complex in the tropics, where high diversity results in multiple physiological responses to a given driver (Koven *et al.*, 2019) and where high rates of wood decomposition quickly remove signs of the killing agent (Gora et al. 2019). Large-scale and frequent monitoring of tree death with explicit consideration of the likely factors involved is needed to improve estimates of tropical tree mortality.

Biomass losses within living trees contribute significantly to biomass loss in tropical forests (Chambers et al. 2001; Chave et al. 2003). Biomass losses result from mechanical damage (e.g., wind), physiological stress (e.g., dieback by drought), and natural self-pruning. The assumption that a tree is intact as long as a diameter is reported leads to overestimation of total biomass and underestimation of biomass turnover rates (Clark et al. 2001). Damage also impacts tree growth and survival due to the loss of structural support, hydraulic conductivity, photosynthetic capacity, and increased exposure to pathogens/pests (Clark & Clark 1991; King et al. 2005; Rutishauser et al. 2011;

Dyer et al. 2012; Arellano et al. 2019). Assessment of tree damage coupled with identification of factors associated with tree death should lead to improved understanding of the causes of tree mortality and estimates of biomass fluxes in tropical forests.

We designed a standardized field protocol to evaluate tree vigor, biomass loss, and factors likely to be associated with future tree death. The protocol minimizes the effort required at each tree to allow the frequent assessments of more trees. First, we describe sampling design challenges and trade-offs considered. Second, we present operational definitions of tree attributes for objective protocol implementation. Finally, we describe the specific observations for individual tree assessment in the field. Although this protocol focuses on large forest plots, it can be easily applied to any forest mortality survey. The ForestGEO website contains the protocol and example datasheets (https://forestgeo.si.edu/node/146527/).

2. ForestGEO annual mortality survey (AMS) sampling design

Annual mortality and damage surveys (AMS) were initiated in 2016 in eight ForestGEO plots across the tropics (Anderson-Teixeira et al. 2015) following the protocol herein. The AMS follows ~5,000 trees with diameter at the breast height (DBH) ≥ 1 cm in each plot. Here, we describe the main trade-offs considered, as a guide to establishing mortality surveys in other sites. Note that we implement the AMS on already existing plots of great spatial extent; to take advantage of that, while sampling a reasonable number of stems, we implemented a stratified sampling targeting different areas for different size classes. This and other decisions have consequences for data analyses described in Appendix S1. The application of the suggested protocol itself does not depend on the sampling design, so much simpler sampling designs are possible and even desirable depending on the circumstances of each research team.

2.1 Spatial distribution of monitored trees

A key decision is whether to sample randomized individuals or all individuals within contiguous subareas. Monitoring randomly distributed trees is ideal for detecting shifts in mortality (McMahon et al. 2019), and does not have the statistical problems associated with spatial autocorrelation. However, a randomized design does not allow for spatially explicit analyses, which can provide important insights. For example, competition affects trees of similar sizes/characteristics in the same locations (e.g., Pillet *et al.*, 2018), Janzen-Connell effects result in mortality of aggregated conspecifics, and abiotic drivers (e.g., gaps) result in aggregated deaths regardless of species. Other causes of mortality may result in less aggregated spatial patterns (e.g., mortality caused by water stress). Besides, a spatially contiguous sampling design requires much less field effort than visiting randomly distributed trees. Based on these considerations, the ForestGEO AMS adopted a sampling design based on contiguous areas.

2.2 Stratification by habitat and tree size

Tree mortality rates often decline with tree size, how trees respond to climate stressors is often size-dependent, and large tree deaths have bigger impacts on forest carbon stocks (Coomes & Allen 2007; Bennett et al. 2015; Pillet et al. 2018). The ForestGEO AMS uses 35 DBH classes to stratify sampling, with limits exactly evenly distributed on a log(DBH) scale: e^x, $x \in \{\ln(10) + 0z, \ln(10) + 1z, \ln(10) + 2z, \ldots, \ln(10) + 34z, \infty\}$ and $z = (7 - \ln(10))/34$, in mm. The size classes were the same in all sites. To maximize the number of species and functional strategies included and enable tests of resource-related effects on tree mortality, we also stratify by 3-5 habitat classes defined independently for each plot based on topography and/or soil properties.

In our overall design, each habitat contains a series of nested square quadrats, with increasingly smaller trees in the smaller quadrats. All stems above a given size are sampled in these quadrats (plus all the stems of those individuals, even if not large enough). The area used for each size class varies between sites, as it is determined so that the number of stems per size class is roughly the same in the final selection of stems at that site. For classes >10 cm DBH we use one quadrat per size class and habitat. For classes ≤10 cm DBH we use five smaller quadrats per size class and habitat. This results in a loss of spatial contiguity at those scales, but it minimizes the chances of losing particular combinations of size class and habitat with one disturbance event (e.g., a single large tree fall). No effort was made to incorporate under-represented species (e.g., pioneer species concentrated in gaps) as the range of likely responses is expected to be captured by the forest habitats. Appendix S2 contains a simplified example and some other details of the spatial stratification. In any case, the areas invested for each size class are fundamental metadata that needs to be carefully and permanently stored and distributed along with the data.

Besides, the AMS opportunistically includes additional trees that are being frequently monitored for growth or other attributes (e.g., dendrometer bands). While measuring DBH every year for all stems would be too time-consuming for the AMS, adding stems with frequent growth measurements creates opportunities for understanding links between tree performance and the likelihood of mortality. If DBH is not measured regularly it would be advisable to measure it every 5 years or so (at intervals long enough to detect DBH change over the measurement error). In general, monitoring trees that are already being monitored for other reasons allows for the accumulation of more detailed observations on a subset of stems, and should facilitate more detailed analyses in the future.

2.3 Cohort vs. population

Annualized mortality rate estimates are expected to decline with increasing time intervals (Sheil & May, 1996; Zens & Peart, 2003; Kohyama *et al.*, 2018). In the absence of recruitment, a series of surveys will observe the increasingly biased sample of "the most resistant trees" each year, and we should expect lower mortality rates as the study progresses. When designing a long-term mortality study, it is important to acknowledge this sampling artifact and to consider whether to follow the same initial sample of trees (i.e., cohort) or to replace the dead trees with new recruits each year (and follow a population instead of a cohort). The population approach is desirable as it is not affected by the survival bias, but the cost involved in locating, tagging, mapping, and identifying additional trees during each annual survey is significant. The cohort approach may cause statistical problems but the lower cost allows larger sample size. The ForestGEO AMS uses a hybrid of these approaches. Trees for the AMS were selected from the last full census of the large plot and are monitored as a cohort during the time period between full plot censuses (~5 years in ForestGEO plots). At each full census, the population included in the AMS is increased to include new recruits that reach the defined size classes within the defined target areas.

3. Operational issues and definitions for protocol implementation

In ForestGEO plots, the AMSs are usually performed by one or several teams of two persons per team. One has the map of the targeted trees and the other manages the data form. At each tree, both persons examine the tree while walking around it for ~30 seconds. They conduct a visual examination of the tree, without binoculars, looking for immediately visible factors affecting the tree. Then they record each variable and register the observations in the data form. For large trees, more time is generally required to assess canopy-related variables. Such a rapid assessment technique is likely to miss relevant factors, particularly cryptic ones such as hollow stems, tiny bore holes of insects, etc., especially if far from the tree base. However, alternative detailed screening would require much more work in the field, and currently there is limited information about pest/pathogen impacts in tropical forest species to make good use of symptoms that are not immediately visible.

An objective way to assess each tree is key. Our operational definitions excluded, as much as possible, considerations about function or biological meaning (e.g., we purposely avoided the distinction between "lateral branch below 1.30 m" and "secondary but independent stem"). For consistency and repeatability, it is important that these operational definitions are fully discussed with the field crew before and during data collection. In the ForestGEO sites, for example, we allocated two weeks to field crew training during the first (and sometimes second) AMS in order to reduce the observer bias and obtain standardized data across sites.

POM: Point of measurement of stem diameter, typically 1.30 m above ground. In buttressed trees, or in presence of deformities, it can be at a different height (Condit 1998).

Individual: An individual consists of all woody stems *and* anything else (e.g., non-woody resprouts) that arise from the same root system. Stems with a reasonably obvious aboveground or belowground connection are assigned to the same individual. In the case of clonal species, two stems within 1 m of each other are likely the same individual, but different rules exist depending on the biology of the species. In the ForestGEO AMS, we include individual trees with one or more stems ≥1 cm DBH at the *POM* in the previous full census of the plot.

Stem: Most individual trees are composed of a single stem. If there are multiple woody shoots bifurcating below the POM and reaching ≥1 cm DBH, each of them is considered a "stem" in our protocol. Note that, in some species, stems can be produced at or below ground level. Branches (including those arising horizontally from an obvious main trunk) are also treated as separate stems (not "branches"; see below), as long as they bifurcate below the POM and reach ≥1 cm DBH (Condit 1998). In this protocol each stem is assessed separately, so rows in the data forms correspond to *stems*, not individuals.

Main axis: Not all woody plants have an obvious main trunk from base to tip. We use an operational definition to allow consistent measurements during the censuses. The goal is to split any given stem into a *main axis* and *branches* in a way that is repeatable. For any given stem, the *main axis* extends from the rooting point (height = 0) to the apex of the stem, passing through the POM (Figure 1a). From the POM to the top of the stem, we follow the thickest part at each bifurcation or branching, no matter if it is alive (Figure 1b) or dead (Figure 1c). If the bifurcation involves two parts of exactly the same size, we follow the living one (Figure 1d). If both are alive, we follow the most upright (Figure 1e). If both are equally upright, we follow the longest (Figure 1f). From the POM to the rooting point, the stem is usually obvious. Only if in doubt, we follow the shortest line connecting the POM with a rooting point through living tissues (see Figure 2 for main axis definitions in multi-stemmed individuals, and Appendix S4 for examples of main axis definitions).

Branch: Branches are woody shoots connected laterally to the *living length* (Section 4.3 below) of the main axis above the POM. Woody shoots connected to the main axis below the POM are by definition stems, not branches (e.g., Figure 4, Appendixes S3 and S4). If they are ≥1 cm DBH at the POM, they would carry a tag and would be evaluated independently, since we include all the stems ≥1 cm DBH of any included individual.

Crown: The set of all branches on a stem.

Damage: Damage includes any physical harm that leaves the inner wood exposed at the time of assessment. If the inner wood is hidden by sap or latex but we assume the wound is recent and still open, it is also considered damage (e.g., stem #25 in Appendix S4). Previously damaged areas that are covered with bark (e.g., sealed wounds, case 1 in Appendix S3, stem #1 in Appendix S4) are no longer considered damage because they do not represent current risks for the tree. Scars of old branches are not considered damage or branch loss (*e.g.*, Figure 4). This definition of damage is used to decide whether the main axis is broken or not (Section 4.2), to estimate crown damage (Section 4.4), and to record wounds along the main axis (Section 4.9). Note that the distinction between "there is inner wood exposed" and "there is not any inner wood exposed" is crucial for this protocol, since it represents the operational definition of "time". It is our only criterion to determine whether something happened recently or too long ago to be considered.

4. Protocol variables collected at each tree

The data forms are designed to be filled out completely for each stem. If it is not possible to assess a given aspect of a particular stem, we suggest using "?" (i.e., "the field crew cannot tell in 30 seconds without binoculars"). Besides, we record people and dates, which are particularly relevant when the survey duration is substantial, relative to the time between surveys (Kubo et al. 2000).

4.1. Survival status (OK / A / D / X / NF)

Although mortality is an individual-based process, we record this field for every *stem* (each row in the data form). This redundancy is useful for data-cleaning and quality control assessment. If the tree is found, there are three possibilities (Figure 2):

- A: *stem* alive, which necessarily implies *individual* alive. "A" should be registered when there is any living tissue on the *stem*. For example, a small segment of the stem or a resprout. Note that, even if there is no green shoot, a tree could be alive if the cambium is visibly green and the twigs flexible.

- D: dead *individual*, which necessarily implies dead *stem(s)*. There is no sign of living tissues anywhere in the *individual*. Not in this stem, not in any other stem, not in anywhere else.
- X: This case applies to stems entirely composed of dead tissues above ground ("living length = 0 m", see below) in a living individual (i.e., an individual that has living tissues somewhere else, but not on this stem). Given that this protocol only focuses on aboveground components and stems are defined from the ground level, the "X" case applies only for stems with a resprout at the ground level or for multi-stemmed trees that bifurcate at or below the ground level (Figure 2). Since this case is not very common, it is possible that field crew write "D" instead of "X". In this case, comments on other stems of the same individual or codes for the same stem in previous or subsequent surveys should be checked. An alternative way of coding a "X" stem is "status = A" along with "living length [of the stem] = 0 m" (see Section 4.2 and Appendix S4).

Besides, we use two more codes for convenience:

- NF: Stem not found and tag not found. This case can be interpreted as D (or X, depending on the case) in data analyses, unless the stem is found alive in subsequent surveys.
- OK: Is a shortcut for a healthy and undamaged stem. "OK" means that the focal stem is alive, has a standing and complete main axis (not uprooted, not broken; Section 4.2), has living tissues from the base to the tip of the main axis (Section 4.3), and has a complete crown (90% of remaining crown or more; Section 4.4). This code saves a lot of time.

4.2. Mode (S, B, U)

This field describes the mode of death (if status = "D") or damage (if status = "A" or "X"). If the tree is dead, the goal is to infer what may have killed the tree. If the tree is alive, the goal is to record damage that reduces total aboveground biomass and may impact future survival.

- S: standing. The main axis is complete and retains physical continuity or integrity. It does *not* imply the stem is vertical. This code applies even if the main axis is composed of partially or entirely dead tissues (the proportion of dead tissue is estimated as a "living length"; see Section 4.3). Damaged or dead trees will always be incomplete to some degree as dead wood decays. In those potentially unclear cases, if we estimate that the tree died or decayed while standing, we note "S". This is one of the few instances where some biological or contextual reasoning is needed when applying this protocol (see below).

- B: broken. The main axis is snapped, incomplete, but some of it is still standing (may be meters or just centimeters). The broken section of the main axis typically has splinters. Unlike "S", "B" mode generally implies an external force acting on the stem.
- U: uprooted. The tree has tipped over with the roots aboveground. This code means that roots that were belowground are now aboveground. Like "B", "U" generally implies an external force acting on the tree. Stilt or aerial roots, or roots exposed by soil erosion, do not qualify as "U". Uprooted does not necessarily mean that the main axis is on the ground. If Mode = "U", we always fill out the "Leaning" field (Section 4.6).

All damaged or dead stems coded "S" will become "B" later (Figure 3). It is important to try to record the standing damage (if it happened) before wood decay hides it. A tip that has proven useful in the field to differentiate between an initially "B" tree and an initially "S" tree that later decayed is to examine splinters and woody debris on the forest floor (Figure 3). In the S → B trajectory, the tree often starts to die and decompose from the top. In time, branch and trunk sections fall down in relatively small and non-continuous pieces (Gale & Hall 2001). On the other hand, the B → B trajectory implies an external, mechanical force acting on the stem (e.g., storms, other tree falls). When a tree breaks, the remaining stem has splinters, and the snapped part is generally found as continuous and more obvious sections that take longer to decompose. If still unclear, write "S/B?" or record the uncertainty in the "comments" field.

If the stem is uprooted (U) and broken (B), both codes can be used in the same field. "B" and "S" are not compatible. When the tree is dead and only the tag can be found, we fill "Mode = ?".

4.3. Living length (m)

The *living length* field provides an estimate of the proportion of remaining living tissues in the main axis of the stem when the stem is alive but broken (mode = "B"), or in the process of dying standing (mode = "S") (Figure 3). Nothing is recorded when there are living tissues along the entire main axis of the stem. The amount of biomass loss within a living tree can be estimated by comparing the estimated biomass up to the living length with the "idealized" biomass of the complete tree (e.g., based on allometries). In addition to being a direct estimate of biomass mortality, the living length is also a major factor influencing future mortality (Clark & Clark 1991; Arellano et al. 2019).

The total living length is estimated in meters along the main axis. Usually the living length corresponds to the basal part of the stem. However, a tree may fall, resprout along its trunk, and survive and grow from there, and its original base could die (Appendix S4, case #15) or stay alive (Appendix S4, stem #26). In those cases, we include the total, accumulated living length along the main axis. In most cases, we will be able to identify short living lengths, particularly if the tag remains attached. In some other cases the tag will not be there and we will not be sure about the former structure of the individual, in which case we record "<POM" in this field.

4.4. Remaining crown within the living length (%)

We characterize damage to the tree crown that remains within the living length. As with the living length estimate, the remaining crown field contributes to the estimation of biomass loss within living individuals, and relates to future tree survival (e.g., Arellano *et al.*, 2019). The remaining crown (%) is assessed only within the remaining living length because the living length variable already discounts some dead crown sections (see Appendix S3 for detailed cases). Branches below the POM are not considered branches in this protocol, so they play no role in the remaining crown assessment. Note that we include every multiple stem of each included individual and ForestGEO sites use a cutoff of 1 cm DBH, so we leave out things <1 cm DBH. Forest inventories based on a larger DBH cutoff may include branches below the POM in the remaining crown assessment.

The remaining crown assessment is based on evidence of dead branches still attached or broken branches leaving inner wood exposed (Figure 4). Fallen branches that disappeared so long ago that the scar does not leave inner wood exposed are not included in this estimate. In this field, 100% means that there is no evidence that branches have been lost within the living length, whereas 0% means a tree that lost all the crown within the living length (Figure 4). If there were no branches within the living length in the first place, we write "NA", not "0%". It is sometimes difficult to distinguish dead branches from living branches without leaves. Often living branches show abundant twigs that are absent from dead branches (Figure 4). We do not assign low % to naturally sparse and open crowns with very few branches (e.g., *Cecropia*), or abnormal/asymmetrical crown growth that do not involve recent branch loss. For palms, we can assess the loss of leaves in this field, with caution during data analyses.

This variable cannot be interpreted by itself as the absolute remaining crown. A tree with "100% of remaining crown" may have lost most of its crown, if the crown was originally above the remaining living length (case 6 in Appendix S3). We use a conditional variable (% remaining crown within the living length) because it is almost impossible to estimate what a broken tree looked like before, and breakage along the main axis is the most common cause of absolute crown loss. The applicability of this variable in accounting for biomass loss, or absolute crown loss, depends almost entirely on the existence of a model describing the amount of crown above/below a given height (e.g., Ver Planck & Macfarlane 2014).

4.5. Crown illumination, CI (levels from 1 = no light to 5 = full light)

Light is an important limiting factor related to tree performance but its relationship with mortality remains unclear, possibly due to interactions with tree size and age (Rüger et al. 2011; Arellano et al. 2019). The CI field describes how much light the tree can access, including all leaves above or below the POM, regardless of whether they are covered with lianas or epiphytes (Figure 5) (Clark & Clark (1992) adapted from Dawkins & Field (1978)):

- CI = 5: tree leaves completely exposed to vertical light and to lateral light within the 90 degrees inverted cone encompassing the crown.
- CI = 4: full overhead light. ≥90% of the vertical projection of the crown exposed to vertical light.
- CI = 3: some overhead light. 10-90% of the vertical projection of the crown exposed to vertical light.
- CI = 2: lateral light. <10% of the vertical project of the crown exposed to vertical light, but the crown receives some light laterally.
- CI = 1: no direct light. The crown receives only light filtered through the crowns of other trees.

4.6. Leaning (°)

Leaning stems may be more prone to breakage or uprooting due to the twisting force exerted by its weight and have been found to affect tree survival in temperate (Brewer & Linnartz 1973; Bragg & Shelton 2010) and tropical forests (Shenkin et al. 2015). This variable may also help to identify if domino effects are important in forming gaps (e.g., van der Meer & Bongers, 1996). This field records the deviation of the stem from vertical; the leaning angle measured in degrees from the base through the POM (Figure 6). If the stem is curved, we evaluate this attribute in the basal part of the main axis, between the rooting point and the POM (see case 27 in Appendix S4).

4.7. Liana, stranglers (L, S)

Lianas can kill trees by competing for water and light or by strangling the tree and causing xylem damage (McDowell et al. 2018). Here we use "liana" in a broad sense, recording data on any liana, strangler fig or (hemi)epiphyte plant growing on the tree that may be affecting its vitality. If >50% of the crown is covered by a liana (s.l.), the tree is coded "L". If the liana or strangler appears to limit the diameter growth of the main axis, the tree is coded "S". Both codes are compatible. The 50% cut-off was used as it has been shown that growth and survival decline significantly only with liana loads in >50% of the crown (Visser et al. 2018); more nuanced estimation of liana infestation may be required in the light of new evidence.

4.8. Fungi (presence/absence)

The extent to which the presence of fungi affects tree survival remains unclear. We interpret the presence of fungi as a symptom of decaying wood and thus increased risk of mechanical failure, at the very least. This field is checked (√) if there are visible fungi on the trunk that might affect the inner wood. We do not record the presence of fungi living superficially in the bark or of lichens on the bark or leaves.

4.9. Wounded main axis (levels: 1 = small, 2 = large, 3 = massive)

Tree damage can occur without much biomass loss, but significantly affecting future tree survival (Mattheck 1995). For example, collateral damage from neighbor trees can cause severe longitudinal damage to trunks or lightly scrape the bark leaving inner wood exposed to pathogens and other killing agents. This field records the presence and degree of damage to the wood or bark on the surface of the stem that leaves inner wood exposed. It is assessed within the living length of the main axis and it does not refer to the branches (Figure 7):

- 1 = small wound, smaller than an area of dimensions DBH × DBH.
- 2 = large wound, greater than an area of dimensions DBH × DBH but not affecting >50% of the basal area or living length.
- 3 = massive wound, affecting >50% of the basal area (i.e., a very deep and extensive wound; Figure 7c) or >50% of the living length (Figure 7d). These are cases of main stem breakage in which the breakage is not complete and the broken part is still connected and alive, and trunks that have been longitudinally split in two.

We do not record in this field anything associated with hollow trunks or hollow bases; we use the "comments" field for this information.

4.10. Canker, swelling, deformity (levels: 1 = small, 2 = large, 3 = massive)

Plant tumors are morphologically distinct structures caused by an uncontrolled growth of abnormal cells. Although plant tumors do not metastasize due to the rigidity of the cell wall (Doonan & Sablowski 2010), they have been associated with poor performance in tree crops and mangrove trees (Tattar et al. 1994; Pike et al. 2006). Their effect in most tropical forest species remains unknown. In practice, a quick survey cannot differentiate between a canker and any other swelling, deformity or gall (Dodueva et al. 2020). Abnormal woody growth, in any case, may represent a risk of mechanical failure or be a sign of infection or disease. In this field, deformities are recorded regardless of the possible cause. In these cases, the inner wood is not exposed.

- 1 = small deformity, smaller than an area of dimensions DBH × DBH.
- 2 = big deformity, larger than an area of dimensions DBH × DBH but not affecting >50% of the basal area or main axis length.
- 3 = massive deformity or canker, affecting >50% of the basal area or >50% of the main axis length.

4.11. Rotting trunk (levels: 1 = small, 2 = large, 3 = massive)

Advanced decay in a tree trunk can cause serious structural damage, weakening the tree. This field records the degree of active rotting wood. Rotting can be identified as a change in color, structure, and strength of the wood. If the wood was previously rotted and has disappeared, for example leaving a wound in the trunk with inner wood exposed, then that would be considered a "wounded main axis" (Section 4.9). Rotting precedes hollow trunks in most cases, but it is recorded as "rotting trunk" only if active rotting is taking place during the survey.

- 1 = small rotting area, smaller than an area of dimensions DBH × DBH.
- 2 = big rotting area, larger than an area of dimensions DBH × DBH but not affecting >50% of the basal area or main axis length.
- 3 = massive rotting, affecting >50% of the basal area or >50% of the main axis length.

4.12. Leaves (%)

The percentage of leaves remaining on the stem is estimated when there is immediately visible evidence of ongoing defoliation. To distinguish between defoliation and deciduousness, it can be useful to conduct surveys in the wet season. Local deciduous species can be treated differently during data analyses. This field applies only to remaining branches or sprouts within the living length. We do not discount leaves on dead branches or parts of the main axis that are dead or gone. As described in Section 4.4, a tip to differentiate dead branches from living branches without leaves is that, usually, living branches show abundant twigs that often disappear quickly from dead branches (Figure 4). For palms, leaves (%) and remaining crown (%) correspond to the same assessment.

4.13. Leaf damage (presence/absence)

The loss of leaf area can negatively impact tree survival and has been correlated with future mortality in tropical trees (e.g., Eichhorn *et al.*, 2010). This field is checked (√) if, despite the retention of leaves, there is immediately visible leaf damage, including >25% lamina loss, obvious presence of abnormal leaf spots, blotch, etc. We do not record light leaf damage (which is ubiquitous in a natural forest), so this field should remain empty in a majority of cases. If branches have burnt tips this might be a symptom of a lightning strike (see “L” code below).

4.14. Comments and other status indicators

In this last field, any additional information on factors likely to negatively affect or increase the risk of tree mortality are recorded. This might include specific comments such as gap size estimations or unique observations linked to the tree status. Each site/study can develop its own codes for fieldwork efficiency. Some codes that we used are:

- Animals. If there is immediately visible damage by animals, or animal structures (e.g., big ant or termite nests) that may damage the main axis or be a symptom of poor health, then this is recorded, e.g., “ant nest”, “termites”, “borer beetle”.
- L = Lightning. To identify lightning damage in trees, field crews should look for patterns of flashover as the primary diagnostic clue. This includes burnt tips of branches around the focal tree, burnt tips of branches from different trees facing each other, particular palms damaged around the focal tree (they seem to be more sensitive to lightning), or wilting, blackened epiphytes (see Yanoviak *et al.*, 2017 for a detailed description).
- G = Gaps (we record estimated disturbance driver and impact if possible).
- F = Fire (stem charred, fire scars on bark).
- H = Hollow trunk.
- HB = Hollow base of the stem.
- R = Root damage.

- S = Slope failure, evident landslide even if small.
- W = Wind-throw.

Figure 8 summarizes the most common cases and provides examples on how to encode them in order to improve understanding of the most important variables in the protocol. Specific cases are provided in Appendixes S3 and S4.

5. Discussion

During the preparation of this protocol we studied published and unpublished protocols that have been used in temperate and tropical forests. The resulting protocol unifies different practices and is general enough as to be comparable with other damage/mortality assessments to a large degree. We generalize as much as possible. Instead of recording mode (standing, broken or uprooted) depending on the dead/alive status (Phillips et al. 2002; Chao et al. 2009), we record mode and survival status independently, allowing all possible combinations (including the compatibility of broken and uprooted modes within the same tree). Instead of using categories for crown damage (Muller-Landau & Dong 2010; Gonzalez-Akre et al. 2016; Arellano et al. 2019) we use % of remaining crown (as in the FIA Program of the U.S. Forest Service protocol). Instead of recording trees leaning >45 degrees (e.g., Zimmerman et al. 1994) we record the degree of leaning. The flexibility of this protocol implies some nuisances when comparing the data obtained using other protocols. In particular, our protocol records breakage even if it affects a minimum part of the main axis (whether the broken part is large or small will be determined by the "living length" variable, in the case of living trees). Many other protocols record "broken" only when substantial portions of the stem have broken (e.g., "major trunk or crown loss", Chave et al. 2003). This has consequences when making comparisons with data obtained with other protocols (e.g., Uriarte et al., 2019); our advice is to re-interpret the "B" code in the light of the "living length" field when making such comparisons. Some narrow categories in other protocols, such as "stem broken at ground level without uprooting" (Zimmerman et al. 1994, Chao et al. 2009) can be easily recovered from our variables. Overall, our protocol has more similarities than differences with other protocols applied in tropical forests. It has been adapted to quickly evaluate the impact of the hurricanes María and Irma on tree damage and mortality across Puerto Rican forests (Hall et al. 2020), allowing comparisons with data from past hurricanes in Puerto Rico (Zimmerman et al. 1994; Uriarte et al. 2019). It has also been successfully integrated with other protocols to add extra information during repeated surveys focused on growth (Muller-Landau & Dong 2010).

From 2016 to date, the AMS protocol has been applied in 24 surveys in several ForestGEO sites, making 154,000 observations (stem × time) for over 62,000 stems in total. In most sites, there were around 5,000-7,000 stems included. Whether this number is enough will depend on the use or application for the data. When designing a long-term monitoring program, it is important to include as many trees as possible but it finally depends on the budgets, annual plans, and amounts of available workforce of each site. Table 1 includes a summary of our progress in different ForestGEO sites as a reference. In any case, these surveys represent a useful baseline for any other and more detailed assessment (e.g., sap flow, carbohydrate content, etc.). For example, instead of selecting species arbitrarily or based on life history or functional traits, we can use our basic covariates to identify trees with contrasting probabilities of death and make physiological measurements on them. Importantly, the application of this protocol in large plots with geolocated trees facilitates studies from remote sensing required for a better understanding of tree mortality from local to landscape and regional scales (McDowell et al. 2015). The "crown illumination" variable in particular can be a useful indicator if the dead tree is potentially visible from above. The data obtained with this protocol is also important to calibrate the remote sensing information by taking into account the difference between dead standing trees and leafless living trees, broken trees whose crown is no longer visible but that remain alive, etc.

Given the increasing need to better quantify and monitor tree mortality and biomass loss (Brodribb et al. 2020; McDowell et al. 2020), standardized protocols are a crucial step to providing large-scale, comparable data on the underlying causes and consequences of tree mortality.

6. Acknowledgements

We thank to Helene Muller-Landau, Krista Anderson-Teixeira and Erika Gonzalez-Akre (for general discussion about the feasibility and scope of the fieldwork); Sabrina Russo, Stephen Hubbell and James Lutz (randomized vs. aggregated design); Marco Visser and Joe Wright (liana impact); Sean McMahon (survival bias); and Rolando Pérez and Salomón Aguilar (fieldwork implementation).

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Appendix S1. Analytical considerations.

Appendix S2. Details on Forest GEO's spatially stratified sampling design.

Appendix S3. Assessment of remaining crown within (decreased) living length.

Appendix S4. Encoding and explanation for particular cases of tree damage and death assessment.

Site and survey	Number of stems	Field crew	Range of dates (days)	Days worked per week	Hours in the plot per day	Stems per day of fieldwork	Stems per hour per team
A-1	5373	2 teams × 2-3 persons each	34	5	7.5	221	15
A-2	4908	2 teams × 2 persons/team	26	5	7.5	264	18
A-3	4908	2 teams × 2-3 persons each	21	5	7.5	327	22
A-4	4909	2 teams × 2 persons/team	27	5	7.5	255	17
B-1	8622	2 teams × 3-4 persons/team	56	5	4	216	27
B-2	8805	2 teams × 3-4 persons/team	87	5	4	142	18
B-3	8696	2 teams × 3-4 persons/team	51	5	4	239	30
B-4	8610	2 teams × 3-4 persons/team	53	5	4	227	28
C-1	5492	2 teams × 3 persons/team	22	6	6	291	24
C-2	5497	2 teams × 3 persons/team	23	6	6	279	23
C-3	5497	3 teams × 3 persons/team	14	6	6	458	25
C-4	5317	3 teams × 3 persons/team	14	6	6	443	25
D-1	5356	1 team × 4 persons/team	22	6	7	284	41
D-2	5357	1 team × 4 persons/team	28	6	7	223	32
D-3	5356	1 team × 4 persons/team	18	6	7	347	50
E-1	5065	1 team × 5 persons/team	27	6	6	219	36
E-2	5065	1 team × 5 persons/team	26	6	6	227	38
E-3	5065	1 team × 5 persons/team	27	6	6	219	36
F-1	18272	5 teams × 3-4 persons/team	53	5	6	483	16
G-1	5932	1 team × 4 persons/team	69	4	4	150	38

H-1	5653	2 teams × 3 persons	25	4.5	7	352	25

Table 1. Summary of sample sizes and fieldwork times invested in multiple ForestGEO tropical sites. The sample included was a subset of the many stems included in large ForestGEO plots, which were already tagged, mapped, and identified. The sites are anonymized to avoid gossip. All were located in tropical or subtropical sites but local conditions (access, weather, topography, etc.) were very varied. Experience of local workers also differed substantially, from teams composed by supervised volunteer students working for the season to teams composed by long-term staff living inside the forest for the most part of the year. A survey that involved the installation of dendrometers (much more time-consuming) has been excluded from this table. None of these surveys took place during the COVID-19 pandemic.

Figures

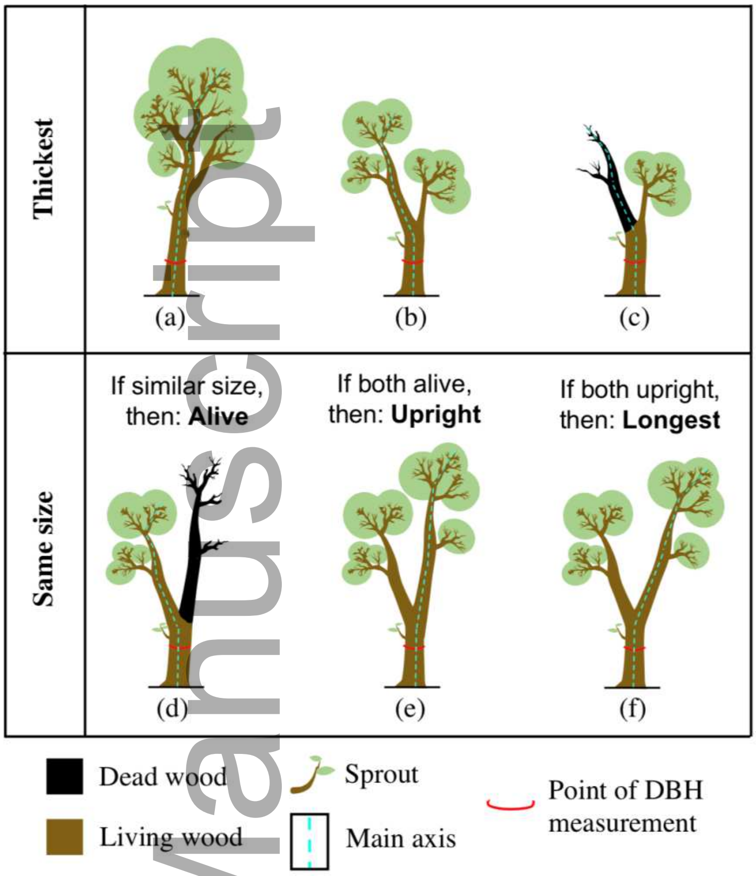


Figure 1. Main axis definition (light blue dashed line) in single-stemmed tree individuals (i.e., only one DBH) used in the annual mortality survey protocol. The main axis criteria are, in order of importance, the thickest, alive, upright and longest tree sections (see Section 3). The thickest part always takes priority (a) even when it is not the most upright (b) or the longest (c) part. Only when two parts in a bifurcation are of the same size, we follow the alive part (d) or the most upright (e) or the longest (f). Main axis definitions for multi-stemmed tree individuals are presented in Figure 2.

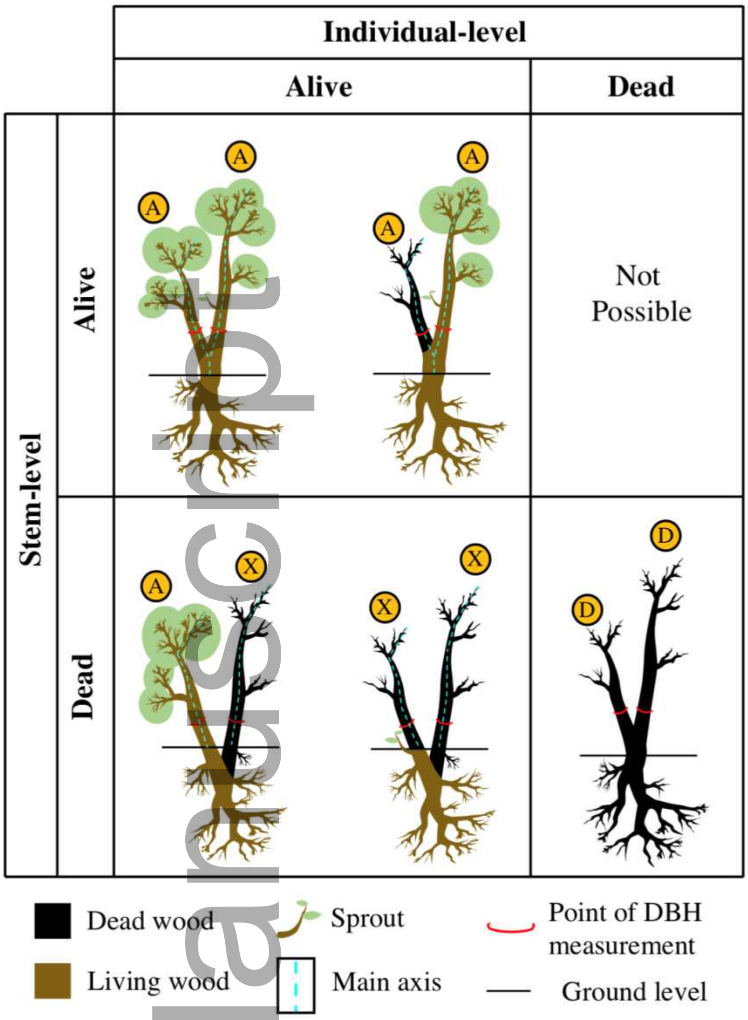


Figure 2. Three unique possibilities to encode the survival status of every *stem* in the mortality survey form: "A", "D", or "X". Note the sprout in the base of the tree in the second "Status = X" case: stems entirely composed of dead tissues above ground in living individuals. This figure additionally provides examples of the *main axis* definition (light blue dashed line) in multi-stemmed individuals.

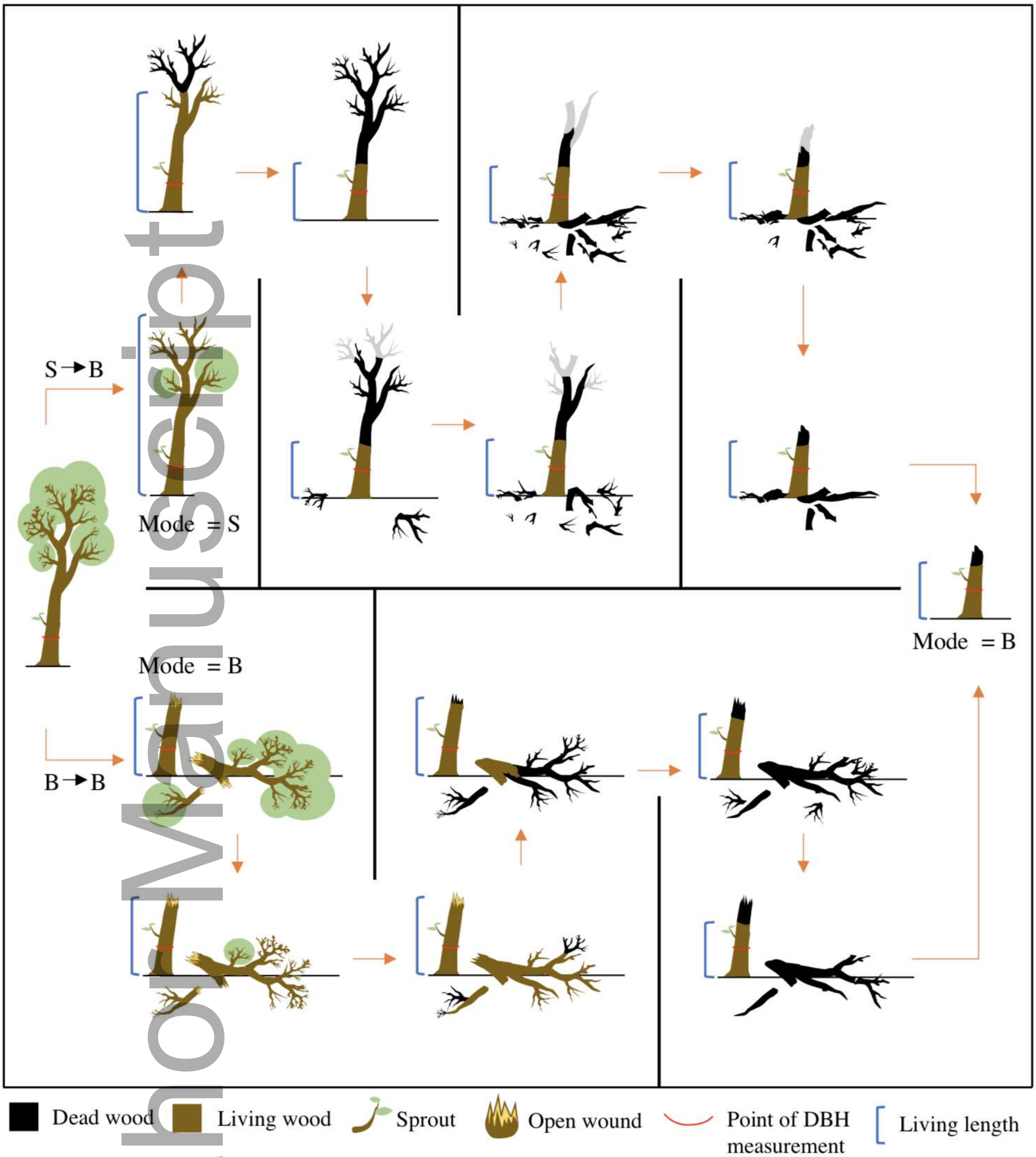


Figure 3. Two different causes may lead to the same recorded *mode* in a stem after some time: from a tree that was broken in the first place (B→B) to a tree that appear broken only after a damage occurs and the wood decays (S→B). Note that, in these cases, splinters and woody debris can provide valuable clues on the mode of damage (or mortality if the tree finally dies). Specifically, the lack of prominent splinters in the stem as well as the presence of softer boles, smoothened segments in the forest floor may indicate that the transition was S→B.

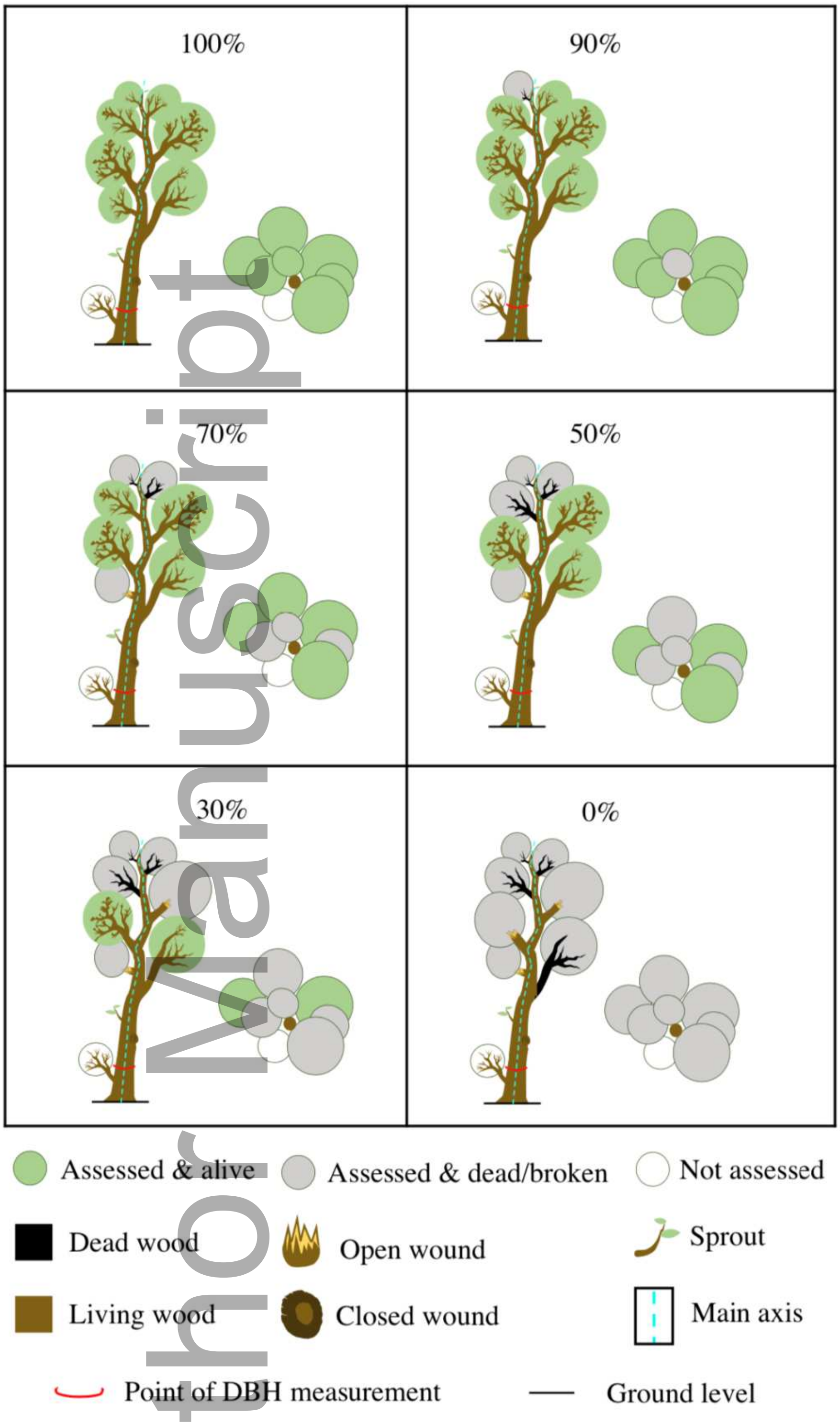


Figure 4. *Remaining crown (%)* assessment, with lateral and vertical projections of standard cases. Green circles correspond to alive branches that are assessed (i.e., they are connected to the living length of the main axis above the POM). Grey circles correspond to dead branches that can remain attached to the main axis (black branches) or that may have fallen recently (open wound). Empty circles refer to structures that are not assessed for "remaining crown" because they are connected below the POM (crown is defined above the POM). Structures larger than 1 cm DBH below POM would be assessed independently, as they would receive an independent tag in common forest inventories; however, they are included in the crown illumination, leaves (%), and leaf damage

assessments (see next sections). Note that dead branches, even if not broken, are stripped from small twigs; this is a useful visual clue to detect them in many cases. See Appendix S3 for a more complex case of remaining crown assessment with decreasing living length.

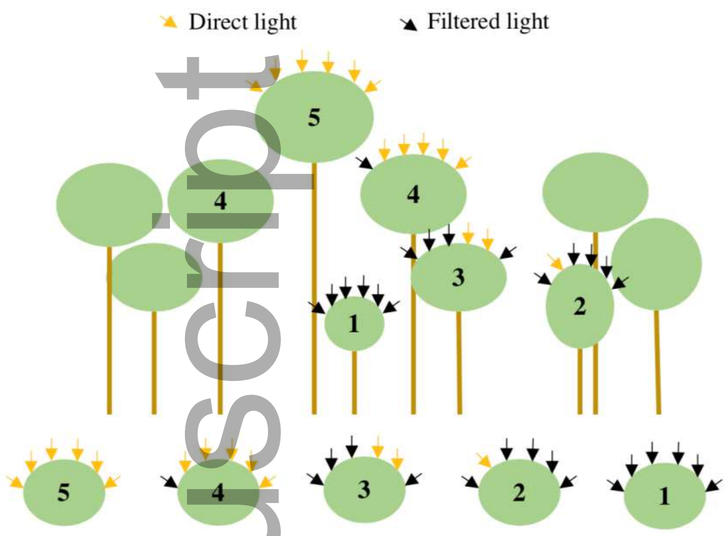


Figure 5. Schematic representation of the *crown illumination* (CI) index.

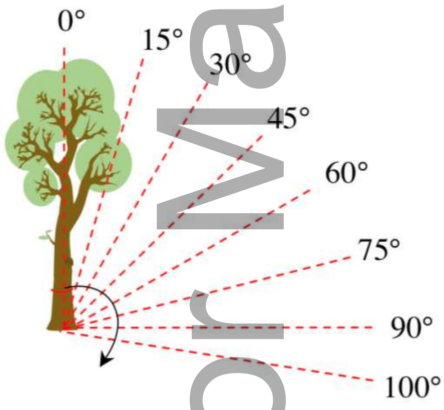


Figure 6. Tree inclination from the vertical, *leaning* (°). Note that for trees lying on slopes or creeks the leaning may be >90°. The inclination always refers to the straight line connecting the rooting point and the POM.

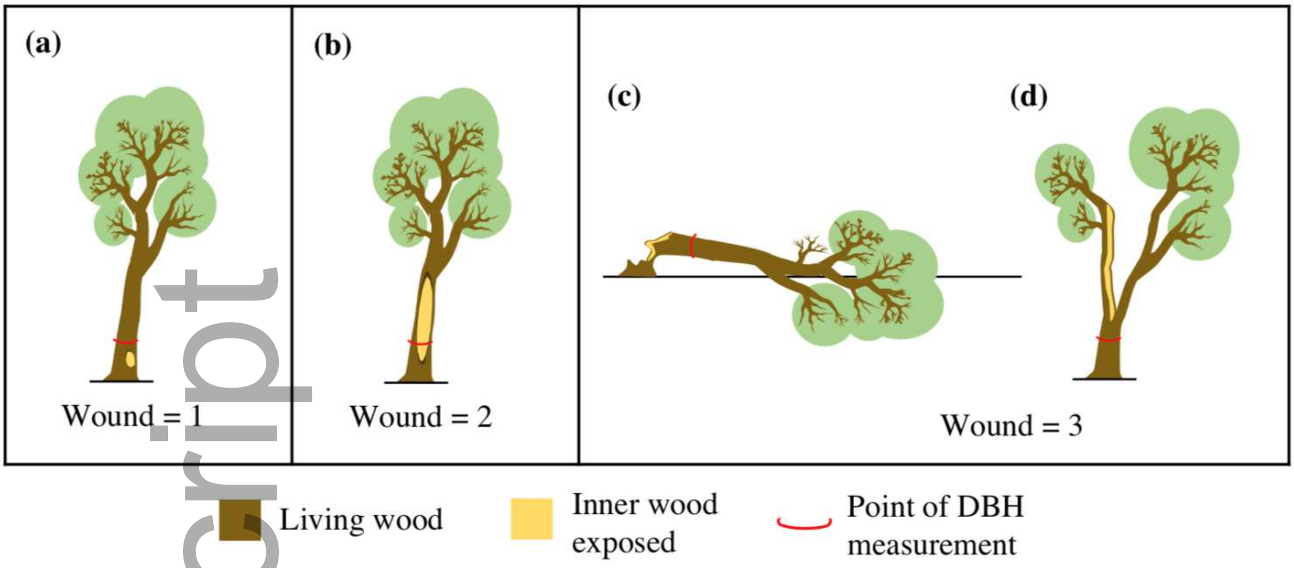
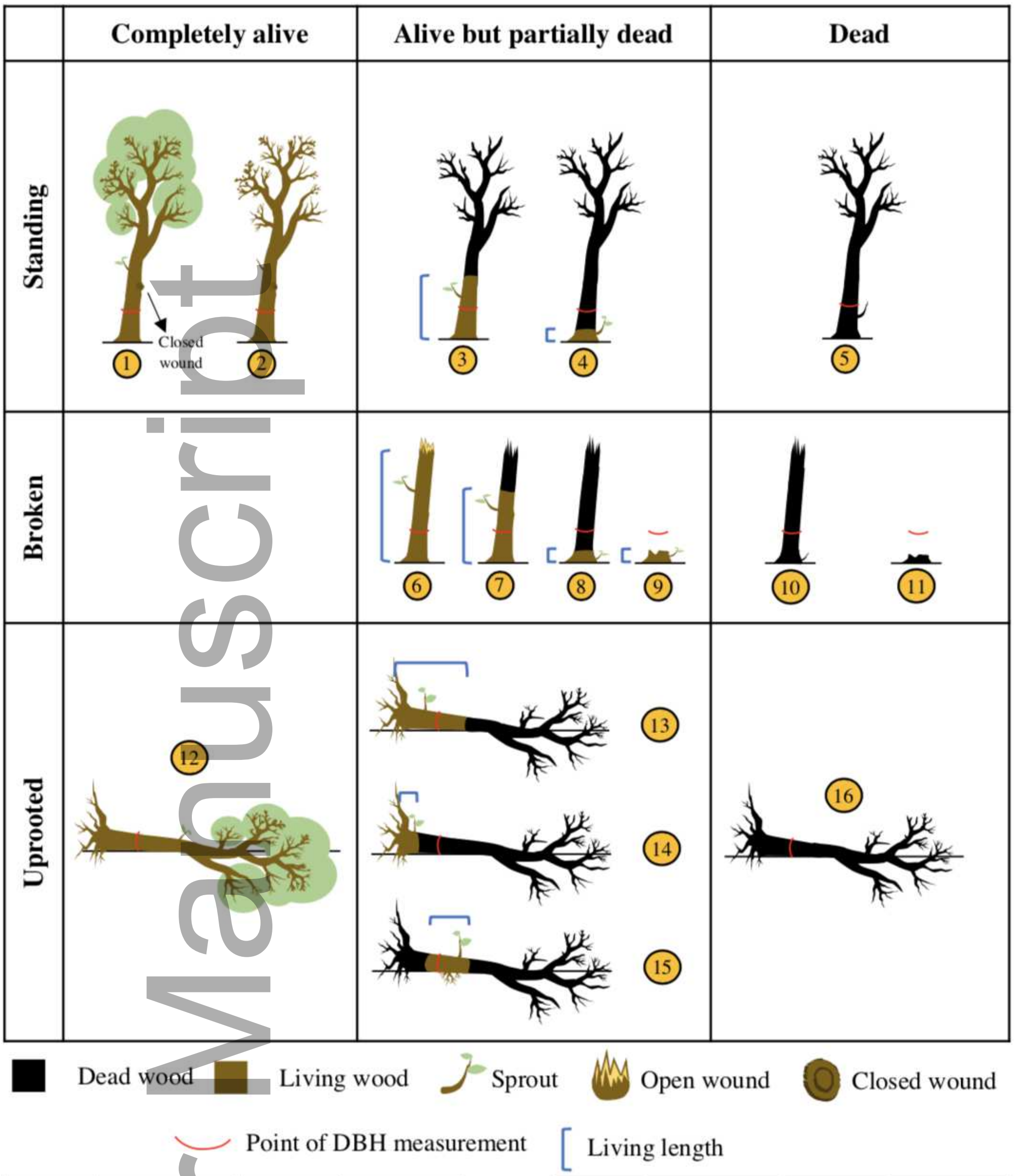


Figure 7. Levels of *wound* assessed on the main axis: (a) smaller in area than DBH × DBH; (b) larger in area than DBH × DBH; (c) massive damage affecting >50% of basal area; (d) massive damage affecting >50% of living length (a stem almost completely split in two longitudinally along its main axis). Areas represented by inner wood in (a) and (b) can be used as examples when referring to tumors or rotting trunk in Sections 4.10 and 4.11.

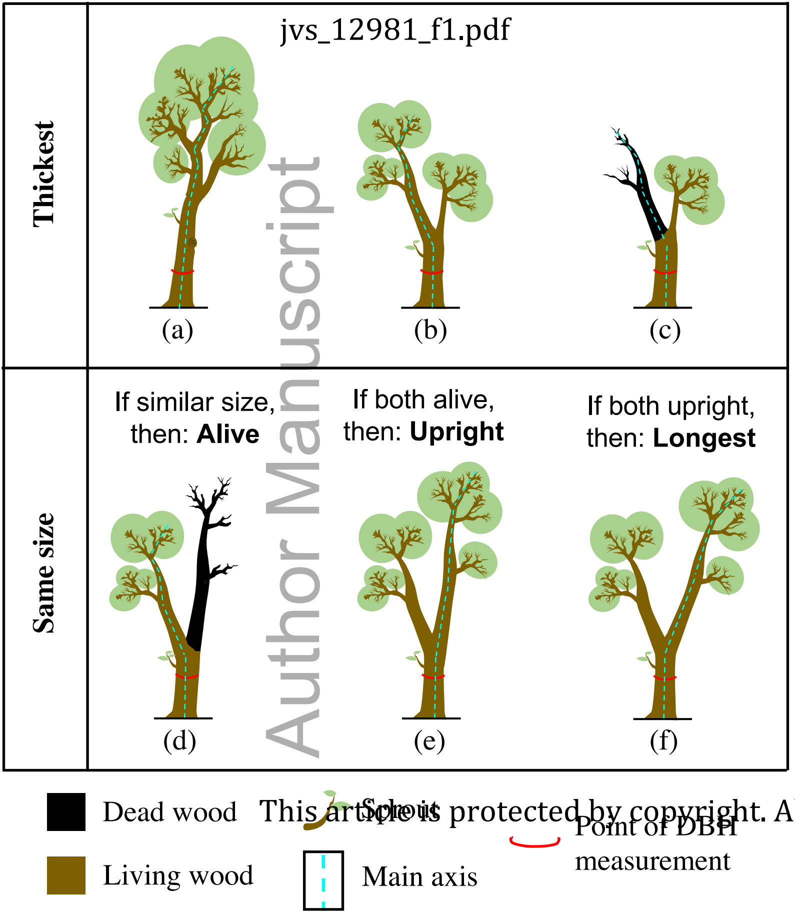


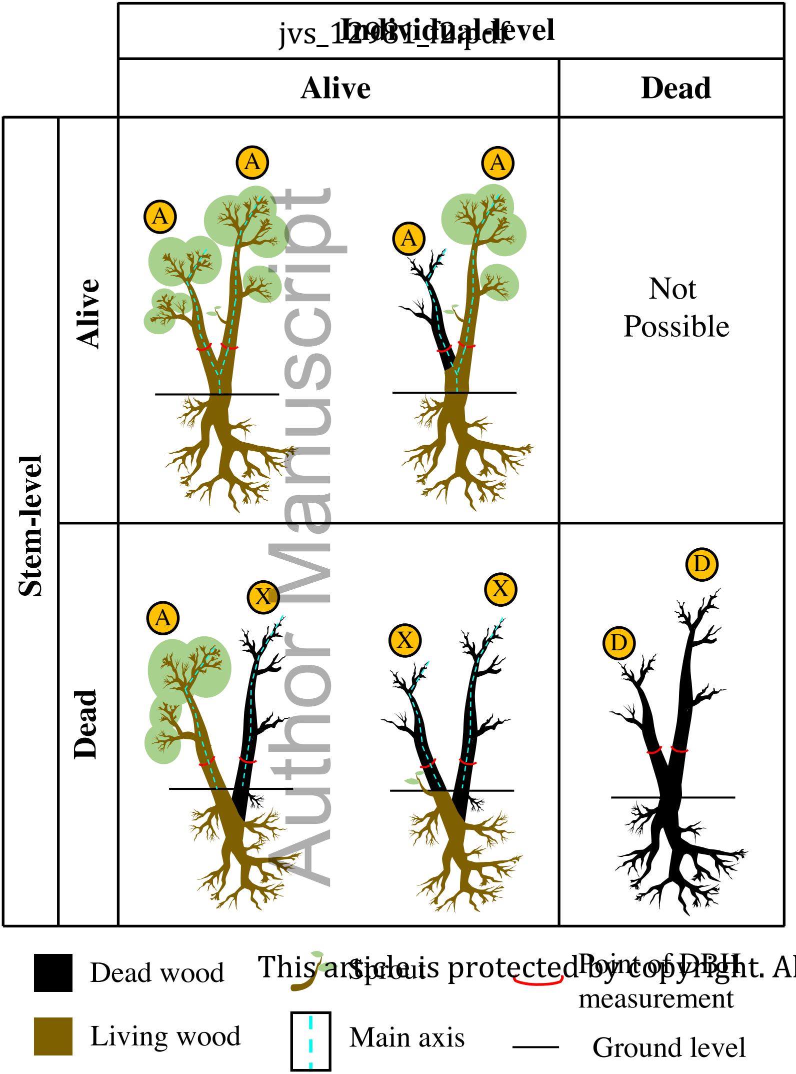
Case	Survival Status	Mode	Living length
1	A	S	-
2	A	S	-
3	A	S	3
4	A	S	0.4
5	D	S	-
6	A	B	5
7	A	B	3
8	A	B	0.4

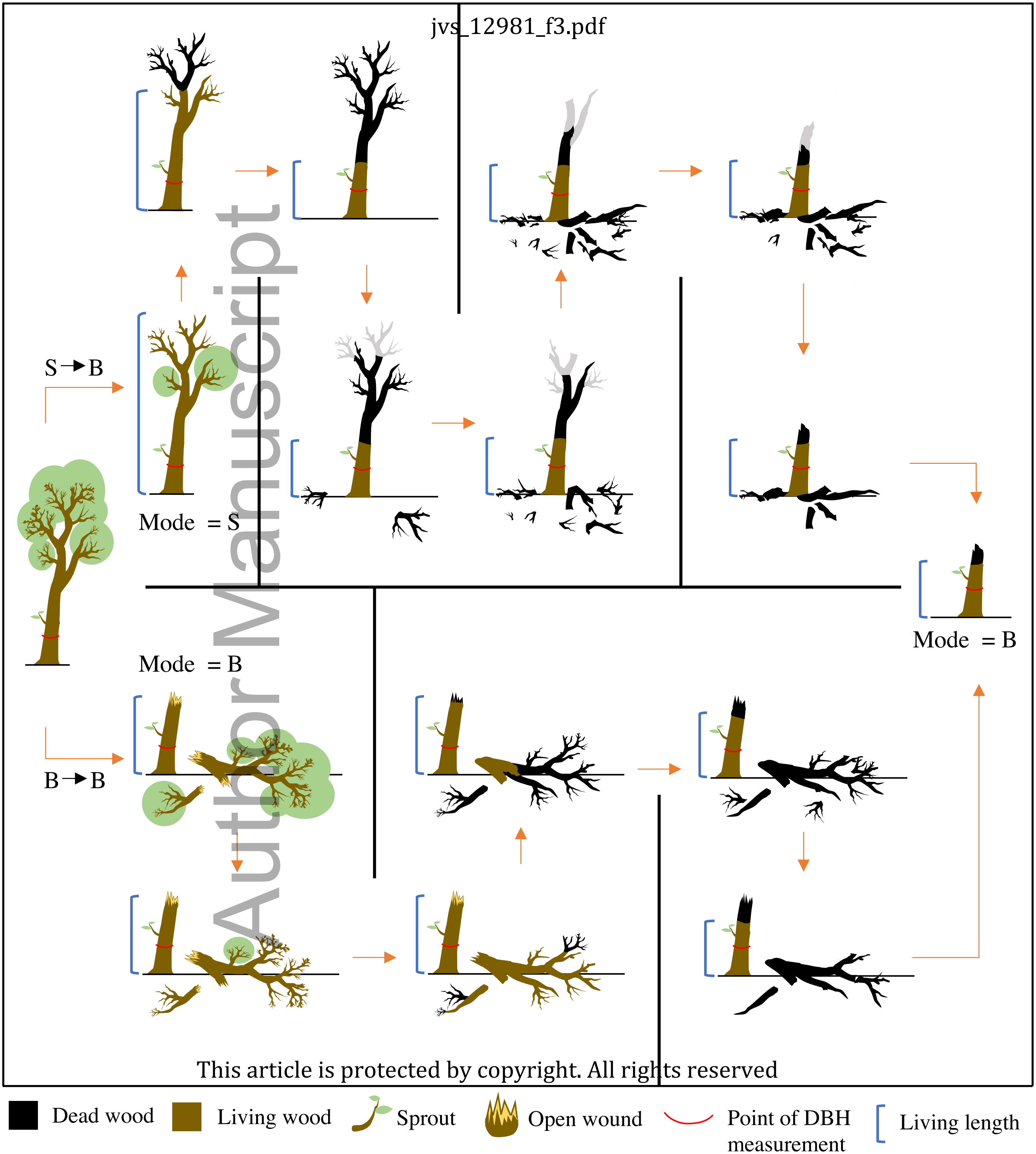
Case	Survival Status	Mode	Living length
9	A	B	0.4
10	D	B	-
11	D	B	-
12	A	U	-
13	A	U	5
14	A	U	0.4
15	A	U	1.5
16	D	U	-

Figure 8. Common cases recorded in the annual mortality surveys. Note that the length of the living length in a stem (blue open bracket '[') can be greater than the height of the last sprout (e.g., in case 2, for a completely defoliated tree). Living length in 'completely' alive trees is assumed to be the total height of the tree; in these cases, we rely on allometric models to avoid estimating heights of every tree in the field. The numeric value in the "living length" column is hypothetical, just an

example of a possible living length expressed in meters.


Thickest
(a)
(b)
(c)
Same size
If similar size, then: Alive
If both alive, then: Upright
If both upright, then: Longest
(d)
(e)
(f)
Dead wood
Living wood
Sprout
Main axis
Point of DBH measurement


Individual-level
Alive
Dead
Stem-level
Alive
Dead
A
A
A
A
Not
Possible
A
X
X
X
D
D
Dead wood
Living wood
Sprout
Main axis
Point of DBH
measurement
Ground level


S → B
Mode = S
Mode = B
B → B
Mode = B
Dead wood
Living wood
Sprout
Open wound
Point of DBH measurement
Living length

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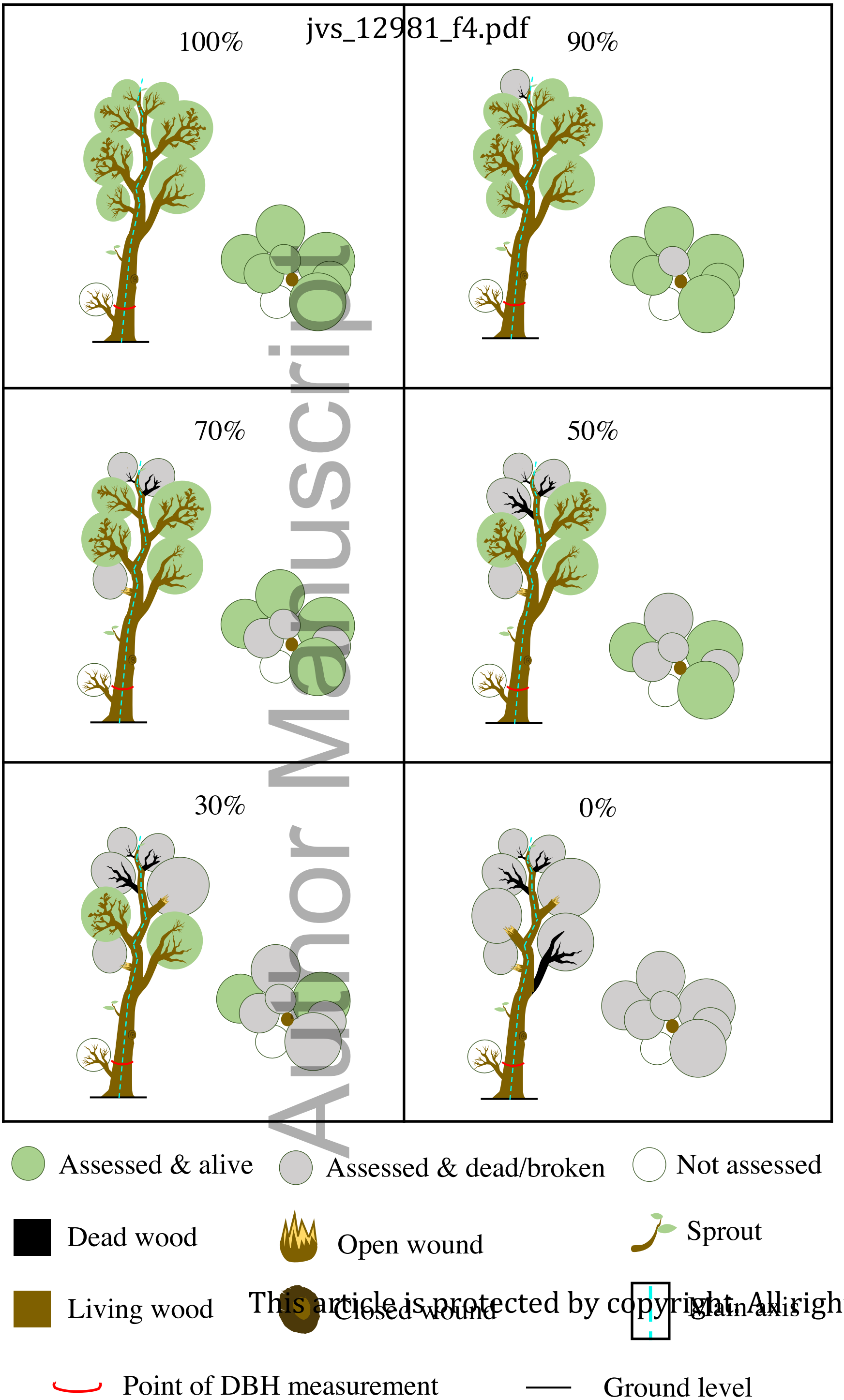

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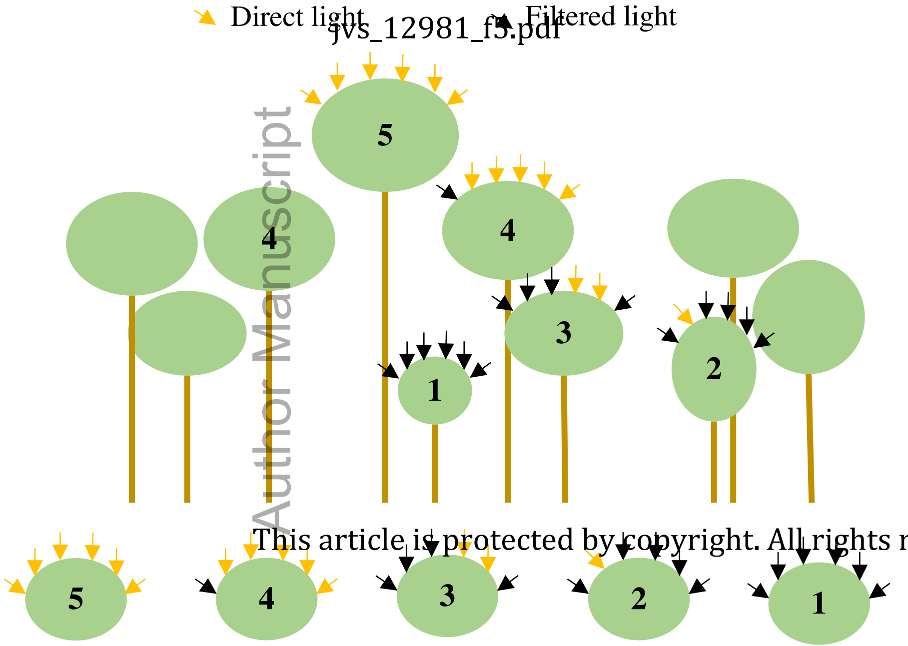

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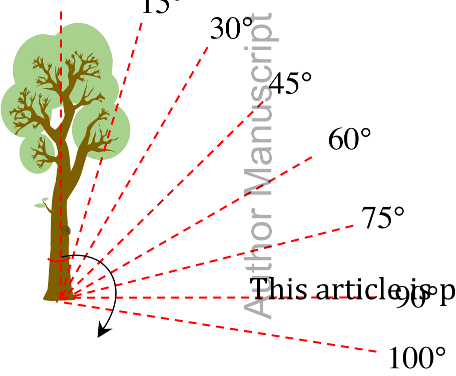
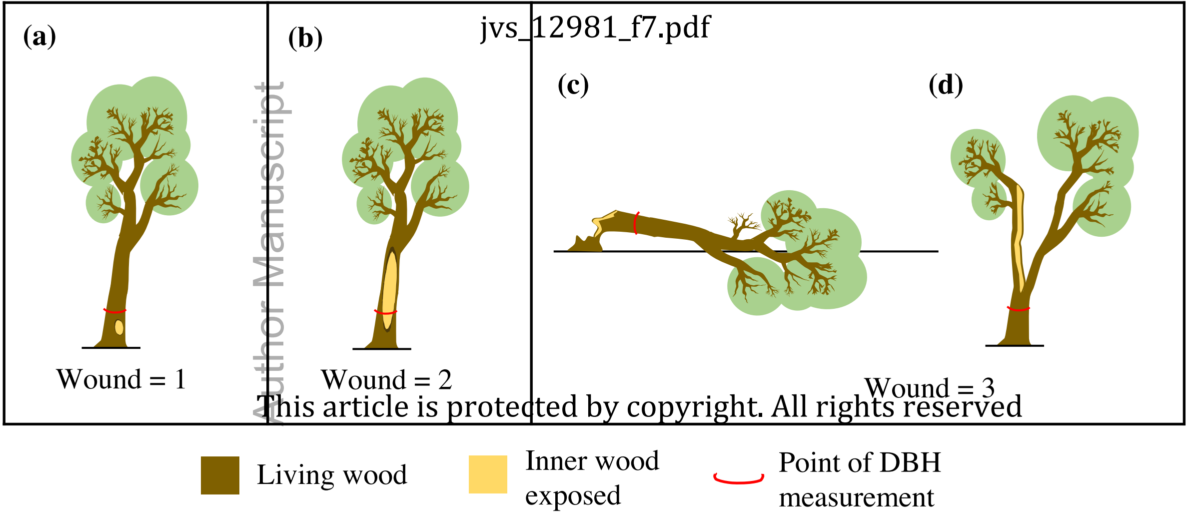
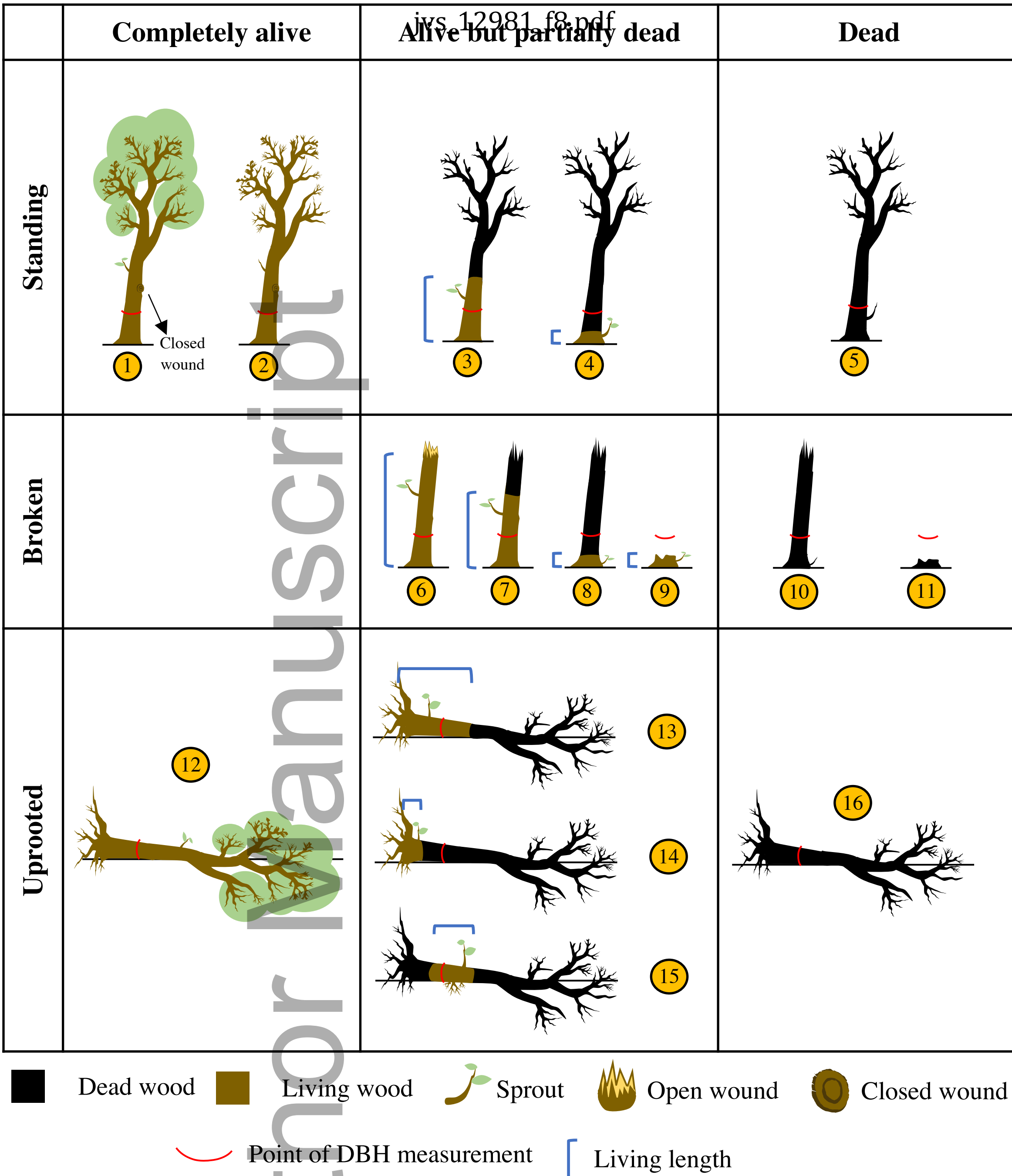



(a)
Wound = 1
(b)
Wound = 2
(c)
(d)
Wound = 3
Living wood
Inner wood exposed
Point of DBH measurement



Case	Survival Status	Mode	Living length
1	A	S	-
2	A	S	-
3	A	S	3
4	A	S	0.4
5	D	S	-
6	A	B	5
7	A	B	3
8	A	B	0.4

Case	Survival Status	Mode	Living length
9	A	B	0.4
10	D	B	-
11	D	B	-
12	A	U	-
13	A	U	5
14	A	U	0.4
15	A	U	1.5
16	D	U	-