


## REVIEW

# Handbook for the measurement of macrofungal functional traits: A start with basidiomycete wood fungi

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## Funding information

Svenska Forskningsrådet Formas, Grant/Award Number: 2016-00461 (to MJ); Sveriges Lantbruksuniversitet, Grant/Award Number: Postdoctoral researcher funding (to SD); Suomen Akatemia, Grant/Award Number: 209581 (to OO) and 250444 (to OO); Academy of Finland, Grant/Award Number: 250444 and 209581

Handling Editor: Miranda Hart

## Abstract

1. Functional traits are widely recognized as a useful framework for testing mechanisms underlying species community assemblage patterns and ecosystem processes. Functional trait studies in the plant and animal literature have burgeoned in the past 20 years, highlighting a need for standardized ways to measure ecologically meaningful traits across taxa and ecosystems. However, standardized measurements of functional traits are lacking for many organisms and ecosystems, including fungi.
2. Basidiomycete wood fungi occur in all forest ecosystems world-wide, where they are decomposers and also provide food or habitat for other species, or act as tree pathogens.
3. Despite their major role in the functioning of forest ecosystems, the understanding and application of functional traits in studies of communities of wood fungi lags behind other disciplines. As the research field of fungal functional ecology is growing, there is a need for standardized ways to measure fungal traits within and across taxa and spatial scales.
4. This handbook reviews pre-existing fungal trait measurements, proposes new core fungal traits, discusses trait ecology in fungi and highlights areas for future work on basidiomycete wood fungi.
5. We propose standard and potential future methodologies for collecting traits to be used across studies, ensuring replicability and fostering between-study comparison. Combining concepts from fungal ecology and functional trait ecology,

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methodologies covered here can be related to fungal performance within a community and environmental setting.

6. This manuscript is titled “a start with” as we only cover a subset of the fungal community here, with the aim of encouraging and facilitating the writing of handbooks for other members of the macrofungal community, for example, mycorrhizal fungi.

#### KEYWORDS

characteristics, community ecology, lignicolous, methods, protocols

## 1 | INTRODUCTION

Functional traits are widely recognized as a useful framework for answering some of the core questions of community ecology (Götzenberger et al., 2012; Keddy, 1992; Weiher & Keddy, 1995). Using functional traits has several advantages: It allows generalization across scales through the use of traits as the response rather than species; it can provide mechanistic insights into community functioning and it enables deeper understanding of community assembly processes (Brown et al., 2014; Carmona, Bello, Mason, & Lepš, 2016; Ovaskainen et al., 2017; Weiher & Keddy, 1995). The plant functional trait (or trait-based) ecology literature has been steadily increasing since the 1990s and is now recognized as a major field of plant ecology (Shipley et al., 2016). Although research into fungal functional traits is only beginning, the past five years has seen several papers both advocating and implementing functional traits in analyses (Aguilar-Trigueros et al., 2015; Bässler, Ernst, Cadotte, Heibl, & Müller, 2014; Crowther et al., 2014; Nordén, Penttilä, Siitonen, Tomppo, & Ovaskainen, 2013). Recently, there have been calls for development of a fungal functional trait handbook (Aguilar-Trigueros et al., 2015; Halbwachs, Simmel, & Bässler, 2016); however, the broad range of fungal species makes writing a single trait handbook impossible. This handbook is intended to be the first in a series that introduces protocols for fungi, and focuses on basidiomycete wood decay fungi. While some traits may be transferable to other fungal groups (e.g., mycorrhizal, ascomycetes), reasoning behind their selection and environmental conditions of interest will change. Throughout the handbook when “fungi” is used, it is intended to refer only to basidiomycete wood fungi; ectomycorrhizal fungi (EMF) is used where EMF research provides evidence relevant to the trait being discussed.

This handbook uses the framework set out by the plant trait handbooks (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013) to organize the structure and subdivide traits. The protocols (Supporting Information Appendix S1) include reasoning and methodologies for specimen selection and sampling, traits for the whole fungus, fruit body, mycelium, and reproductive and conservation-related aspects. However, given the infancy of fungal trait studies, we are unable to propose a standard methodology for all traits. Some traits have several proposed methods and others have outline methodologies, both of which will need to be refined in the future (all fall under “potential future methods”). We include traits with non-standard methodologies as we believe

they are core traits to fungal functioning. In the manuscript, we consider environmental variables and outline some basics for trait measurement in fungi. We also highlight areas where we believe future research is of the highest importance. In addition to the protocols in Supporting Information Appendix S1, we also cover statistical techniques for analysing traits (Supporting Information Appendix S2) and briefly introduce trait theories (Supporting Information Appendix S3). Given the infancy of fungal trait ecology, we emphasize that any trait measurements represent significant advances. While some of the traits presented have been previously measured and studied, others have been conceived especially for this handbook and only theoretical underpinning exists for their inclusion. The empirical evidence demonstrating ecological importance of most traits is generally lacking in the fungal literature (Aguilar-Trigueros et al., 2015; Crowther et al., 2014; Halbwachs et al., 2016), and basidiomycete wood fungi is no exception. New traits were inspired by complementary plant traits or theoretical assumptions about fungal ecology, but require future studies to validate their inclusion in common fungal traits. The handbook is intended as a first effort towards a unified protocol for measuring functional traits in fungi and to stimulate discussion of additional traits to include in handbooks for other fungal groups.

### 1.1 | What is a trait?

Traits can include a wide variety of characteristics surrounding a living organism, and there are several ways to define “functional trait” (McGill, Enquist, Weiher, & Westoby, 2006; Pérez-Harguindeguy et al., 2013; Violle et al., 2007). We have chosen to follow the definition provided by the plant trait handbook (Pérez-Harguindeguy et al., 2013): “we consider *fungal* functional traits to be any morphological, physiological or phenological feature, measurable for *an individual fungus*, at the cell to the whole organism level, which potentially affects its fitness.” As this implies, a functional trait should be linked to the fitness of an individual, with performance being a direct measure of fitness (e.g., biomass; Violle et al., 2007; Shipley et al., 2016). For brevity, we use trait throughout this manuscript to indicate functional traits. Functional traits can also be classified based on their interaction with the environment. For example, a *response trait* varies with

changes in environmental conditions, while an *effect trait* changes an aspect of environmental or ecosystem conditions (e.g., secondary compounds; Violle et al., 2007; Shipley et al., 2016). This division of trait categories can be useful when considering hypotheses of trait–environment interactions. The selection of both traits and environmental gradients should be undertaken carefully, with specific hypotheses proposed (Abrego, Norberg, & Ovaskainen, 2017; Shipley et al., 2016).

In addition to functional traits, we have included a short section on conservation-based non-functional traits. While these traits do not directly relate to fungal fitness, they are of interest in applied research and conservation (e.g., Red List status; Nordén et al., 2013). Such traits can be thought of as “attributes” under the framework of Violle et al. (2007). The underlying issue with including non-functional traits in trait-based analyses is that they may explain variation in the data which is more properly explained by a functional trait. Section 7 of the protocols includes some methods for avoiding this problem, but anyone using non-functional traits should be aware of, and account for, this in their analysis.

In the plant trait literature, measured traits can be divided into “hard” and “soft” traits. Hard traits are difficult to measure, often requiring experimental studies, but generally provide a clearer or closer mechanistic understanding (e.g., relative growth rate; Walker & Langridge, 2002; Violle et al., 2007). On the other hand, soft traits are easier, faster measurements and can be conducted on many specimens in the field. Soft traits, although providing useful data, may be more difficult to link to an exact mechanism than hard traits (Shipley et al., 2016; Walker & Langridge, 2002). For example, specific leaf area is often used in plants, but is affected by soil nutrients, competition and light availability, making it difficult to attribute changes in this trait to a particular cause. While we propose both hard and soft traits, we realize that in general, the majority of studies will use soft traits, similar to the plant literature (Shipley et al., 2016). Nevertheless, the importance of collecting hard traits should not be underestimated for gaining a complete understanding of fungal trait functions within a community.

In addition to traits themselves, there are numerous concepts, theories and hypotheses underpinning functional trait ecology and its use to gain mechanistic understanding of community assembly and ecosystem function. These have been developed over time and can be followed in many scientific articles, books, etc. (Götzenberger et al., 2012; McGill et al., 2006; Moles, 2017; Shipley et al., 2016; Violle et al., 2007; Weiher & Keddy, 1995). Some foundational concepts in functional ecology are still being developed and tested in plants (Shipley et al., 2016). We suggest those undertaking fungal functional trait studies familiarize themselves with relevant theories and concepts regarding their study. For example, an examination of the role of traits in community composition requires familiarization with community assembly theory and assumptions (Götzenberger et al., 2012). For

mycologists new to functional trait ecology, Appendix S3 gives a very brief overview of theories referenced in the protocols.

## 1.2 | Why are trait handbooks on fungi needed at all?

While fungal trait research is only beginning, we believe it is an ideal time to publish a handbook of common traits and their collection methodology. The plant trait handbook was published after many years of research and included evidence to support the selection of each trait (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013). Although this evidence does not exist yet for most of the fungal traits and methodologies we propose, this handbook is a starting point for identifying traits and using standard methods from the outset.

This handbook focuses on morphological and physiological approaches for collecting trait data on basidiomycete wood fungi. Identifying and measuring fungi, particularly wood decay fungi, can be complicated by fungal lifestyles, which is perhaps why fungal ecology lags behind plant ecology in trait approaches. However, these complications can be overcome in a number of ways using laboratory studies, measuring in the field or running laboratory tests on samples taken from the field. We do not cover traits that can be measured with molecular methods in this handbook, as the approach would require its own handbook for adequate coverage. These two types of methods can be viewed as complementary, with morphological and physiological methods focusing on those species that colonize a large enough proportion of the substrate to reproduce/dominate and molecular methods providing information on all species occurring (or the percentage of OTUs that can be identified, which may be limited) in a very small substrate sample. While there are some traits or aspects of fungi that cannot be measured with morphological and physiological methods, a large amount of knowledge can still be gained.

The standardization of methods when undertaking trait studies is important to advance the field and build evidence required for generalizations between communities (Pérez-Harguindeguy et al., 2013; Shipley et al., 2016). Standardized measurements enable the combination and comparison of results from multiple studies and/or locations. In addition to standardized trait methods, the standardized measurements of environmental gradients are also important for generalizing studies (Shipley et al., 2016) as is information on sites and sampling effort (Halme & Kotiaho, 2011). In this handbook (Appendix S1), we include both traits where standardized methods are presented in full detail and traits where methods are summarized but not fully described either due to more research being required to refine methods or if methodological explanations require substantial technical detail (e.g., stable isotope measurement or enzyme assays), in which case we reference an appropriate source. If trait-based fungal studies use standardized methods now, at the beginning of this field, there

will be greater ease in the future when comparing and interpreting patterns across regions and ecosystems.

### 1.3 | Why is this handbook on basidiomycete wood fungi?

Basidiomycete wood fungi affect and regulate critical ecosystem processes in forest environments world-wide and encompass a great amount of biodiversity (Heilmann-Clausen et al., 2015). As the main agents of wood decomposition, basidiomycete wood fungi are crucial to nutrient cycling, soil formation and carbon budgets (Lonsdale, Pautasso, & Holdenrieder, 2007). Many vertebrates, invertebrates, bacteria, plants and other fungi are directly or indirectly dependent on the basidiomycete wood fungi, as a food source or a location for reproduction (Jonsell, Nordlander, & Jonsson, 1999). Further, in many forest ecosystems, basidiomycete wood fungi account for a large amount of the biodiversity in dead wood (Dahlberg & Stokland, 2004). These fungi are generally confined to dead wood resource units, which are spatially and temporally discrete at the local scale (e.g., a log, a stump or a still-attached dead branch). This means their dispersal and colonization traits are of particular interest when considering community and population dynamics (Abrego, Bässler, Christensen, & Heilmann-Clausen, 2015; Jönsson, Edman, & Jonsson, 2008; Nordén et al., 2013). As a consequence of deforestation, logging activities and land-use change, however, many basidiomycete wood fungi species are now threatened, which also threatens the vital ecosystem services they provide (Valentín et al., 2014). There has been a subsequent rise in research into these communities and attempts to understand the impact of management actions (Junninen & Komonen, 2011).

Of the published studies examining basidiomycete fungal traits, almost all use values sourced from the literature, typically identification handbooks (Abrego et al., 2017; Bässler et al., 2016; Kauserud et al., 2010; Nordén et al., 2013; Ottosson et al., 2015). However, online trait databases are increasingly compiled (e.g., Kattge et al., 2011) and used to provide easy access to trait values for different organism groups, including fungi, for example, the UNITE database (Kõljalg et al., 2013) and the Fun<sup>Fun</sup> database (<https://github.com/traitecoevo/fungaltraits>). Given the paucity of site-specific data on fungal traits currently available, mean trait values in fungal and regional databases are often the only option available; however, there are some major drawbacks to these approaches. These include lack of information on trait measurement methods, replicate numbers, environmental conditions and quantification of intraspecific variation. This last is of particular concern as, if basidiomycete wood fungi have large intraspecific variation (the same as mycorrhizal fungi; Cairney, 1999; Behm & Kiers, 2014), interpretation and strength of results may change (see Section 1.4 below). As shown for plants, accuracy of traits retrieved from a database may also depend on the level of the study (lower accuracy at community-level than habitat-level studies), the trait (lower accuracy in plastic traits) and the habitat type (lower accuracy in extreme habitats; Cordlandwehr et al., 2013). Although some important categorical traits are already known and will not vary between environments (e.g., fruit body type), we hope

presenting a greater range of traits and methods for measurement will encourage more trait quantification in the field.

### 1.4 | Intraspecific trait variation in fungi

Intraspecific variability, the within-species variation for a given trait, can provide information on species niches, response to environmental gradients, degree of specialization and other factors important for understanding species ecology (Behm & Kiers, 2014; Cairney, 1999; Jung, Violle, Mondy, Hoffmann, & Muller, 2010). Genetic variability and phenotypic plasticity (leading to local adaptation) are the sources of intraspecific variation. Fungi have been recognized as having high intraspecific variability for a number of traits, but there is very little empirical evidence (except for mycorrhizal fungi; Behm & Kiers, 2014; Aguilar-Trigueros et al., 2015). While studies using mean values can examine larger communities, it is important to understand the expected range of trait values within species. Intraspecific variability has ecological relevance, for instance in niche and trait overlap, and not including it can lead to significant difficulties in interpreting results (Cairney, 1999; Shipley et al., 2016; Violle et al., 2012). Many plant species have lower intraspecific than interspecific variation in trait values, and this is assumed to be the case for most plants (McGill et al., 2006; Violle et al., 2012). This larger inter- to intraspecific variation assumption has provided the basis for using mean trait values when applying trait theories and conducting trait-based analyses (McGill et al., 2006; Violle et al., 2012). However, this approach has been criticized both theoretically and empirically, and there are increasing calls for greater inclusion of intraspecific variation in trait studies (Jung et al., 2010; Shipley et al., 2016; Violle et al., 2012).

There is no empirical evidence that wood fungi have higher inter- than intraspecific variation of any functional trait. It is of paramount importance that this difference is explored, particularly in studies focusing on coexistence and community assembly mechanisms (Aguilar-Trigueros et al., 2015; Behm & Kiers, 2014; Cairney, 1999). If intraspecific variation is being studied, the guidelines around finding healthy specimens in optimum environments (protocols, Section 1) can be relaxed, as the aim is to capture as much variation as possible (Violle et al., 2012). A typical example is *Hypoholoma fasciculare*, a wood fungus that can develop mature caps with diameters ranging from ca. 20 to 75 mm (Ludwig, 2001). Finding the extent of intraspecific variation requires either (1) sampling as many random individuals as possible along well-defined and unconfounded environmental gradients (Violle et al., 2012) or (2) an experimental approach manipulating micro- or mesocosms.

## 2 | FUNGAL FUNCTIONAL TRAITS

The traits proposed, their measurement protocols, potential issues and hypotheses of community and/or environmental relevance can be found in Supporting Information Appendix S1. Section 1 of the appendix covers sampling methods of fungi, including the collection of spores, spatio-temporal concerns and replicate measurements. Table 1 below presents each group of traits, the relevant section number and the categories or measurement units. It is difficult to recommend a specific

**TABLE 1** Functional traits considered in the protocols, including the measurement method and unit of measurement

Section and number	Suite of traits	Trait	Measurement method	Measurement unit
<b>Lifestyle</b>				
2.1		Life strategies (trophic)	*Categorical Enzyme production *Growth and microscope Isotopic analysis "Omics" methods	Saprotrophy, necrotrophy, parasitically, mycorrhizas Enzyme Unit (U) per weight unit Occurs/does not occur Concentration of isotopes, for example, <sup>14</sup> C and <sup>15</sup> N Methods; genomics, transcriptomics, proteomics, metabolomics
2.2		Decay strategy	*Categorical Enzyme assays "Omics" methods	Physical: white, brown, soft, non-lignocellulose (e.g., stain); timing: primary, secondary, tertiary Enzyme Unit (U) per weight unit (possibly per time unit as well) Assay lignocellulose decay profile; genomics, transcriptomics and proteomics
2.3	Life history and life span	Persistence of vegetative and resting structures	*Sampling over time/space and pairing on agar	Persistence of individual across area/over decay stages
2.3.1			DNA analysis along timeline	Number of years individual persists
			*Observation	Presence of sclerotia and/or chlamydospores
2.3.2		Persistence of fruiting structures	*Categorical	Annual/perennial
			*Observation	Months/years
2.3.3		Metabolically active period	Respiration (CO <sub>2</sub> or O <sub>3</sub> ) Gas chromatography Enzyme assays	CO <sub>2</sub> or O <sub>3</sub> concentration Gas concentration (ppm) Enzyme unit (U) per weight unit (possibly per time unit as well)
2.3.4		Species-specific time to sexual reproduction	Inoculation and monitoring	Years
2.4		Fruit body: mycelium mass ratio	Mass at relevant time	mg, with the fruit body expressed as the fraction of the weight (0–1)
2.5		Relative Growth Rate	Dry weight over time	mg g <sup>-1</sup> day <sup>-1</sup>
2.6		Mating systems	*Categorical *Pairing single basidiospores	Homothallic, heterothallic unifactorial or heterothallic bifactorial, or homothallic, bi- or tetra-polar heterothallic Observe mating type (after clamp connections occur)
2.7		Wood decay rate	*Change in mass over time	mg/day
2.8		Respiration rate	CO <sub>2</sub> production over time	R <sub>mass</sub> : CO <sub>2</sub> produced per dry mass of fungus
2.9		Carbon-use efficiency	Relative change in biomass	CUE %

(Continues)

**TABLE 1** (Continued)

Section and number	Suite of traits	Trait	Measurement method	Measurement unit	
<b>Mycelia</b>					
3.2	Mycelial differentiation and hyphal characteristics		*Observe and measure under microscope	Categorical: for example, colour and texture. Occurrence: deposits on walls, terminal structures, etc. Measure: diameter, wall thickness, etc.	
3.3		Aggregated mycelial structures	*Presence of structures  Observation and mapping	Occurrence of mycelia cords, rhizomorphs, pseudorhiza, sclerotia, pseudosclerotial plates  Presence and distribution of mycelia cords and rhizomorphs in litter layer around resource units	
3.4		Mycelial biomass (density)	*Direct observation (SEM or measuring ground samples), *PLFA or assaying chitin or ergosterol	mg/g, mg g <sup>-1</sup> day <sup>-1</sup> , mg mm <sup>2-1</sup> SEM: scanning electron microscope PLFA: phospholipid fatty acids	
3.5		Radial extension rate	*Observation and measure	mm/day	
3.6	Mycelia area, hyphal coverage and space filling		*Image analysis	Various measures: for example, hyphal coverage, mass fractal dimension and surface (or border) fractal dimension	
3.7	Mycelial network parameters		Network architecture developed from image analysis	Various summary statistics: number of tips, branch junctions and edges; total hyphal/cord length, area and volume; distribution of side branch angles and length between branches	
3.8	Interspecific competition strategy	Volatile organic compounds (VOCs)	For each strategy:		
		Hyphal interference	*Categorical	Species uses strategy, preferred/dominant strategy, number of strategies engaged	
		Mycoparasitism	Quantification	Production of VOCs measured, amount and type of enzymes/non-enzymic toxins produced, measuring mycelial growth (see Section 3.6 and 3.7)	
		Gross mycelial contact			
3.9		Tissue composition	Quantification of elements	Mass spectrometry or high-pressure liquid chromatography	
<b>Fruit Body</b>					
4.1		Fruit body type	*Categorical	Major fruit body types: agaricoid, resupinate corticioid, discomycetoid, pileate corticioid, pileate polyporoid, resupinate polyporoid, ramarioid, stromatoid, tremelloid	
4.2	Fruit body size and biomass	Fruit body dimensions	*Ruler measurement	mm (length, depth, width) and calculated mm <sup>3</sup>	
4.2.1.1					
4.2.1.2				*Image analysis, 2D	mm <sup>2</sup> and calculated mm <sup>3</sup>
4.2.1.3			Image analysis, 3D	mm <sup>3</sup>	
4.2.2/4.2.3		Fruit body biomass	*Fresh weight/dry weight	mg	
4.2.4		Density	*Biomass per volume unit	mg/mm <sup>3</sup>	

(Continues)

**TABLE 1** (Continued)

Section and number	Suite of traits	Trait	Measurement method	Measurement unit
4.2.5		Biomass proxies	*Cap area index	mm <sup>2</sup> used to estimate mg
4.3	Hymenium traits	Hymenophore type	*Categorical	Smooth (resupinate), poroid, labyrinthine (mazy), lamellate, denticulate (hydroid), gasteroid and irregular
4.3.1		Hymenophore surface characteristics	*Presence of structures	Presence of sterile structures, for example, cystidia and setae
4.3.2		Relative investment in sporophore tissue structures	*Measure trama and sporophore thickness	Ratio of trama thickness to sporophore thickness
4.3.3		Hymenophore size	*Ruler measurement (regular shaped fruit bodies) Image analysis, 2D or surface area calculations	mm (length, width) and calculated mm <sup>2</sup> mm <sup>2</sup>
		Density of gills	*Categorical	Narrow, intermediate, distant
		Density of gills/pores/spines	*Counted from photos	Number per cm <sup>2</sup>
4.4	Texture, mitic systems, water retention	Toughness	*Penetrometer measurements	g/mm <sup>2</sup>
		Texture characteristics	*Categorical	Tough, soft, fleshy, gelatinous and fragile or brittle
		Mitic systems	*Categorical (via microscopy)	Monomitic, dimitic, trimitic
4.5		Pigmentation	*Categorical	Named colours, for example, brown and yellow
			*Digital photography and colour extraction	Average RGB (red/green/blue) value or similar
			*Spectrophotometry	RGB, ultraviolet, hue, etc.
4.6		Velum and surface (pileus) structures	*Categorical	Type of pileus characteristic, for example, glabrous, hirsute, scaly, tomentum and trichoderm
			Measure structure size	For example, average hair length and thickness of epidermis
4.7		Fruit body phenology (timing and duration of fruiting and sporulation)	*Surveys or combining multiple surveys	Time of year fruiting and/or sporulation occurs by season, months, Julian date, number of days, etc.
4.8		Spore production rate	*Count spores collected	Number of spores per unit time and unit area
4.9		Fruit body height above ground	*Measured	cm
<b>Secondary Metabolites</b>				
5.1		Scent-related and other VOCs	*Categorical	Occurs/does not occur
			Mass spectrometry and/or gas chromatography	Production of VOC and/or amount produced

(Continues)

Section and number	Suite of traits	Trait	Measurement method	Measurement unit
5.2		Taste	*Categorical  Identification of responsible metabolite and quantification	Bitter taste occurs/does not occur; or degree of taste: slight, moderate, very  Production of metabolite and/or amount produced
5.3		Luminescence	*Visual assessment; categorical  *Digital photography  Bioluminescence assay	Occurs/does not occur  Occurs/does not occur (according to set luminescence standard)  Intensity and spectra of luminescence
<b>Spores</b>				
6.1		Spore type	*Categorical	Sexual/asexual (conidia/oidia/chlamydo-spores)
6.2		Spore size	*Measured length and width	$\mu\text{m}$ and calculated $\mu\text{m}^3$
6.3		Spore shape	Categorical  *Quantified	Cylindric, allantoid, lunate, navicular, oblong ellipsoid, etc.  Ratio of length/width (Q)
6.4		Spore wall thickness	*Categorical  Measured (SEM)	thin <0.2 $\mu\text{m}$ <thick and if double walled  $\mu\text{m}$
6.5 6.5.1	Spore surface	Ornamentation	Categorical	Reticulose, russuloid, spiny, verrucose, rugose, etc.
6.5.2		Germ pore	*Presence of pore	Occurs/does not occur
6.5.3		Plage and hilum	*Presence of depression/indentation	Occurs/underdeveloped/does not occur
6.6		Spore pigmentation	*Categorical  *Spore print image analysis and colour extraction	Named colours, for example, brown and yellow  Average RGB value or similar
6.7 6.7.1	Dispersal distance	Dispersal distance	Long-distance spore capture and genetic comparison	km
6.7.2			Bulk distance	m to km
6.7.3		Aerodynamic diameter	Measured with an aerodynamic particle sizer	$\mu\text{m}$
6.7.4		Terminal velocity	Stokes' law and aerodyn. dia.	m/s
6.7.5		Insect-mediated dispersal	SEM of insect exoskeleton and/or DNA sequencing	Occurs/does not occur; average spore load per insect
6.8		Dormancy	*Germination rates over time	Percentage of viable spores per time unit passed
6.9		Germinability under environmental stress	*Germination rates under environmental stress, for example, solar radiation	Percentage of germination occurring under stressor relative to control group without stressor
<b>Conservation Attributes</b>				
7.1		Frequency and conservation status	*Categorical	For example, Red List status



**TABLE 1** (Continued)

Section and number	Suite of traits	Trait	Measurement method	Measurement unit
7.2		Native/exotic/ invasive	*Categorical	Native/exotic/invasive

*Note.* Some traits can more properly be grouped into a “suite of traits” which can be broken into several components; where applicable, these suites are listed. Trait measurement methods with an \* next to them are those where we propose standardized methods in detail in Supporting Information Appendix S1, and others are those where we propose several methods, outline potential future methods (both of which need more research) or reference appropriate material as the measurement requires substantial technical detail or specialized training.

number of replicates, as this will depend on questions of interest (e.g., how a species vs. how a community varies across a gradient) and the variability of the trait in question. This is exacerbated by the limited information on the variability of most traits for basidiomycete fungi. Further, similar to stoloniferous plants and clonal animals, it is important to ensure that replicates come from distinct genetic individuals, rather than pseudo-replicates of the same individual (see Sections 1.4 Appendix S1). The issue of pseudo-replication applies to traits measured in the field (e.g., fungal colonies) as well as laboratory settings (e.g., growing the same isolate on several plates). Where mean trait values for each species in a community are required, the number of replicates will be smaller than if a single species is the focal interest, and power analyses could be used to determine the appropriate number. The handbook includes a wide range of lifestyle traits, mycelial traits, fruit body traits, secondary metabolites and spore traits. The last section focuses on non-functional traits, or conservation attributes, relevant for evaluating fungal status and management.

### 3 | FUNGAL TRAIT SAMPLING

In this section, we introduce some basic principles for undertaking fungal trait measurement in basidiomycete wood fungi. This includes procedures for sourcing trait values, choosing between field- and laboratory-based measurements, collection of host tree details, and species selection and coverage. These are presented here as they apply to all studies undertaking fungal trait measurement.

#### 3.1 | Sourcing or measuring traits

##### 3.1.1 | Literature and database mining

Traits from the literature can be divided into two broad categories: those based on research and associated publications, versus those found within species descriptions of taxonomic keys.

Research articles can be mined for species-specific traits at the individual level, meaning they can capture variance in traits along environmental, geographic and genetic gradients. An advantage of these data sources is that they represent fungi beyond a single mean value and can provide indications of intraspecific variation. Disadvantages include the following: the complexity of integrating multiple studies with different control and experimental variables; reconciling different techniques and measures; being aware of all studies as well as

potential limitations to the measures; and combining sufficient data to accurately represent the true variance of the trait.

Species descriptions and components of regional keys can also be mined for trait data. In this case, a range for the trait can be provided, but ultimately this range may not reflect the true individual variance across the entire range of the species. A major advantage of this form of literature-based traits is the large diversity in species that regional keys contain; that is, far more species are included than any single research publication can. A major disadvantage is that individual variability is questionably represented, even when ranges are provided. Some values are also applied at the genus or greater ranking.

A challenge for both methods is changes in taxonomy of species through time. Reconciling this is a continual challenge that can only be partly alleviated through updated species taxonomy. The splitting and lumping of species causes further difficulty that requires highly specialized taxonomists, a group of people themselves “threatened with extinction” and the loss of valuable scientific knowledge. Further, incorporating for phylogenetic signal in analyses (Appendix S2) can be useful for species and trait relatedness.

##### 3.1.2 | Field- and laboratory-based measurements

The protocols presented here provide methods for taking field- and laboratory-based measurements. Direct measurements of traits are preferable when study questions involve environmental gradients or site-specific matters (e.g., evaluation of conservation actions) or where large intraspecific variation means site-based measurements better answer study questions than values extracted from databases. Laboratory-based measurements provide a controlled setting where measurements can be made on life stages (e.g., mycelia) that are difficult to quantify in the field and where standard conditions or many replicates of a single species are required. Field measurements are better suited to studies of community composition or studies examining in situ conditions. As with literature-sourced traits, taxonomy is important and efforts should be made to ensure that the taxonomy is consistent with current international nomenclature (The Index Fungorum; [www.indexfungorum.org](http://www.indexfungorum.org)).

#### 3.2 | Hosts (taxonomy and conditions)

To the novice eye, and even to experienced workers examining well-decayed wood, the determination of the hosts of wood fungi

can be challenging, but host species and qualities are important in determining fungal species composition and diversity (Heilmann-Clausen & Christensen, 2004). Taxonomic characteristics of the wood are often seemingly lacking; for example, bark may have mostly or completely sloughed off, and the wood structure has turned into a pulpy or cubical disintegration of lighter or darker coloured wood, but these are all actually clues that should be recorded and used to retain as ecological trait characteristics related to the fungal species.

Host groups will fall broadly into angiosperms (flowering trees) or gymnosperms (conifer trees), which often can be identified based on minimal knowledge of the site history as well as the current tree composition. Any remnant bark on the wood, the branching pattern and tree diameter are all clues that will help group the decaying wood into one of these main groupings. Further characteristics of the hosts may also be useful, for example, whether host tree dead wood is comprised of a bole (main stem of a tree), a stump (mechanically cut or naturally fallen tree as a result of age, storm, fungi or insects), or a cut or fallen log or large branch. Fungi that grow within the interior of a tree, carving out a hollow (itself important for biodiversity, e.g., Remm & Löhmus 2011), are referred to as “heartrot” fungi. Fungi that decay and fruit towards the base of a tree are distinguished from those fruiting or found higher up on the bole or at the top of the tree. Fungi on branches are often different from those on boles, with gradations based on the diameter of the branch. When sampling, attention should be paid to the lower surfaces of fallen host material, as a different microhabitat is created here due to higher moisture retention, limited sun exposure and proximity to soil and ground vegetation. The cause of host tree death and decay stage are also of utmost importance (Lisiewska, 1992; Ovaskainen, Hottola, & Siitonen, 2010; Nordén et al., 2013; Ottosson et al., 2015): Has the tree died standing, been blown over or cut? Is the host still alive (look for leaves along branches)? Is the bark still on the branch or bole surface, or has it completely sloughed off? Are the wood fibres stringy, often bleached and pulp-like, or cubical and darker stained? Are rhizomorphs or mycelial cords (see Section 3, Appendix S1) readily visible along the host surface? See Section 2.2, Appendix S1 for further discussion related to decay stage.

### 3.3 | Fungal species

The selection of fungal species is determined by the studies' questions (Pérez-Harguindeguy et al., 2013) and the life stage sampled (mycelium vs. fruit body). The majority of published fungal trait studies investigate trait variation along environmental or disturbance gradients (e.g., Nordén et al., 2013; Crowther et al., 2014; Halbwachs et al., 2016). Often the aim is to gain insights into how environmental gradients shape community composition, characteristics and change (McGill et al., 2006). For environment–trait studies focused on the whole community, we suggest that covering 80% of the cumulative relative abundance or biomass of fungal communities is appropriate, after plant trait literature (Garnier et al., 2004; Pérez-Harguindeguy et al., 2013), although we recognize this is challenging for mycelia.

However, if rare species are of specific interest (e.g., in comparison with the common ones), additional sampling above the 80% abundance guide may be needed. The scale at which abundance and traits are measured (resource unit level, plot level, stand level, forest level) will largely depend on the environmental gradient studied and the ecological relevance of scales. Studies with broader foci, examining general strategies (resource use, trade-offs, etc.; Kausarud et al., 2010; Bässler, Heilmann-Clausen, Karasch, Brandl, & Halbwachs, 2015) across larger, local-to-global scales, need to sample from as wide a range of environments or phylogenetic groups as possible (Pérez-Harguindeguy et al., 2013). In contrast, studies with a more singular focus (e.g., local processes, microgradients or single species) will have a small range, offset by a requirement for greater replication. Whether the mycelium or the fruit body is being studied will also depend on the research questions and the resources available; for example, expensive and time-consuming molecular analyses are needed for mycelial surveys, whereas mycologists adept at species identification are necessary for fruit body surveys.

## 4 | STANDARDIZED ENVIRONMENTAL MEASUREMENTS

Defining generalities from trait-based approaches requires comparable studies in all measurements used, that is, not only traits, but environmental covariates as well (Shipley et al., 2016). If a generalization, rule or mechanism is postulated in one study, it needs to be presented in a way that other studies (experimental or observational) can confirm or refute in other sites, species, communities or environments. While this handbook primarily deals with trait standardization, it is important that environmental gradients measured and sampling methods used are also clearly described and similar, if study comparisons are to be made (Shipley et al., 2016). Such protocols have been lacking in plant trait ecology and are essential for linking traits with environmental gradients influencing trait selection (Shipley et al., 2016). If standard environmental measurements are implemented in fungal ecology from a relatively early stage, we will be able to test for and understand trait–environment interactions across scales more quickly and efficiently.

Below we cover methods for the most commonly measured environmental gradients. Some measurements already exist, and continued use will enhance past/future comparability. For example, many studies classify decay stage using the McCullough (1948; e.g., Söderström, 1988; Renvall, 1995) or National Forest Inventory methods (e.g., Riksskogstaxeringen, 2016). Other gradients dependent on landscape context, such as disturbance history, may be harder to standardize.

### 4.1 | Decay stage

There are three methods for measuring wood decay stage: an ordinal classification system based on several physical aspects of the decaying tree (e.g., McCullough, 1948), an approach measuring the force

required to pierce wood with a penetrometer on a continuous scale (e.g., Kubartová, Ottosson, Dahlberg, & Stenlid, 2012), and finally by direct measures of wood density in the laboratory, based on wood samples (Kubartová et al., 2012). Ultimately one system should be used, and as classification methods (although vulnerable to subjectivity) have been and continue to be regularly used, we recommend this approach. If a study is particularly interested in decay stage effect, quantification methods are more appropriate, but classification could also be reported, as it is fast and easy to record.

## 4.2 | Resource unit size

The resource unit size may be important for the fungal community composition found within it (Edman, Kruys, & Jonsson, 2004; Juutilainen, Mönkkönen, Kotiranta, & Halme, 2017). This is especially so for rare or red-listed species, which appear to be confined to larger logs (Edman et al., 2004; Nordén et al., 2013). Resource unit volume can be estimated by taking log length/snag height, maximum and minimum diameter and, assuming a frustum/truncated cylindrical cone, using the calculation:

$$\pi L/3(R^2 + r^2 + Rr)$$

where  $L$  is length/snag height,  $R$  is radius at maximum diameter and  $r$  is radius at minimum diameter. This volume should be expressed in metres cubed, following common reporting. Where tree-species-specific volume equations are available (e.g., Laasasenaho, 1982; Näslund, 1947) for the area and habitat type, these are preferred as they take into account more explicitly tree shape depending on species and site characteristics.

Diameter of the resource unit may be a more appropriate tree size measure than volume in studies that focus on the ecology of individual species which prefer trees with a large or small diameter, to some extent irrespective of tree length and therefore volume (Juutilainen et al., 2017; Nordén et al., 2013). The microclimatic conditions, physical and chemical characteristics, life span and the biotic environment of the dead tree change with tree diameter (Boddy & Heilmann-Clausen, 2008), affecting what fungal functional traits are favourable in trees of different sizes.

## 4.3 | Disturbance history

Fungal communities may be impacted by historical disturbances, both natural and anthropogenic (Josefsson, Olsson, & Östlund, 2010; Nordén et al., 2018). This is not limited to, but could include, fire, bark-beetle outbreaks, clear-cutting and selective logging. Measurement of disturbance history is often complicated by lack of data (especially in pre-satellite era) and by difficulty in clearly defining disturbances. In general, the ideal disturbance dataset would have disturbance dates and a measure of disturbance intensity. The latter could include the size of the area affected, magnitude of living tree death or removal, or measures of changes in forest edges (i.e., increased edge effects).

Historical disturbances can be detected and dated using a range of methods, including dendrochronology or geospatial analyses. Increment core samples from living trees in which the radial growth pattern may reveal growth release events that indicate gap-creating or stand-replacing disturbances (Groven, Rolstad, Storaunet, & Rolstad, 2002). The causes of disturbances can be classified with the help of a survey of old cut stumps, fire marks or soil charcoal, and records of past management, storm and insect outbreak events (Kasin, Blanck, Storaunet, Rolstad, & Ohlson, 2013; Nordén et al., 2018). Similarly, other signs of human impact such as culturally modified trees (Josefsson et al., 2010) can be dated with the help of increment core samples. Older dominant trees, as well as the presence of large well-decomposed logs that may take decades or centuries to form, are indications of long forest continuity (Josefsson et al., 2010; Nordén et al., 2018). Additionally, historical maps, aerial photography and satellite imagery can all be used in geospatial analyses to identify disturbances and evaluate landscape scale changes over time.

Depending on the disturbance regime in the study ecosystem and the aim of the research, any of the above disturbance measures may be appropriate. Although we are unable to prescribe a specific method, also a common problem in the plant trait literature (Shipley et al., 2016), we recommend any study incorporating disturbance history clearly explains and justifies selection and quantification methods.

## 4.4 | Climate and elevation data

Climate data primarily consist of temperature and precipitation, both of which can be influential in mycelial growth and fruit body development, and thereby also fungal community and trait dynamics (Andrew et al., 2016). All climate data should be expressed in the metric system and sourced from either the nearest local weather station or interpolated climate grids produced by scientific research or national weather organizations. The increasing availability of open-source metadata, for example, WorldClim (<http://www.worldclim.org>; Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) and E-OBS (Haylock et al., 2008), provides excellent sources to link climate to fungal ecology (Andrew et al., 2017).

## 4.5 | Habitat (patch) area and edge measurement

Species show varying responses to habitat area and edges (e.g., positive, negative or neutral) depending on edge-to-interior resource gradients and microclimatic conditions (Ewers & Didham, 2006). The taxonomic coverage in edge-effect studies has been uneven, but the few studies available for basidiomycete wood fungi indicate forest stand size and edge effects affect fungal occupancy and viability (Ruete, Snäll, Jonsson, & Jönsson, 2016; Siitonen, Lehtinen, & Siitonen, 2005). Stands covering larger areas can support more diverse and larger populations of fungi, which are more resistant to stochastic extinctions. Many old-growth forest indicator fungi are sensitive to edge effects and generally occur in the interior of forest

stands (e.g., Ruete et al., 2016). Therefore, forest area and edge effects are important metrics to consider when testing for habitat effects on traits and dynamics of single species populations or community composition.

Forest stand area (hectares or square kilometres), shape and edge metrics can be quantified using desktop spatial analyses at different spatial scales. Distances of 50–100 m are often considered sufficient to reach forest interiors, where edge effects no longer apply (Ruete et al., 2016; Siitonen et al., 2005). However, the magnitude of the edge influence may also vary for different types of forest edge (Ruete et al., 2016).

#### 4.6 | Forest type

Site characteristics and descriptions of resource amounts, including the number, volume and quality of resource units (dead trees, branches, etc.), are necessary for comparison across studies. Sample-plot-based surveys are preferred over survey-time-based surveys. Fungal species often subsist on decaying logs of only coniferous or deciduous trees, or only one tree species, although there are also many generalists (Nordén et al., 2013; Ryvarde & Melo, 2017). Therefore, the community found in a spruce log will be very different from the community in an oak log. Additionally, studies of fungal communities tend to focus on logs of one species, dominant in that forest. As such, it is important to record which tree species are studied and what other tree species may be present in the forest.

#### 4.7 | Microsite conditions

Fungi can be strongly affected by microsite conditions, as shown by some studies (e.g., Krah et al., 2018, Pouska, Macek, & Zíbarová, 2017), and others which are still hypothesized to affect fungi. While we cannot cover all microsite conditions or their measurement methodology here, it is important to be aware of their effect and, where possible, measure those relevant to study questions. Microsite conditions could include factors such as soil moisture, microsite humidity and temperature, soil type, leaf cover, exposure to light and the elements, and shade cover percentage. These factors are only occasionally recorded with observational fungal data; however, some conditions have been captured with studies of fungal communities in gradient edge habitats (e.g., Crockatt & Bebbler, 2015, Ruete et al., 2016). Substrate-level microclimatic conditions such as moisture and temperature both inside and immediately surrounding wood have been shown to substantially influence fungal community assembly (e.g., Fukasawa, Osono, & Takeda, 2008, Pouska et al., 2017). Red-listed species have been shown to respond differently to microclimate conditions compared to non-red-listed species (Pouska et al., 2017). Measuring different microsite conditions and linking them with fungal traits could provide much information on fungal niches. Further such studies in different habitats are needed to establish general microsite-related patterns for fungi.

## 5 | AREAS IN NEED OF FUTURE RESEARCH

Fungal trait ecology is in its infancy, and there is a vast amount of work yet to be done. Many functional traits and corresponding environmental relationships proposed in this handbook are theorized and require supporting data. Of trait-based analyses conducted thus far, the majority rely on values from fungal taxonomic texts and the literature, which have limitations (Section 1.4). While these studies launch our understanding of fungal traits and help identify traits of interest, they need to be supplemented with field or laboratory measurements if we are to fully understand trait ecology and community governing processes.

One of the largest stumbling blocks in describing basidiomycete wood fungal traits is our limited ability to observe mycelia within resources and to identify mycelia species in the field. In most cases, this divides the setting for trait measurements into macroscopic characteristics of fruit bodies in the field, microscopic measurements from field samples and mycelial measurements from specimens in a laboratory. The use of laboratory or field approaches will be largely determined by the trait being examined and the study questions. Some traits can be measured in the field relatively easily (e.g., fruit body traits) or can only be measured in the laboratory (some mycelial traits). Further, laboratory conditions enable researchers to isolate variables of interest, with all other conditions standardized, whereas field measurements integrate effects of all of the factors affecting the fungi. Advances in molecular methods and DNA sequencing show promise, and when a larger proportion of species are sequenced, these methods may become more applicable in trait studies (Somervuo, Koskela, Pennanen, Nilsson, & Ovaskainen, 2016). Studies able to link mycelial traits measured in the laboratory with surveys undertaken in the field (i.e., the same species and environmental conditions) are particularly desirable. In the same vein, if fruit body traits could be measured in conjunction with mycelial traits, it would enhance understanding of whole-of-fungus dynamics.

Genomics of fungal communities, while not covered in this handbook, is playing an increasingly large part of fungal ecology. This subdiscipline utilizes various sequencing methods to identify species present and link these with or measure certain traits (Aguilar-Trigueros et al., 2015; Crowther et al., 2014). While these methods can yield powerful insights, we chose not to cover related traits here, as the methodology is completely different and would likely require its own trait handbook. Such a review of the potential genomic traits and links that can be made between the fungal traits presented here represents a key knowledge gap.

There has been almost no work conducted on intraspecific variability of traits within basidiomycete wood fungi. Trait literature in general over the past 20–30 years has largely ignored intraspecific variability and focused on interspecific variation, as a consequence of using mean trait values in analyses (Bolnick et al., 2011; Violle et al., 2007). Indeed, McGill et al. (2006) emphasized the importance of inter- over intraspecific variability and the

requisite for interspecific variability to be the larger of the two. Many of the theories and assumptions underpinning the use of mean traits are based on greater inter- than intraspecific variability (Violle et al., 2012). Intraspecific variation, however, is important in community ecology for a number of processes involving evolution, species niche breadth and phenotypic traits (Bolnick et al., 2011; Violle et al., 2012). When only using mean trait values, knowledge gaps in trait functions occur through underestimation of niche overlap and of the species ability to withstand competitors, and misrepresentation of species resource use and environmental constraints (Jung et al., 2010; Violle et al., 2012). This leads to decreased predictive ability and misinterpretations of results in functional community ecology (Bolnick et al., 2011; Violle et al., 2012). These issues are especially pertinent in fungal trait ecology as intraspecific variability is often likely to be large, possibly larger than interspecific variability, and the bulk of existing studies use mean traits (Aguilar-Trigueros et al., 2015; Halbwachs et al., 2016). Therefore, it is essential that future studies of basidiomycete wood fungi traits begin to incorporate, or at least consider, intraspecific variation.

Many of the traits we propose in this handbook have either limited methodologies or we propose several alternative methodologies. The former case are mostly new traits that we are proposing and future studies are needed to refine the methods. For the latter case, several methods of measurement are included for one trait, as there are several viable options and studies are needed to evaluate whether there is a best approach or if the approach will be question-dependent.

Finally, in plant trait ecology, trait databases have been extremely useful when amalgamating studies to provide data to examine generalities, mechanisms and intraspecific variation (e.g., TRY and LEDA databases; Kleyer et al., 2008; Kattge et al., 2011). These databases act as repositories where researchers can deposit trait values, locations and environmental conditions measured in their studies. These databases have some requirements in terms of quality control, and researchers can stipulate how their data can be disseminated (e.g., permission requirements before the data are shared, co-authorship agreements). These databases have proven valuable for a range of plant community ecologists, both data submitters and users. Such a database for fungi would provide fungal trait ecologists with equivalent opportunities and may be easier to build now, before fungal trait research expands (Aguilar-Trigueros et al., 2015; Halbwachs et al., 2016). Several databases already exist which contain information on some traits, although they have the same limitations as fungal taxonomic texts. Such databases include the Artfakta (<http://artfakta.artdatabanken.se/>; curated by Artdatabanken, Sweden) and MycoBank (<http://www.mycobank.org/>; curated by the International Mycological Association). These databases and others, such as the UNITE database and Fun<sup>Fun</sup> database, could be expanded to include many fungal trait measurements per species in the future as the structures and support already exist.

## 6 | CONCLUSIONS

Fungal trait-based research is a relatively new field, with much work needed. There are many lessons that can be learned from plant trait literature, both in knowledge accumulated and in identified knowledge gaps. Here, we have proposed a series of core basidiomycete wood fungi functional traits and methods for quantifying them. These traits and methods are by no means the only traits or measurement methods available. We consider this handbook as a starting point for conducting fungal trait measurements, which will be improved over time as new methods are developed and a stronger understanding of fungal functional traits emerges.

## ACKNOWLEDGEMENTS

The authors would like to acknowledge and thank colleagues for discussions surrounding fungi, traits and trait theory during the development of this handbook. The authors would also like to thank the four anonymous reviewers for their constructive comments. The study was funded by the Swedish Agricultural University to S.K.D., FORMAS Grant 2016-00461 to M.J. and the Academy of Finland (Grant Nos. 250444 and 209581 to O.O.).

## AUTHORS' CONTRIBUTIONS

S.K.D. conceived and organized the study, organized the protocol structure, conceived traits, led the writing of the manuscript and edited the manuscript; L.B., H.H., M.J. and C.B. organized the protocol structure, conceived traits and wrote and edited the manuscript; C.A., T.W.C., J.H.-C. and J.N. conceived traits and wrote and edited the manuscript; and O.O. wrote statistical sections and edited the manuscript.

## DATA ACCESSIBILITY

No data were collected for this manuscript.

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## SUPPORTING INFORMATION

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**How to cite this article:** Dawson SK, Boddy L, Halbwachs H, et al. Handbook for the measurement of macrofungal functional traits: A start with basidiomycete wood fungi. *Funct Ecol*. 2019;33:372–387. <https://doi.org/10.1111/1365-2435.13239>