A protocol for reproducible functional diversity analyses

Facundo X. Palacio[1,2], Corey T. Callaghan[3], Pedro Cardoso[4], Emma J. Hudgins[5], Marta A. Jarzyna[6,7], Gianluigi Ottaviani[8], Federico Riva[5], Caio Graco-Roza[9], Vaughn Shirey[10], Stefano Mammola[4,11*]

[1]División Zoología Vertebrados, Museo de La Plata, Universidad Nacional de La Plata, La Plata, Argentina.

[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), La Plata, Argentina.

[3]German Centre for Integrative Biodiversity Research (iDiv) - Leipzig, Halle, Jena, Puschstraße 4, 04103 Leipzig, Germany.

[4]LIBRe–Laboratory for Integrative Biodiversity Research, Finnish Museum of Natural History Luomus, University of Helsinki, Helsinki, Finland.

[5]Department of Biology, Carleton University, Ottawa, ON, Canada.

[6]Department of Evolution, Ecology and Organismal Biology, The Ohio State University, Columbus, OH, United States.

[7]Translational Data Analytics Institute, The Ohio State University, Columbus, OH, United States.

[8]Institute of Botany, The Czech Academy of Sciences, Třeboň, Czech Republic.

[9]Department of Geosciences and Geography, University of Helsinki, Finland.

[10]Department of Biology, Georgetown University, Washington, DC, United States.

[11]Molecular Ecology Group (MEG), Water Research Institute (IRSA), National Research Council of Italy (CNR), Verbania Pallanza, Italy.

*Corresponding author: stefano.mammola@helsinki.fi

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Abstract

1. The widespread use of species traits to infer community assembly mechanisms or to link species to ecosystem functions has led to an exponential increase in functional diversity analyses, with >10,000 papers published in 2010–2019, and >1,500 papers only in 2020. This interest is reflected in the development of a multitude of theoretical and methodological frameworks for calculating functional diversity, making it challenging to navigate the myriads of options and to report details to reproduce a trait-based analysis. Therefore, the study of functional diversity would benefit from the existence of a general guideline for standard reporting and good practices in this discipline.

2. We devise an eight-step protocol to guide ecologists in conducting and reporting functional diversity analyses. We do so by streamlining available terminology, concepts, and methods, with the overarching goal of increasing reproducibility, transparency and comparability across studies. The protocol is based on the following key elements: identification of a research question, a sampling scheme and a study design, assemblage of community and trait data matrices, data exploration and preprocessing, functional diversity computation, model fitting, evaluation and interpretation, and data, metadata and code provision.

3. Throughout the protocol, we provide information on how to best select research questions and study designs, and discuss ways to ensure reproducibility in reporting results. To facilitate the implementation of this protocol, we further developed an interactive web-based application (*stepFD*) in the form of a checklist workflow, detailing all the steps of the protocol and providing tabular and graphical outputs that can be merged to produce a final report.

4. The protocol streamlined here is expected to promote the description of functional diversity analyses in sufficient detail to ensure full transparency and reproducibility. A thorough reporting of functional diversity analyses ensures that ecologists can incorporate others’ findings into meta-analyses, the shared data can be integrated into larger databases for consensus analyses, and available code can be reused by other researchers. All these elements are key to push forward this vibrant and fast-growing field of research.

Resumen

1. El amplio uso de los caracteres de las especies para inferir mecanismos que estructuran las comunidades o vincular especies a funciones ecosistémicas, ha producido un crecimiento exponencial en los análisis de diversidad funcional, con > 10.000 trabajos publicados en 2010–2019, y > 1.500 publicaciones únicamente en 2020. Este interés se ve reflejado en el desarrollo de una multitud de enfoques teóricos y metodológicos para calcular la diversidad funcional, lo que hace desafiante navegar la miríada de opciones y reportar los detalles necesarios para reproducir un análisis basado en caracteres. Por lo tanto, el estudio de la diversidad funcional se vería beneficiado con la existencia de lineamientos generales para el reporte estándar y de buenas prácticas en esta disciplina.

2. Diseñamos un protocolo de 8 pasos para guiar a ecólogos en el proceso de llevar a cabo y reportar análisis de diversidad funcional. Para esto, sintetizamos terminología disponible, conceptos y métodos, con el objetivo primordial de aumentar la reproducibilidad, transparencia y comparabilidad entre estudios. Este protocolo se basa en los siguientes elementos clave: identificación de la pregunta de investigación, de un

diseño de muestreo y de estudio, construcción de matrices de comunidades y caracteres, exploración y preprocesamiento de datos, cálculo de la diversidad funcional, ajuste, evaluación e interpretación de modelos, y suministro de datos, metadatos y código.

3. A través de este protocolo, brindamos información sobre cómo elegir las preguntas de investigación y el diseño de estudio, y discutimos formas para garantizar la reproducibilidad en el reporte de los resultados. Para facilitar su implementación, desarrollamos una aplicación web interactiva (*stepFD*) en forma de flujo de trabajo, detallando todos los pasos del protocolo y proporcionando tablas y gráficos, que pueden ser combinados para producir un reporte final.

4. Se espera que este protocolo promueva la descripción de análisis de diversidad funcional con el suficiente detalle para asegurar una completa transparencia y reproducibilidad. Un reporte riguroso de los análisis de diversidad funcional garantiza que los ecólogos puedan incorporar los hallazgos de otros en meta-análisis, que los datos compartidos puedan integrarse en grandes bases de datos para análisis de consenso, y que el código disponible pueda ser reutilizado por otros investigadores. Todos estos elementos resultan clave para impulsar este campo de investigación vibrante y de rápido crecimiento.

Keywords

Biological diversity, ecosystem functioning, replicability, Shiny, standardized protocols, trait-based ecology

Introduction

Failure to reproduce many results in the published literature is causing discussions among scientists about poor research practices (Baker, 2016; Fanelli, 2018). A lack of reproducibility (Glossary) hinders our ability to falsify results and to reduce the misuse of statistics. Poor reporting of experimental protocols and pipelines (Munafò et al., 2017), limited data and code sharing (Tenopir et al., 2011; Culina et al., 2020), and other issues (e.g., cherry picking statistically significant results, *p*-hacking, hypothesizing after the results are known; Fraser et al., 2018) all lead to a lack of reproducibility. Transparent practices are gaining attention across many domains of science as a solution to these issues.

Similar concerns over transparent practices in ecology (Fidler et al., 2017; Fraser et al. 2018; Eckert et al., 2020; Culina et al. 2020) have prompted the development of protocols to enhance and achieve best standards in data acquisition, analysis, and result reporting. For example, pipelines and protocols are available for collecting trait data (Cornelissen et al., 2003; Moretti et al., 2017; Klimešová et al. 2019), conducting regression-type analyses (Zuur & Ieno, 2016), modelling species distributions (Araújo et al., 2019; Feng et al., 2019; Zurell et al., 2020), and performing phenotypic selection analyses in evolutionary ecology (Palacio et al., 2019).

Conversely, discussions about reproducibility are still incipient in trait-based ecology (Glossary). Trait-based studies have increased exponentially in the last 20 years (Figure 1), advancing our understanding of the impact of global change on biodiversity (Newbold et al., 2020), ecological resilience (He et al., 2011; Pausas et al., 2016), and determinants of assembly rules (Mouillot et al., 2021). As a result, functional diversity has emerged as one of the core constructs in trait-based ecology at the community level (Petchey & Gaston, 2002), prompting the development of an array of methods and metrics (see Mammola et al., 2021 for an overview). This myriad of options has led to confusion when selecting appropriate methods for answering specific ecological questions (Carmona et al., 2016; Mammola et al. 2021), and made it difficult to keep track of, and navigate, an ever-growing flood of new concepts and approaches. The field of functional diversity would thus greatly benefit from having general guidelines for standard reporting of all steps of a trait-based study.

Here, we developed an eight-step protocol to maximise reproducibility in functional diversity analyses (Figure 2). We suggest that trait-based studies should start with the conceptualization of an ecological question, generally ingrained in a theoretical hypothesis-driven framework (Step 1). A clear ecological rationale then informs an appropriate experimental design (Step 2). Next, occurrence (Step 3) and trait (Step 4) data for individuals or species—the raw material of any trait analysis—are collected. Data exploration (Step 5) precedes the core of the analysis to estimate functional diversity (Step 6), and the validation, interpretation, and reporting of results (Step 7). The last step considers all the procedures to maximise the clarity and reproducibility of the proposed pipeline (Step 8).

Preface: three general principles for the sharp functional ecologist

Three main principles should be considered in all the steps of the protocol.

i) The question(s) and hypothesis(es) dictate analytical and conceptual choices. One should always fine-tune the selection of the study design, traits, and methods to most effectively answer the proposed research questions. Knowing the strengths and limitations of the different frameworks and methods prior to analysis is essential, as each might provide different answers to the same questions and data (e.g., trade-offs between predictive power and extrapolation). Importantly, the limitations of the approach selected should be acknowledged (Mammola et al. 2021).

ii) The peculiarities of the organisms/ecosystems under study should be considered when determining the questions, choice of traits, and methodology. Understanding how a system functions is crucial to making sound methodological choices—though admittedly this is not always possible, and may in itself represent one of the research targets. This requires collecting all the available information on the study system, and often collaborating closely with experts on the taxa assessed to reveal different information gaps before testing hypotheses under a functional diversity framework.

iii) All conceptual, analytical, and computational choices made to answer the research question(s) should be clearly justified and concisely documented. For example, rather than stating "*... we used the trait probability density approach (Carmona et al., 2016) to analyse the data*" we suggest to briefly justify the reason—"*... we selected the trait probability density approach (Carmona et al., 2016) because we were interested in a probabilistic representation of the trait space and because this approach allowed us to take into account intraspecific variation in traits*".

Step 1. Identify an appropriate research question

Since any scientific study begins with a question or hypothesis, establishing a salient and feasible one prior to collecting data is critical. Because resources are often limited, one should also ensure that the question addressed has theoretical and/or applied relevance, while being methodologically (e.g., computationally) and logistically (e.g., time- and money-wise) feasible. The academic community currently rewards ‘novel’ contributions (Mammola, 2020). However, authors might also be interested in an exploratory analysis (Yanai & Lercher, 2020), in addressing questions not novel *per se* but that still provide a valuable applied perspective (e.g., for conservation or management), or evaluating previous inferences with confirmatory studies (Nilsen et al., 2020). Once a salient question is established, it is important to determine whether a trait-based approach is relevant to answering it. For example, if the research question involves understanding the drivers of temporal change in community composition of a given system, researchers must evaluate whether employing a trait-based approach might provide more in-depth (or complementary) insights into that question than taxonomic or phylogenetic approaches.

There are two main tenets in answering scientific questions: the hypothetico-deductive (formulating hypotheses first, and then testing these hypotheses by collecting data) and inductive (collecting empirical observations first, and then generating hypotheses based on those observations) paradigms (Mentis, 1988). In the context of hypothetico-deductive approaches, ‘strong inference’ (i.e., devising a set of competing hypotheses, obtaining data and designing experiments to test these hypotheses) emerged to address the complexity of natural systems by exclusion of alternative hypotheses (Platt, 1964). Many have argued that a hypothetico-deductive scheme has led to more advancements in scientific understanding (Platt, 1964; Betts et al., 2021), but the inductive scheme also plays an important role in creating foundational knowledge (Mentis, 1988). In trait-based ecology, the choice between hypothetico-deductive and inductive frameworks is often guided by the taxa under study and the scale of analysis. For instance, plants and microorganisms are relatively easy to experimentally manipulate in terms of their abundance and trait values at small spatio-temporal scales, and thus allow easier implementation of the hypothetico-deductive scheme. By contrast, trait-based analyses in animals across large spatial and temporal scales analyses often fall under an inductive scheme because correlation techniques, instead of experimental manipulation, are most often employed. Finally, testing a hypothesis is not always necessary or desirable. For example, one might be interested in describing or predicting an ecological response with the highest accuracy for practical reasons (e.g., conservation planning), in which case predictive power overcomes the ability to interpret ecologically a model (Currie, 2019; Betts et al., 2021).

Step 2. Identify an appropriate experimental design

The choice of the study design—observational, experimental, or simulation—should be dictated by the research question(s) (Step 1). Observational studies facilitate insights into ecological patterns, but their ability to disentangle the mechanisms underlying a pattern is limited because many factors often interact to produce the observed patterns (de Bello et al., 2012; Spasojevic & Suding, 2012). Even though methods to model this complexity are available (e.g., structural equation models), observational studies can rarely distinguish correlation from causation. In contrast, experimental studies allow controlling for major confounding factors inherent to natural settings. In the context of trait-based ecology, for example, an experiment allows isolating the role of biotic interactions (e.g., competition) in determining functional diversity at smaller scales, whereas observational data could reveal macroecological patterns of trait diversity across larger spatio-temporal scales. In parallel, simulations can be used to link patterns revealed from observational studies with putative processes to evaluate conditions in which a given process might result in an observed pattern. Simulations can also pinpoint numerical properties and statistical artifacts, which is especially important in trait-based ecology where subjective choices, e.g., on the number, types and measures of traits, are routinely made (McPherson et al., 2018; Step 4).

In addition to these decisions a researcher should make when designing the study, there are also limitations based on the type of available data (Steps 3 and 4). Available databases vary in relation to their spatial coverage and extent, with spatio-temporal resolution typically decreasing with spatial extent (Hulbert & Jetz, 2007). Occurrence and trait data sources (opportunistic, historical or collected/experiment) are a primary consideration when designing a study, and community science datasets (Callaghan et al., 2021) and museum/herbarium collections are becoming increasingly important in trait-based ecology (e.g., Perez et al., 2020).

The identification of an appropriate sampling design is a crucial next step after the study design has been chosen. This should be primarily driven by the research question (Step 1), and secondarily by the scale of the focal ecological phenomenon (McGill, 2010) and the level of organization at which functional diversity will be assessed (e.g., individuals within a population, populations forming an assemblage; Violle et al., 2014).

Step 3. Assemble a community data matrix

Once the data collection has been conducted following the selected experimental design (Step 2), acquired data need to be tabulated in a meaningful way to explore functional diversity.

Observations are organised in a community data matrix **C** holding occurrence data. In the most general case, this is a matrix of S rows × N columns, where rows ($i = 1, 2, \ldots, S$) represent sampling units (e.g., sites, plots, transects) and columns ($j = 1, 2, \ldots, N$) represent taxonomic entities of interest (typically species, but also individuals or higher taxonomic ranks) found within each sampling unit. This basic matrix can be expanded to a set of temporal replicates or a set of individuals when accounting for intraspecific variation. In describing the matrix **C**, one should specify taxonomic resolution, sample sizes (i.e., number of sampling units, temporal replicates), number of recorded taxa, and sampling effort.

Occurrence data may take multiple forms with different ecological meanings, which should be clarified. Incidence (presence/absence) and abundance (number of individuals) data have historically been most commonly used in community ecology, though presence-only data or model-based estimates of species incidence/abundance have also been used. Other types of data, such as biomass and percent cover in sessile organisms, are often treated as abundance proxies or transformed into incidence data (e.g., Riva et al., 2020).

All these types of data can come from different sources. Besides laboratory/field experiments and traditional observations, rapid progression in monitoring technologies (e.g., remote sensing, acoustic sensors, camera traps, environmental DNA, metabarcoding) has enabled ecologists to automate extraction of massive amounts of biodiversity data from different environmental media (e.g., water, soil, or air), and identify taxa associated with the environment with high accuracy (Tosa et al., 2021). Whilst promising, the use of these data sources is still at an incipient state in trait-based ecology (e.g., Gasc et al., 2013; Schneider et al., 2017; Aglieri et al., 2020; Sigsgaard et al., 2020). Given method-specific technical limitations (e.g., amplification of a large proportion of nontarget sequences and degradation time of DNA), we suggest always reporting whether sampling effort has been adequate to capture taxonomic diversity—e.g., through rarefaction techniques (Roswell et al., 2021).

Step 4. Assemble a trait data matrix

The second key element of any functional diversity analysis is the use of species traits linking species roles in ecosystem functioning. Traits include a variety of morphological, behavioural, physiological, anatomical, biochemical, or phenological attributes that have the potential to impact the individual's fitness (Violle et al., 2007; Sobral, 2021). These traits provide the raw material to build the trait data matrix **T**, a matrix of N rows × p columns where rows ($i = 1, 2, \dots, N$) represent the taxonomic entities of interest (univocally corresponding to the N columns in the **C** matrix), and columns ($j = 1, 2, \dots, p$) represent traits. The matrix **T** can easily accommodate multiple measurements per trait (e.g., when intraspecific variation in traits is of interest, N would then equal the total number of trait measurements). We recommend specifying the functional traits used in the analysis, their nature (continuous, categorical, ordinal or ratio; Pavoine et al., 2009), and sample size per trait.

Most functional diversity studies rely on species' mean trait values—i.e., averaged across traits measurements collected from multiple individuals per species ('mean field approach' *sensu* Violle et al., 2012). This relies on the assumption that among-species trait variation largely exceeds intraspecific trait variation. However, growing evidence challenges this view (Albert et al., 2011; Palacio et al., 2019; Gentile et al., 2021; Wong & Carmona, 2021). For instance, intraspecific trait variation may increase along an environmental gradient due to phenotypic plasticity and/or local adaptation (Günter et al., 2019). As a result, two communities with the same species composition may have different trait distributions and thus different functional diversity. Our protocol therefore calls for a clear statement whether trait data are described by measurements collected from several individuals and averaged at the species level, or if intraspecific variation has been taken into account and at which organization level (e.g., site, populations, species, tree, leaves).

Selecting how many traits to include is also not trivial. For instance, there might be trade-offs between using a low number of traits and having high functional redundancy and limited variability to properly estimate functional diversity, or using a high number of traits and having low functional redundancy leading to many unique combinations of trait values (in the most extreme case, functional diversity may equal species richness; Petchey & Gaston, 2002). A common practice is to reduce the number of multiple correlated traits to a set of a few ecologically meaningful dimensions (Maire et al. 2016), e.g. using ordination methods (Step 5). Mouillot et al. (2021) showed that between 3 and 6 functional axes should be enough to accurately describe the matrix **T** without significant information loss. Yet, there is considerable variation among taxonomic groups (Díaz et al., 2016; Pigot et al., 2020) and this inference was based on a single method for estimating functional diversity—convex hull (Mouillot et al., 2021). Ultimately, the optimal number of axes will be system-, taxon-, method-, and metric-dependent, and often rests upon available computing power.

The ecological rationale for which traits are selected in an analysis is equally important and should be carefully detailed, along with their hypothesized functions (Luck et al., 2012). For instance, Lavorel and Garnier (2002) classified species traits into response and effect traits (Glossary). Response traits indicate the response of organisms to environmental factors, whereas effect traits determine the effect organisms have on ecosystem functioning, though these categories are not mutually exclusive. Another heuristic to classify traits is the ‘soft’ and ‘hard’ traits dichotomy (e.g., Hodgson et al., 1999; Cornelissen et al., 2003; Nock et al., 2016) (Glossary). ‘Hard’ traits are accurate indicators of species functions within ecosystems and are often physiological or ecological traits (e.g., growth rate, phenology). In contrast, ‘soft’ traits are proxies for such functions and tend to be morphological or anatomical (e.g., body size, plant height). ‘Hard’ traits are generally either difficult or expensive to measure in practice (Hodgson et al., 1999) and are thus often substituted by ‘soft’ traits whose collection is less expensive.

Trait data can be also measured directly from individuals (e.g., in the field/laboratory or from museum specimens), or extracted from different sources (e.g., peer-reviewed literature, field guides, online databases; Supporting Information), or a combination of the above. Trait resolution (Glossary) should be carefully considered, particularly when different data sources are combined, as differences in resolution may tangle ecological patterns and bias inference (Cordlandwehr et al., 2013; Palacio et al. 2019; Kohli & Jarzyna, 2021).

Importantly, we recommend detailing the traits used, their nature (e.g., indicating their possible states or range values, the ontogenetic stages of the sampled individuals, whether these are response/effect or soft/hard traits), and their hypothesized ecological function(s). The methods should also contain all relevant information on trait data sources. If trait data are retrieved from online databases, then information on version and access date should be provided.

Step 5. Explore and prepare the data

Data exploration is perhaps one of the most informative, yet often overlooked, steps of analysing an ecological dataset (Zuur et al., 2010). When inspecting the community data matrix (Step 3), one has to carefully check for the existence and potential causes of zero-inflation in occurrence data (these can be true zeros or an artifact due to, e.g., imperfect detection, species misidentification, or poor sampling design; Roth et al., 2018; Blasco-Moreno et al., 2019), dependency structures (e.g., pseudoreplication due to spatio-temporal autocorrelation), and potential problems due to uneven spatio-temporal sampling effort (e.g., Walker et al., 2008; Ricotta et al., 2012). Trait data (Step

4) are often a mixture of numerical, ordered, fuzzy, and/or categorical variables that should be examined for correlation. Trait data can also be characterized by unbalanced levels in categorical traits, outliers in continuous traits, and missing data, all of which might introduce biases into the functional diversity estimation (Step 6), and thus should be closely investigated.

Exploratory analyses for functional diversity datasets are no different from those routinely performed in other ecological research areas (e.g., Zuur et al., 2010). As a general pipeline, we recommend to:

1. Plot the community data matrix (e.g., heatmaps) to check whether there is a high frequency of zeroes (Box 1).
2. Check species sampling coverage (e.g., rarefaction).
3. Plot the distribution of continuous traits (e.g., with histograms, density plots, Cleveland dot plots, correlograms, and boxplots) to check for outliers. Plot categorical traits (e.g., with barplots) to check the balance of levels in fuzzy and categorical variables.
4. Evaluate multicollinearity among continuous traits (e.g., with scatterplots, pairwise correlations) and associations between continuous and categorical traits (e.g., with boxplots).
5. Identify missing trait data (e.g., with barplots or heatmaps); if any, decide how to handle them (Box 2).

These simple steps provide a better understanding into the nature of, and the issues inherent to the data, and thus allow making informed decisions on how to best approach the analysis. Depending on the outcome of initial data exploration, researchers might need to decide: (1) whether statistical corrections, e.g., rarefaction of the data or account for species' imperfect detection, are needed to remove biases in the data (Box 1); (2) how to handle missing data (Box 2); (3) how to deal with collinearity (e.g., remove collinear traits, reduce dimensionality with ordination methods, identify set of correlated traits to define functional groups); (4) how to handle outliers, which might either be of interest to the research question (e.g., Violle et al., 2017; Carmona et al., 2017) or might need to be removed to avoid inflating the outcome of functional diversity estimation; and (5) whether to weight the traits and/or transform them with dissimilarity measures or methods to reduce dimensionality to comply with the assumptions of the implemented technique (Step 6).

The Methods section can include a statement such as '*Data exploration was conducted following the recommendations provided in Palacio et al. (2022)*' together with a brief explanation of the problems and decisions made.

Step 6. Estimate functional diversity

Once the sampling design has been set up and implemented (Step 2), and data assembled (Step 3–4) and cleaned (Step 5), it is time to estimate functional diversity to evaluate whether meaningful patterns exist that can be linked to the primary question of interest (Step 1).

If summarizing or comparing univariate trait characteristics is the principal goal of the study, then raw trait data can often be used without any data transformation. The most common example of a univariate functional diversity metric that uses raw trait data is the community-weighted mean (Garnier et al., 2004; Lavorel et al., 2008), which summarizes the mean trait value of all individuals or species in the population or assemblage (for continuous traits) or the proportion of species that hold a given categorical value of that trait (for discrete traits).

If the focus of the study is on multivariate functional diversity, then this is achieved by first constructing a trait space(s) of the study system(s) from the **T** matrix and then summarising it/them into meaningful descriptive metric(s) after accounting for the information in the **C** matrix (Mammola et al., 2021). The first step in constructing a trait space is creating a trait dissimilarity matrix for all pairs of individuals or species. Caution must be exercised when choosing a dissimilarity metric as well as weights for each of the traits. For highly dimensional trait data, with a combination of continuous, fuzzy coded, categorical, and binary traits, the Gower's distance (Pavoine et al., 2009; de Bello et al., 2021a) is a sound option because it can handle different types of traits and balances the contribution of traits and trait groups to overall dissimilarity (de Bello et al., 2021b). A common practice in trait-based ecology is to assign the same weight to each trait (e.g., Jarzyna et al., 2021), but researchers might choose to weigh their traits differently depending on research goals.

Several methods exist to construct a trait space from the trait dissimilarity matrix, including functional dendrograms (Petchey & Gaston, 2002), convex hulls (Cornwell et al., 2006), and probabilistic hypervolumes (Blonder et al., 2014, Carmona et al., 2016, 2019; Mammola & Cardoso, 2020). Functional dendrograms, often created following a clustering procedure that ensures preserving original distances in the dissimilarity matrix (e.g., UPGMA, Mérigot et al., 2010), represent discrete and categorical trait data fairly accurately, but perform poorly for continuous traits. Convex hulls and hypervolumes represent differences based on continuous traits more accurately and additionally allow accounting for multicollinearity among traits (via an intermediate step of Principal Coordinate Analysis; see Step 5), but are computationally more demanding.

Once the trait space is constructed, one can calculate functional diversity metrics suitable to tackle the research questions at different levels of organisation—individual observations used to construct the trait space, trait space level (alpha FD), pairwise comparisons of trait spaces (beta FD), or the whole system (gamma FD). A comprehensive characterisation of a trait space typically includes quantifying three components of functional diversity: richness, divergence and regularity (Mammola et al., 2021). Functional richness measures the total breadth of functional diversity in a system. For functional dendrograms, functional richness is quantified as a sum of the dendrogram branch lengths (Petchey & Gaston, 2006), sometimes weighted by abundance or detection-corrected probability of species occurrence (Jarzyna & Jetz, 2016). For convex hulls, functional richness is defined as the size of the minimum polygon that encloses all species (Mason et al., 2005), and for probabilistic hypervolumes it is a measure of the volume of the hyperspace (Mammola & Cardoso, 2020). Functional divergence represents how incidence or abundance of species is spread along a functional trait axis, within the range occupied by a given assemblage (Villéger et al., 2008); it is often quantified as the average distance among observations or the mean distance of species to the centroid of their shared trait space (Villéger et al., 2008; Laliberté & Legendre, 2010; Mammola et al., 2021). Lastly, functional regularity can be computed as the regularity of branch lengths in dendrograms (Villéger et al., 2008) or, for hypervolumes, as the overlap between the observed hyperspace and a hypothetical hyperspace where traits and abundances are evenly distributed (Carmona et al., 2016; Mammola & Cardoso, 2020). No approach is currently available for estimating dispersion and regularity of convex hulls (Mammola et al., 2021).

Note that most approaches to study functional diversity can also integrate intraspecific variation in community-level calculations, including functional dendrograms (Cianciaruso et al., 2009, Cardoso et al., 2015), weighted sums of trait probability distributions across organizational levels (Carmona et al., 2016, 2019), or the union of species-level functional hypervolumes (Mammola & Cardoso, 2020; Graco-Roza et al., 2021) (see Step 6).

When obtaining the multiple components of functional diversity, we advise that researchers are consistent in the construction of the trait space, namely using a single trait space representation for all estimations (e.g., either a functional dendrogram or a multivariate space).

Finally, some descriptors of functional diversity (e.g., functional richness) are closely associated with species richness and their interpretation relies on statistically controlling for this association. This is typically done via null models calculating standardized effect sizes (SES) for functional diversity metrics, wherein species richness-controlled values of functional diversity are obtained by randomizing species incidence or abundance values while keeping species richness constant (Mason et al., 2013; see Götzenberger et al. (2016) for an in-depth discussion on null models).

Step 7. Validate and interpret the results

Depending on the primary research question (Step 1), functional diversity metrics (both absolute and those corrected for species richness) might be further used in statistical analysis to link functional diversity with different ecological predictors. A vast number of models are available in the literature, yet most statistical approaches relate functional diversity metrics through space or time with different environmental variables [e.g., generalized additive or linear (mixed) models, structural equation models, machine learning algorithms, null models]. Regardless of the approach, key elements to report include effect sizes, uncertainty estimates (e.g., standard errors, credible intervals) and model support (e.g., Information Criteria, variance explained, discriminatory power). Providing an absolute measure of model goodness-of-fit is crucial to assess how well it explains or predicts the ecological response(s) (Mac Nally et al., 2018). How to report statistical models is beyond the scope of this paper, and we refer the reader to Zuur and Ieno (2016) for presenting results in regression-types analyses.

After model fitting, researchers may desire to determine the generality in their results through validation. Validation determines how a model performs across contexts, either through the application to a novel (or partly novel) dataset, or through the comparison of the model’s performance with one based on simulations of settings where the process of interest is eliminated, i.e., null models. Validation can help determine the limitations of an analysis in terms of its ability to explain phenomena or to extrapolate to new scenarios.

Validation of results in functional diversity analyses should follow standard statistical procedures, which depend on the type of question and model. It is often required to use independent training, validation and testing datasets when the goal is predicting beyond the range of values in the data (e.g. future predictions). Resampling methods such as jackknife or cross-validation are often needed when data are limited or present autocorrelation structure (Roberts et al., 2017), particularly for extrapolation.

After results have been validated, they must be interpreted in order to understand the implications of the analysis. The same trait might represent different processes for different taxa or in different contexts. As an example, larger body size might imply a limitation of resource availability for animals, but may allow plants to outcompete others in the search for light. Similarly, the same function might be represented by different traits in different taxa. For example, dispersal ability is represented by the ratio between wing and body size and shape for many insects (Lancaster & Downes, 2017), the ability and propensity to balloon for spiders (Bonte et al., 2003), the seed size and dispersal modes for aquatic plants (de Jager et al., 2019), and the tendency to be entrained in long-distance transport vectors in invasive species (Hastings et al., 2005).

Plots are often the most effective way to present information in science (Krause & O'Connell, 2012) and, whenever possible, we recommend visually presenting the results to aid interpretability. There are many guidelines that can help thinking creatively about impactful and clear figures (e.g., Rougier et al., 2014; Crameri et al., 2020). Graphical visualisation of results also helps in validating the results, e.g., to detect errors and interpret patterns.

Step 8. Ensure reproducibility

Proper data curation, management, and archival standards should be followed to maximise the transparency and theoretical reproducibility of a study. The FAIR guiding principles for scientific data management suggest that data should be *Findable*, *Accessible*, *Interoperable*, and *Reusable* (Wilkinson et al., 2016). Below, we outline mechanisms that could help the field of trait-based ecology conform to these guiding principles.

Findable data, metadata, and code, should be properly documented and referred to by a unique identifier. One straightforward way of accomplishing this is through the deposition of data and code used in analyses into an archival/repository service which provides digital object identifiers (DOIs). Static repositories such as Zenodo, Dryad, and FigShare are useful for preserving the state of the code used in analysis at the time of publication. GitHub does not automatically provide a DOI itself for repositories, but does facilitate linkages to Zenodo to archive specific versions of code used in research. Research is *accessible* through the sharing of these data, metadata, and code, typically achieved by linking these to the paper via a Data Availability Statement. While there are inevitable limitations in the types of data that can be shared freely, the use of sample data that is sufficient to reproduce the analysis, or the use of anonymized data when there are confidentiality concerns is encouraged within existing data license agreements (e.g., Tulloch et al., 2018). Moreover, whenever possible, open-source protocols should be used ensuring the research is *accessible* in the future. Creating a research data management plan (Supporting Information) before beginning a functional diversity analysis can ensure that contributors have an understanding of the storage requirements and data privacy considerations for the project well in advance of publication.

For data files, fields that contain information should be summarized by metadata that describe the type of data and their origin. These metadata should be provided with the original, archived data file. This is particularly important for functional diversity, where it is common practice to obtain trait information from many sources. The original sources of data should be properly referenced and identified allowing for *interoperability* and *reusability* in the future, and database versions wherein download dates should be clearly specified. Code utilized in the analysis should be well documented, including in-line comments (Culina et al., 2020). Additionally, code authors should consider the versions of various software and packages used in analysis and how changes to those versions may impact reproducibility over time. We also recommend citing the software, library, or R packages used, and their version. Correct citations can be obtained via the R command *citation()* or other tools that facilitate retrieving and formatting references to packages, such as grateful (https://github.com/Pakillo/grateful). Note that we refer here to R (R Core Team 2020) as it is the most common analytical environment in ecology (Lai et al., 2019), but the same logic applies to any other software or programming language used for the analyses.

Many researchers find themselves thinking about reproducibility after a project is completed—even here, we have included reproducibility as the final step!—but we stress that FAIR practices should be implemented from a project’s inception. The Open

Science Framework provides an online platform to link data and code storage systems (including Dropbox, OneDrive, GitHub, and their own cloud storage). This architecture allows the merging of hosting platforms more suited for code with more visually-oriented project wiki pages for protocols, methodology, and analysis. The use of these stable cloud storage platforms by research groups also ensures long-term availability of all project components within a lab in spite of researcher turnover.

Web application

To aid researchers and students in the task of performing trait-based analyses, we developed a Shiny web app that goes through the proposed protocol. The *stepFD* app allows users to check the requirements needed at each step to fully reproduce their study, as well as to explore their data through statistical summaries and interactive plots (e.g., heatmaps and rarefaction curves for community data, correlations and multivariate trait spaces for trait data, functional diversity metrics computation, cross-validation tools). Given the plethora of available metrics to compute functional diversity, we arbitrarily relied on probabilistic hypervolumes as a unified framework to estimate the richness, divergence and regularity facets of functional diversity (Mammola & Cardoso, 2020). The decisions made at each step may be submitted to the app to create a final report. We stress that the app is intended to aid students and researchers in performing a transparent and reproducible functional diversity analysis, and operates mainly as an exploratory and data visualisation tool. For those interested in more rigorous statistical analyses and computation of other functional diversity metrics, we refer the reader to R packages in Table 1. The Shiny app, including datasets generated for demonstrations, is available at https://facuxpalacio.shinyapps.io/stepFD/, and the source code is available from GitHub (https://github.com/facuxpalacio/stepFD).

Conclusions: what's next?

Our protocol offers a set of simple guidelines aimed at maximizing reproducibility, transparency and consistency of functional diversity analyses (Figure 2). Hoping that the protocol will provide a foundation for a more reproducible and transparent trait-based ecology and beyond, we would like to leave the reader with a few points of reflection.

(1) Be flexible: do not limit yourself. While the protocol structure may appear dogmatic, our goal is not limiting creativity and lateral thinking. To us, this protocol is a flexible tool to aid researchers in navigating functional diversity and in remembering key pitfalls and steps to document transparently a trait-based study. However, some of the steps presented here may not apply under specific circumstances—e.g., there are cases where it is not advisable to share sensitive data (Tulloch et al. 2018)—and specific research questions may require that one violates some of our recommendations (e.g., night science; Yanai & Lercher 2020).

(2) Be a giant: offer your shoulders. The correct reporting of methods and statistics, as well as sharing data and codes, provides the foundation for other scientists to build upon your work. A thorough description of sample sizes, statistics, and model estimates ensures that others can incorporate your findings into meta-analyses (Gerstner et al. 2017); the shared data can be integrated into larger databases for consensus analyses (e.g. Mouillot et al. 2021, Graco-Roza et al. 2021); and available code can be reused by other researchers. Whether one sees this altruistically, as a collaborative effort to

advance science as a whole, or opportunistically, as a way to increase one' own citations and credibility in the field, the long-term benefits are undisputed.

(3) Be informed: find your way through the jungle of metrics. As we have shown, functional ecology is a vibrant and fast-growing field of research (Figure 1). We have touched upon examples of methods and metrics based on the current literature, but new tools and approaches are being developed continuously, and one must keep up with the literature to make the best out of this field (Mammola et al. 2021). Even though new methods will become available and concepts will emerge in the future, we believe that the key underlying philosophy and motivations of this protocol will remain valid and applicable.

(4) *Be permeable: exchange with other disciplines.* Functional diversity represents only one of multiple frameworks within ecology. The constant interaction and integration with other disciplines forming the broader biodiversity research platform (e.g., taxonomy, phylogeny) is fundamental to answer questions and test hypotheses relevant to functional diversity itself.

All in all, we envision our protocol as a set of good practices and starting points (not as a 'research shackle' for ecologists!), and we are convinced that, as other standard protocols did, may boost effective communication and enhanced understanding of upcoming functional diversity research.

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The reproducible code box (Supplementary Box S1) was adapted from Joseph R. Bennett's lab manual (Carleton University; compiled by Jaimie G. Vincent), and heavily influenced by the Open Science Foundation.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Authors' contributions statement

FXP conceived the idea and conceptualised it alongside SM. FXP, SM, GO and CTC wrote the introduction. FR and GO provided most arguments for steps 1 and 2. FXP and MAJ generated most content for steps 3 and 4. CG-R, MAJ, and SM prepared steps 5 and 6. PC conceptualized step 7. CTC, EJH, and VS wrote step 8. FXP, EJH and CG-R developed the Shiny app. SM prepared figures. FXP, EJH and SM curated supplementary content. All authors provided comments and suggestions to the final text.

Supporting information

Supplementary Table S1. Selection of open access online trait databases.

Supplementary Box S1. Reproducible code guidelines.

Data and code availability

Computer code associated with this publication is available in GitHub, namely the R code and data to generate Figure 1 (https://github.com/StefanoMammola/Palacio_et_al_2021_FD_protocol.git) and the source code for the Shiny app (https://github.com/facuxpalacio/stepFD).

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GLOSSARY, TABLES, BOXES, AND FIGURES

Glossary

Day / Night science. Day science refers to a hypothesis-driven mode of scientific research, structured by rigorous assessment of hypotheses through experiments, whereas night science stands for a creative process of scientific research, involving creation of novel hypotheses (Yanai & Lercher, 2020).

Effect / Response trait. Effect traits reflect the role organisms have on ecosystem functioning, whereas response traits indicate the response of organisms to environmental factors (Lavorel & Garnier, 2002). Note that response and effect traits are not necessarily mutually exclusive categories. For instance, body mass in frugivorous birds influences the number of seeds ingested and seed dispersal distances (i.e., it is an effect trait; Godínez-Álvarez et al., 2020), but disturbance of habitat and hunting negatively impacts large-bodied species (i.e., it also acts as a response trait; Galetti et al. 2013). In plants, leaf dry matter content (Cornelissen et al., 2003; Garnier et al., 2004) tends to respond negatively to water availability and positively to temperature (it is a response trait) while greatly affecting ecosystem functioning through litter decomposability and soil nutrient cycling (it is an effect trait).

Functional diversity (= trait diversity, FD). A characterization of life diversity in terms of the diversity of functions (Malaterre et al., 2019). Operationally, any mathematical estimation of the diversity of traits of individuals composing a given group (a community, an ecosystem, and so on), from simple measures of trait distributions (means, standard deviation, coefficient of variation, kurtosis) to the plurality of functional diversity indices developed in the last two decades (refer to Mammola et al., 2021 for an overview).

Hard / Soft trait. Hard traits accurately reflect species functions but are often difficult and/or expensive to measure, whereas soft traits are proxies for such functions and are often easier and/or inexpensive to collect data for (Weiher et al., 1999; Hodgson et al. 1999). The thermal tolerance of a species as estimated via a physiological experiment is an example of ‘hard’ trait, whereas the ‘soft’ version of this trait could be inferring thermal tolerance from the temperature conditions found across the species range.

Intraspecific trait variation. Trait variance of a group of individuals of the same species. It results from phenotypic plasticity or local adaptation of different genotypes along environmental gradients or in response to biotic interactions (e.g., competition or mutualism).

Replicability. The process of replicating a certain study using different datasets and/or model systems. A lack of replicability occurs when qualitatively different results are obtained applying the same analytical approach.

Reproducibility. The process of repeating analyses conducted by others. A lack of reproducibility occurs when different results are obtained when re-analysing the data reported in a paper.

Trait. Any phenotypical entity—morphological, anatomical, ecological, physiological, behavioural, phenological—measured on individual organisms at any scale, from gene to whole organism, and which can be scaled up from individuals to genotype, population, species, community, or ecosystem (Violle et al., 2007; Volaire et al. 2020).

Trait resolution. The coarseness of measured traits, ranging from highest-resolution continuous measurements to lowest-resolution binary categories (Kohli & Jarzyna, 2021). Body size measured on a continuous scale is typically a high-resolution trait, whereas the categorical version of this trait (e.g., ‘small’, ‘medium’, or ‘large’) is a low-resolution one.

Table 1. Examples of R packages and functions (in italics) aiding to implement the eight-step protocol for functional diversity analyses. Note that this list is not exhaustive.

Step	**Description**	**R packages (or *functions*)**
1. Identify an appropriate research question	Literature review and research interest	-
2. Identify an appropriate experimental design	Simulations	*simul.comms()*, virtualspecies
3. Assemble a community data matrix	Occurrence data retrieving	auk, rgbif, spocc
	Data manipulation	base, dplyr, tidyr
4. Assemble a trait data matrix	Trait data retrieving	BIEN, TR8, rfishbase
	Data manipulation	dplyr, tidyr
5. Explore and prepare the data	Data visualization	base, ggplot2, lattice, plotly, visreg
	Collinearity	car, usdm, VIF
	Missing data visualization and imputation	Amelia, BAT, mice, VIM
	Imperfect detection	DiversityOccupancy, unmarked

6. Estimate functional diversity	Data transformation	BAT, FactoMineR, FD
	Functional diversity metrics computation	adiv, cati, BAT, FD, FDiversity, funrar, hillR, TPD
7. Validate and interpret the results	Model fit	bmrs, lme4, nlme, glmmTMB, MCMCglmm, mgcv, lavaan, piecewiseSEM, randomForest
	Cross-validation, bootstrapping and jackknifing	CrossValidate, cvTools, bootstrap
	Data visualization	
8. Ensure reproducibility	Cite the packages above!	*base::citation()*

Box 1. Species detectability and functional diversity estimation

Perfect detection of organisms is rare, often resulting in false species absences or the underestimation of population sizes and biodiversity. Such ‘missed detections’ have significant impact on estimates of functional diversity, though the magnitude and the direction of the impact will depend on several factors. Detectability of functional diversity (*sensu* Jarzyna & Jetz, 2016) results from i) the interaction between the type of functional diversity metric, ii) whether and how species detectability is linked to their functional distinctiveness or certain trait characteristics (including trait resolution), iii) how detectability varies along spatial and environmental gradients, iv) the proportion of undetected species at a site, v) the size of the regional species pool, and vi) the spatial scale (Jarzyna & Jetz, 2016; Palacio et al., 2020).

Recent advances in statistical modelling allow accounting for species’ imperfect detection. Specifically, multispecies occupancy (Iknayan et al., 2014; Denes et al., 2015) and *N*-mixture (Gomez et al., 2018) models allow for estimation of the ‘true’ probability of each species occurrence or for their detection-corrected abundance, which can then be incorporated into functional diversity estimates (Jarzyna & Jetz, 2016; Palacio et al., 2020). Multispecies occupancy and *N*-mixture models can be fitted in either a frequentist or a Bayesian framework (Devarajan et al., 2020). Avoiding excessive detail, if models are fitted in a Bayesian framework that relies on Markov Chain Monte Carlo (MCM) sampling, as opposed to Integrated Nested Laplace Approximations (Rue et al., 2009), it is advised to report initial values for parameter estimation, prior distributions, the number of Markov chains and iterations per chain, burn-in, the thinning parameter, convergence evaluation, and a summary of posterior estimates (e.g., occurrence and detection probabilities).

Box 2. Missing data and data imputation

Because encountering species in the field and measuring relevant traits can be difficult, trait matrices often contain missing data, which can be randomly distributed or not (Nakagawa & Freckleton, 2008). Missing data need to be dealt with in order to compute virtually any method for estimating functional diversity. Three main options are available: (1) omit the individuals/species for which trait data are missing, (2) impute the missing trait data, and (3) convert the trait matrix using a distance measure that allows the presence of missing data (e.g., Gower distance; de Bello et al., 2021b). If omission is the selected strategy, the consequences of removing observations linked to missing trait data should be understood and discussed. Alternatively, one might use imputation methods (Penone et al., 2014; Taugourdeau et al., 2014; Johnson et al., 2021), which are roughly based on two strategies: (1) replacing the missing value with a systematically chosen value from the phylogenetically/functionally most similar species; or (2) predicting the missing trait value, e.g., based on linear models (potentially including a phylogenetic covariance structure; Johnson et al., 2021) or Principal Component Analysis (Podani et al., 2021), where traits are estimated as a function of other variables. Depending on whether the missing data are random or not, different algorithms should be considered for the imputation (Wulff & Jeppesen, 2017). Finally, some simply use ‘average imputation’ (e.g., Kralj-Fišer et al., 2020), calculating the mean or median of the values for that trait based on all the non-missing observations. This has the advantage of keeping the same mean and the same sample size but many disadvantages, and thus we discourage this strategy (Taugourdeau et al., 2014; see also Denny, 2017 for a theoretical discussion).

Figure legends

Figure 1. A) Annual number of published papers using the term 'functional diversity' compared to 'phylogenetic diversity' and 'taxonomic diversity'. **B)** Number of papers using the three terms relativized to the total annual number of published papers, to account for the general growth in scientific literature volume in recent years (Landhuis, 2016). The number of papers was sourced from the Web of Science (Clarivate Analytics) on 10 June 2021, using the queries: TS = 'functional diversity', TS = 'phylogenetic diversity', and TS = 'taxonomic diversity'. The total number of papers published in each year is based on the Dimensions database, accessed on 12 January 2021.

Figure 2. Workflow of the eight-steps protocol proposed in this study. Animal silhouettes retrieved from Phylopics—with open license.

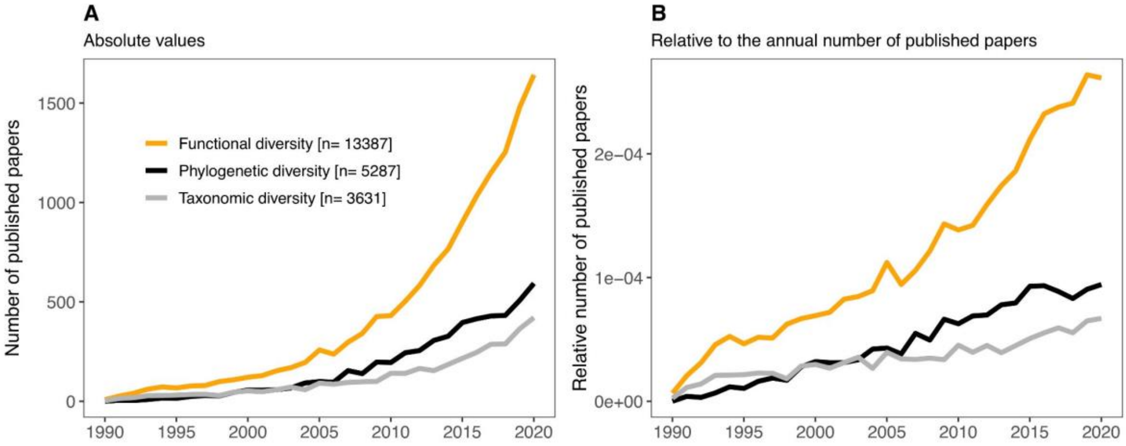
A
Absolute values
Number of published papers
0
500
1000
1500
1990
1995
2000
2005
2010
2015
2020
Functional diversity [n= 13387]
Phylogenetic diversity [n= 5287]
Taxonomic diversity [n= 3631]
B
Relative to the annual number of published papers
Relative number of published papers
0e+00
1e−04
2e−04
1990
1995
2000
2005
2010
2015
2020

Figure 1

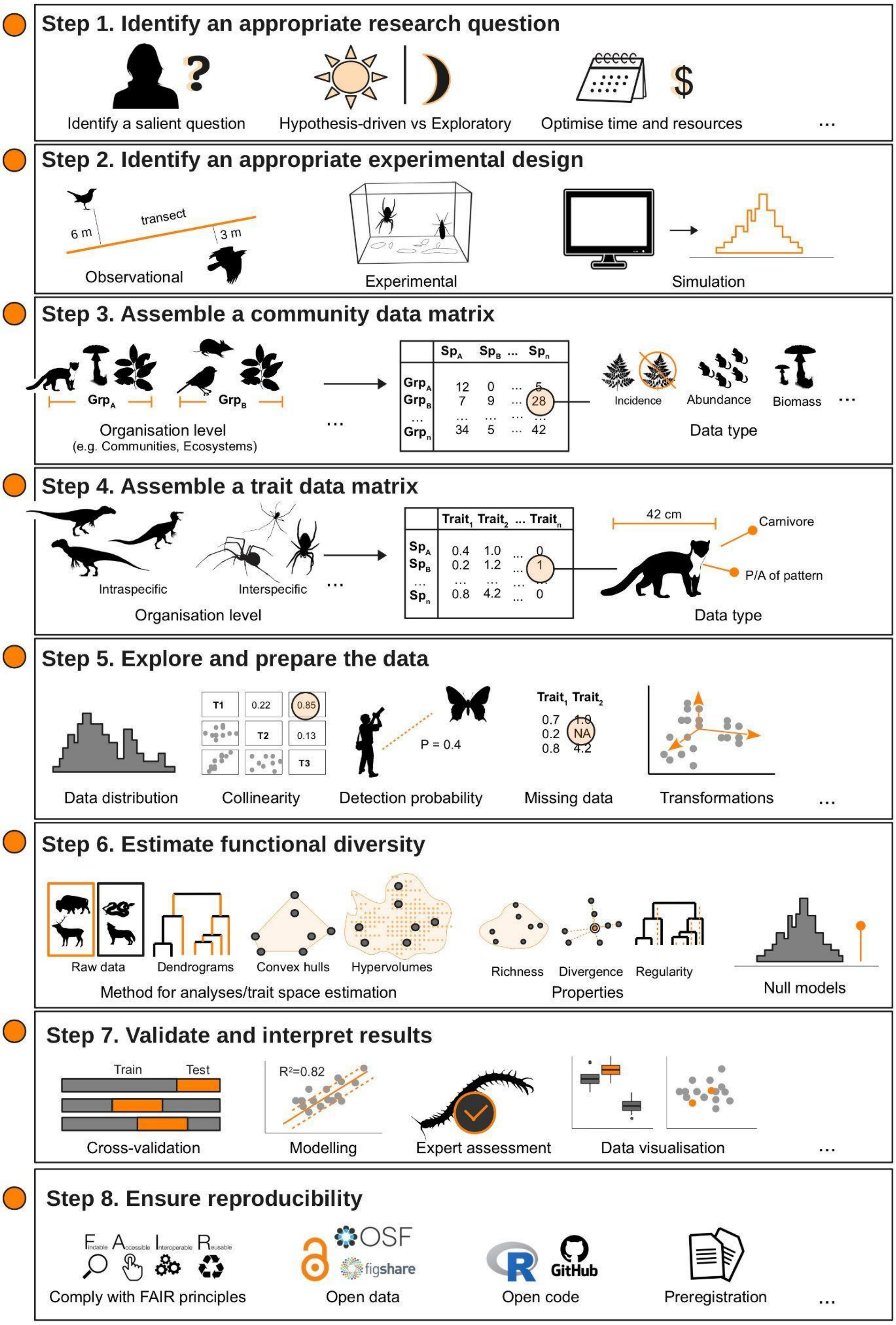
Step 1. Identify an appropriate research question
Identify a salient question
Hypothesis-driven vs Exploratory
Optimise time and resources
...
Step 2. Identify an appropriate experimental design
6 m
transect
3 m
Observational
Experimental
Simulation
Step 3. Assemble a community data matrix
Grp$_A$
Grp$_B$
Organisation level
(e.g. Communities, Ecosystems)
...
Sp$_A$ Sp$_B$ ... Sp$_n$
Grp$_A$ 12 0 ... 5
Grp$_B$ 7 9 ... 28
Grp$_n$ 34 5 ... 42
Incidence
Abundance
Biomass
...
Data type
Step 4. Assemble a trait data matrix
Intraspecific
Interspecific
...
Organisation level
Trait$_1$ Trait$_2$ ... Trait$_n$
Sp$_A$ 0.4 1.0 ... 0
Sp$_B$ 0.2 1.2 ... 1
Sp$_n$ 0.8 4.2 ... 0
42 cm
Carnivore
P/A of pattern
Data type
Step 5. Explore and prepare the data
Data distribution
T1 0.22 0.85
T2 0.13
T3
Collinearity
P = 0.4
Detection probability
Trait$_1$ Trait$_2$
0.7 1.0
0.2 NA
0.8 4.2
Missing data
Transformations
...
Step 6. Estimate functional diversity
Raw data
Dendrograms
Convex hulls
Hypervolumes
Method for analyses/trait space estimation
Richness
Divergence
Regularity
Properties
Null models
Step 7. Validate and interpret results
Train
Test
Cross-validation
R^2=0.82
Modelling
Expert assessment
Data visualisation
...
Step 8. Ensure reproducibility
Findable Accessible Interoperable Reusable
Comply with FAIR principles
OSF
figshare
Open data
R
GitHub
Open code
Preregistration
...

Figure 2