










PERSPECTIVE

Facilitating comparable research in seedling functional ecology

Daniel E. Winkler¹  | Magda Garbowski²  | Kevin Kožič³  | Emma Ladouceur^{4,5,6}  |
Julie Larson⁷  | Sarah Martin^{4,6} | Christoph Rosche^{3,4}  | Christiane Roscher^{4,6}  |
Mandy L. Slate^{8,9}  | Lotte Korell^{4,10} 

¹U.S. Geological Survey, Southwest Biological Science Center, Tucson, Arizona, USA; ²University of Wyoming, Laramie, Wyoming, USA; ³Institute of Biology/Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany; ⁴German Centre for Integrative Biodiversity Research (iDiv) Leipzig-Halle-Jena, Leipzig, Germany; ⁵Institute of Computer Science, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany; ⁶Helmholtz Centre for Environmental Research, Department Physiological Diversity, UFZ, Leipzig, Germany; ⁷USDA Agricultural Research Service, Eastern Oregon Agricultural Research Center (EOARC), Burns, Oregon, USA; ⁸Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado, USA; ⁹Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, Ohio, USA and ¹⁰UFZ, Helmholtz Centre for Environmental Research, Department Community Ecology, Halle (Saale), Germany

Correspondence

Daniel E. Winkler

Email: dwinkler@usgs.gov

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Abstract

1. Ecologists have worked to ascribe function to the variation found in plant populations, communities and ecosystems across environments for at least the past century. The vast body of research in functional ecology has drastically improved understanding of how individuals respond to their environment, communities are assembled and ecosystems function. However, with limited exceptions, few studies have quantified differences in plant function during the *earliest* stages of the plant life cycle, and fewer have tested how this early variability shapes populations, communities and ecosystems.
2. Drawing from the literature and our collective experience, we describe the current state of knowledge in seedling functional ecology and provide examples of how this subdiscipline can enrich our fundamental understanding of plant function across levels of organisation. To inspire progressive work in this area, we also outline key considerations involved in seedling functional research (who, what, when, where and how to measure seedling traits) and identify remaining challenges and gaps in understanding around methodological approaches.
3. Within this conceptual synthesis, we highlight three critical areas in seedling ecology for future research to target. First, given wide variation in the definition of a 'seedling', we provide a standard definition based on seed reserve dependence while emphasising the need to measure ontogenetic variation more clearly both within and following the seedling stage. Second, studies demonstrate that seedlings can be studied in multiple media (e.g. soil, agar, filter paper) and conditions (e.g. field, greenhouse, laboratory). We recommend that researchers select methods based on explicit goals, yet follow standard guidelines to reduce methodological noise across studies. Third, research is critically needed to assess the

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implications of different methodologies on trait measurement and compatibility across studies.

4. By highlighting the importance of seedling functional ecology and suggesting pathways to address key challenges, we aim to inspire future research that generates useful and comparable data on seedling functional ecology. This work is critical to explain variation within and among populations, communities and ecosystems and integrate this most vulnerable stage of plant life into ecological frameworks.

KEYWORDS

functional traits, plant ontogeny, recruitment, seedling, sporophyte

1 | INTRODUCTION

The seedling stage represents one of the most vulnerable and elusive periods of the plant life cycle (Leck et al., 2008). Seedling recruitment can be one of the greatest bottlenecks to population growth (e.g. Eriksson & Ehrlén, 2008) and determinants of conservation and restoration success (e.g. Shackelford et al., 2021). However, despite their outsized importance, small sizes and short developmental timescales of seedlings can make it challenging to measure function and performance. Changes in temperature, water and herbivory pressure can rapidly impact seedlings, making it difficult to pinpoint when and why mortality occurs *in natura*. Further, seedling growth strategies can vary ontogenetically (i.e. at discrete stages of seedling development; Boege & Marquis, 2005; Moles & Leishman, 2008; Garbowski et al., 2021) and respond to abiotic and biotic factors in the surrounding environment (Kitajima & Fenner, 2000). Despite this understanding, our ability to successfully predict seedling dynamics in non-model systems, including most ecological systems and systems in disequilibrium (e.g. disturbed sites), remains limited.

Studying seedling functional traits (i.e. measurable attributes that influence seedling growth, survival and fitness) may improve prediction in multiple ecological and evolutionary subdisciplines. For example, seedling leaf and root traits have been shown to interact with selection pressures and explain variation in recruitment success (e.g. Daws et al., 2007; Harrison & LaForgia, 2019; Khurana & Singh, 2001; Krannitz et al., 1991; Moles & Westoby, 2004). Emerging evidence suggests that a functional perspective on seedling ecology has the potential to shed light on climate change mitigation (e.g. Lewandowski et al., 2021; Walck et al., 2011), restoration and conservation of species and ecosystem services (e.g. Havrilla et al., 2021; Larson et al., 2015) and the eco-evolutionary dynamics of plant life (e.g. Razzaque & Juenger, 2022; Simons, 2009). However, relative to the wide availability of trait data measured from adult plants in global trait databases (e.g. Kattge et al., 2020), there remains a dearth of data and information on seedling traits for most species (Saatkamp et al., 2019).

Practical and methodological challenges associated with studying seedling dynamics may contribute to there being limited

functional trait ecology research on seedlings, particularly for anything other than tree species. The ease of studying and measuring seedlings is likely determined by multiple, interacting research challenges, including access to appropriate experimental resources (e.g. Casad et al., 2021; Cooper & Berry, 2020), existing knowledge about target species (e.g. Buhk et al., 2007) and perhaps most obviously, the small size and relative delicateness of the organisms being studied (e.g. Tang et al., 1992). Because of these challenges, there has been limited research on the functional traits of seedlings outside of agricultural systems (e.g. Cooper & MacDonald, 1970) and model plant species (e.g. Eastmond et al., 2015). While the number of studies on seedling function has been increasing steadily over the past 20 years (Figure 1), the vast majority has been directed towards trees, with only a small portion of seedling trait research centered on herbaceous and woody shrub plant species (26.87%, Appendix 1a).

Our objective is to advance seedling functional ecology research by communicating the value of these data, streamlining decision-making in research design and providing insight on the numerous considerations and challenges to be addressed as this field takes off. First, we define and describe the term seedling and the botanical and ecological characters used to describe seedlings. We then describe why seedling studies are essential to functional ecology and the critical importance of comparable and standardised methods in seedling research. At last, we provide an overview of major steps in the research process to consider when designing a seedling trait study. In doing so, we provide numerous experimental considerations that can influence the outcome of seedling studies and likely mediate establishment trajectories. For example, we explain important ecological and evolutionary considerations when sourcing seeds and describe the environmental and experimental conditions typically required to answer research questions focused on seedling traits. We also define important ontogenetic considerations and approaches to measuring seedlings through time and describe the importance of ontogeny and trait selection. We describe different experimental approaches, their applications and limitations with examples from the existing seedling trait literature. We focus primarily on ecological studies but leverage important insights from evolutionary biology

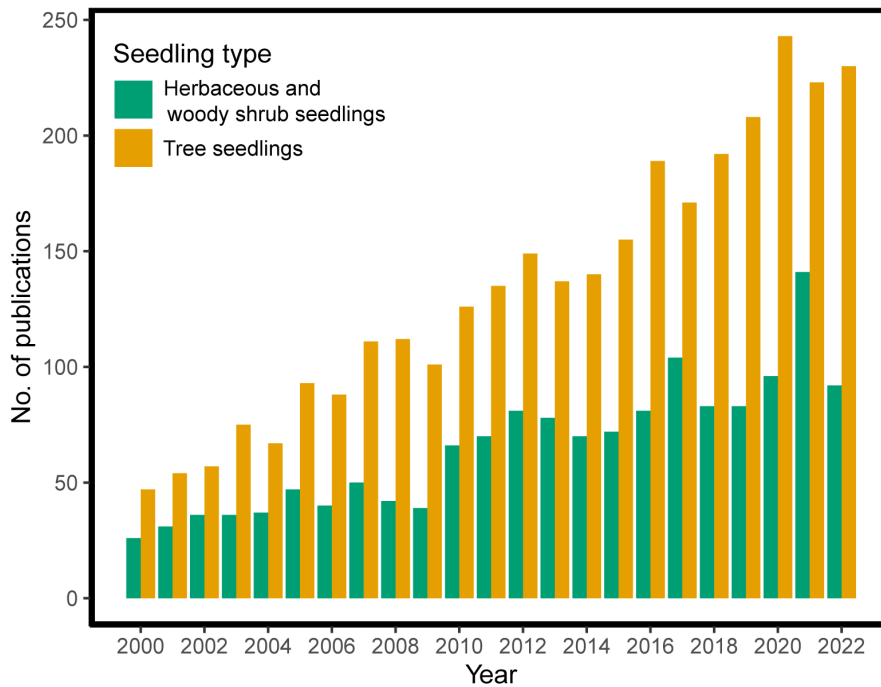


FIGURE 1 The annual number of publications examining herbaceous and woody shrub seedling traits (green bars) vs. tree seedling traits (orange bars) based on a Web of Science search for the period 2000–2022. We excluded agricultural and bioengineering-related seedling studies from our search criteria and identified a total of 5638 articles that included seedling traits across ecological and evolutionary subdisciplines. Full Web of Science search parameters are described in [Appendix 1a](#).

and agricultural science. While experimental design decisions for each study will be determined based on goals of the experiment, we aim to guide future work along established and comparable methodological pathways. With a common vision, the growing literature on seedling functional ecology will be primed to provide more robust insight and solutions to global challenges during the Anthropocene.

2 | DEFINING THE TERM “SEEDLING”

A key challenge in seedling functional ecology is to establish a common and clear definition for the seedling stage, as there are multiple ways seedlings have been defined in the literature. While this stems from practical considerations, lack of clear definitions can lead to confusion about which life stages are being studied, and whether they are comparable across studies. In a general botanical definition, a seedling is a young sporophyte that develops out of a plant embryo from a seed (Leck et al., 2008). In a narrower biological definition, the seedling stage of a plant's life cycle begins when the radicle, or embryonic root, emerges from a seed and develops into a primary root (Figure 2). This initial growth is fueled by seed-based resources contained within the endosperm or cotyledons (i.e. the portion of tissue in the seed that is typically rich in starch, oil and protein used for early growth; Bidlack et al., 2021; Mašková & Herben, 2021). Although there are a wide range of seedling morphologies (e.g. Leck et al., 2008; Tillich, 2007), embryonic shoot or leaf tissue also typically emerges around this time (e.g. as the coleoptile in many monocots, or as the cotyledons or epicotyl in many dicots) and begins to photosynthesise. At this point, the plant is no longer entirely dependent on nutrients in the endosperm or cotyledons but is still considered a seedling until seed energy reserves are fully exhausted (at

which point the plant technically enters the juvenile stage; Hanley et al., 2004).

Given that both seed energy reserves and soil resources influence growth and survival after a seedling emerges above the soil surface (e.g. Hewitt et al., 1954; Krannitz et al., 1991), it is no surprise that the duration of the seedling stage can vary among species, plant functional types (e.g. grasses vs. forbs) and environments, ranging from days to months (e.g. Green & Juniper, 2004; Leck et al., 2008; Peterson et al., 1989; Rodin, 1953) with an endpoint that can be difficult to discern (but see Hanley et al., 2004). In ecological sciences, the term seedling is even used more broadly and, quite often, seedling is a catchall term for any young plant (e.g. a one-year old shrub or a five-year old tree; Antos et al., 2005; Davidson et al., 2019). Thus, a seedling is not easily definable in terms of absolute plant age (e.g. 3 weeks or 2 months), but can be more clearly defined in relation to a distinct morpho-physiological life stage.

We advocate that the term seedling should be used to describe the period during which a developing plant is still dependent on seed energy reserves. Whenever feasible, studies should aim to qualify the seedling stage by the continued presence of seed storage structures (e.g. cotyledons or seed endosperm are still visibly intact), with their disappearance indicating the end of the seedling stage. However, given that it is difficult to determine the exact timepoint when seed reserves are depleted (see Fenner, 1987; Leck et al., 2008; for in-depth discussion), we suggest researchers target an alternative indicator of the seedling stage that is more conservative, but still enables comparability across species and studies. Specifically, when it is not feasible to determine when seed reserves are depleted, we recommend that seedling studies use the maturation of the first true foliage leaf (or pair of leaves)—and consequent beginning of autotrophy—as a broadly applicable indicator of the end of the seedling stage. In practice, this means that studies of seedling

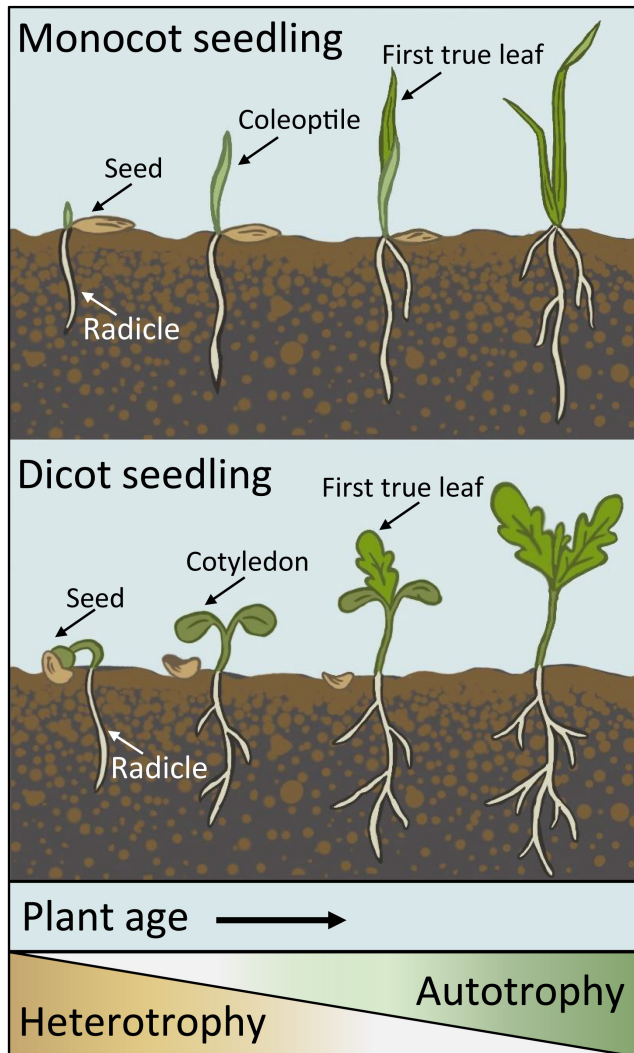


FIGURE 2 Seedling growth begins with the emergence of a radicle and ends when resources within the seed endosperm are depleted. A timing that roughly corresponds with the unfolding of the first true leaf in monocot (upper panel) and dicot species (lower panel). The vulnerability of seedlings as they transition from heterotrophy to autotrophy is discussed in the common methodologies section below.

traits should occur prior to the expansion of the second true leaf, with traits collected after this period more appropriately described as juvenile traits.

3 | STUDYING SEEDLINGS WITH COMMON METHODOLOGIES

The regeneration niche defines the environmental requirements (i.e. the set of abiotic and biotic filters) that enable successful growth and establishment during the seed and seedling stages of the life cycle of a plant (Grubb, 1977; Harrison & LaForgia, 2019; Larson & Funk, 2016). Importantly, the regeneration niche of seedlings can strongly diverge from the environmental conditions adult plants

need for growth and survival despite their role in maintaining population stability and plant biodiversity (e.g. Poorter, 2007). These differences make it critical to understand the conditions that interact with seed and seedling function to drive successful plant regeneration.

Once a seed has arrived at a site, key environmental factors for successful germination include the availability of microsites where small-scale spatial variation in resources and disturbance create suitable space and conditions (Eriksson & Ehrlén, 1992). Germination is often also synchronised with temporal changes in the environment and triggered by seasonal alterations in temperature, day length and/or water availability (Baskin & Baskin, 1998), all of which can impact the timing of seedling emergence and conditions experienced by seedlings (Donohue et al., 2010). Once germination has occurred, access to sufficient resources (i.e. light, water and nutrients) are essential for the seedling's transition to autotrophy (Saatkamp et al., 2019), which makes the period of seedling emergence and establishment highly vulnerable to changes in environmental conditions (e.g. Larson et al., 2020, 2021). Apart from resource limitations (e.g. drought), important biotic filters can also have an outsized impact during seedling emergence and establishment, including herbivory and pathogen infection (Barton & Hanley, 2013; Larson & Funk, 2016; Moles & Westoby, 2004; Wagner & Mitschunas, 2008). Although aspects of the regeneration niche have been studied in some species and systems, we have not yet achieved a cohesive understanding of how the seedling stage differs across niche strategies and likely influences ecological dynamics across systems.

Functional ecology and functional traits provide a pathway to understand aspects of diversity in plant life beyond taxonomic identity and lend themselves to cross-system comparisons and understanding (e.g. Reich et al., 2003; Violle & Jiang, 2009). Syntheses of trait-based research are beginning to increase general understanding around these aspects of diversity by quantifying trends and variability across studies (e.g. Hanisch et al., 2020; Kraft et al., 2015; Siefert et al., 2015). However, meta-analyses or syntheses are only possible if appropriate amounts of quantitative data are available employing similar methods with standardised measurements (Gerstner et al., 2017; Gurevitch et al., 2018; Koricheva et al., 2013). Since seedling functional ecology is a young field, there is an invaluable opportunity to set these standards. Rather than suggesting a single 'best' research design, we advocate for three major actions: (1) Consider working within the parameters of a common set of approaches (described below), making decisions intentionally and explicitly based on research goals. (2) Record robust metadata to allow future assessments of study comparability (see Appendix 2 for recommended metadata in seedling experiments). (3) Pursue qualitative and quantitative methods for evaluating transferability (Spake et al., 2022) to understand how different experimental choices affect outcomes. An open science approach that is transparent across seedling studies can enable and support future knowledge generation, application and overall scientific progress, following suit with other subfields of ecology (Nakagawa et al., 2020).

Once a research question is clearly defined, most researchers will need to evaluate four major methodological questions (Figure 3)

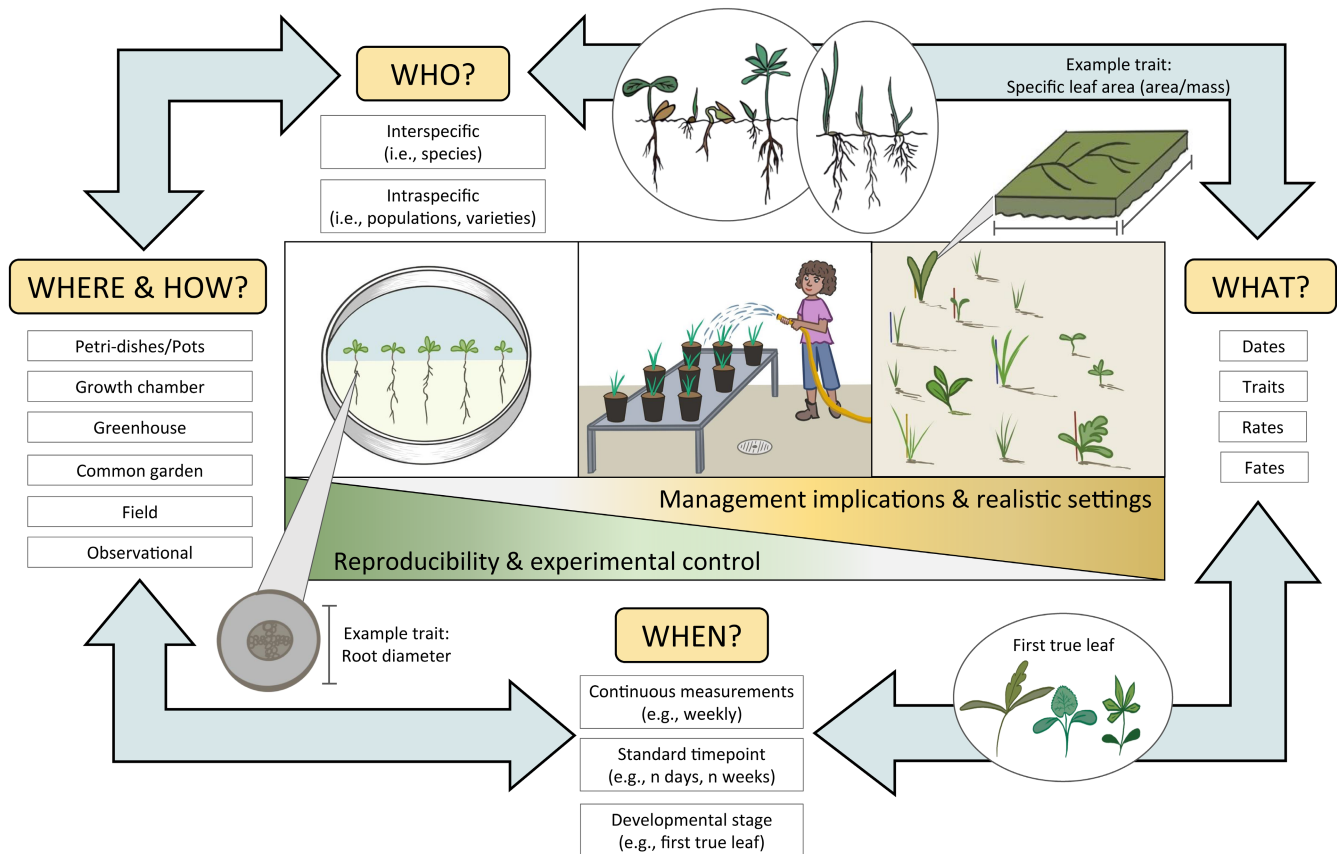


FIGURE 3 The seedling trait ecology research cycle, generalised to illustrate the who, what, when, and where and how to design a seedling trait study. Arrows pointing in either direction highlight that the cycle can start at any of these steps and proceed in either direction. Specific leaf area, or often specific cotyledon area, and root diameter are examples of traits that can be studied at the seedling stage. Additional traits that are often studied are included in Table 1 and example experimental conditions are included in Table 2.

to design an appropriate study: (1) “who” is being studied? (e.g. is the research question focused on intra- or interspecific variation in seedlings and what is the best method to capture variation that best answers the question?); (2) “what” is being studied? (e.g. measuring phenological traits like emergence timing or physiological traits like respiration rate?); (3) “when” is it being studied? (e.g. will seedling trait measurements occur at set time points or developmental stages, such as at emergence, once the first true leaf develops, or at several points through time?); and (4) “where and how” is it being studied? (e.g. will the study take place in a greenhouse or common garden?). The research cycle illustrated and described in Figure 3 is generalised and, although we recommend beginning with a clearly defined question that begins at “who?”, it is possible to begin anywhere in the diagram and use it as a guide to develop any seedling experiment, refining questions and methods in the process.

4 | WHO?—SEEDS USED TO STUDY SEEDLINGS

During question development, a major first step is to consider “who” (i.e. which species, populations and/or individuals) are the key subjects and how seeds will be sourced for a seedling study. Most trait

studies begin by identifying the relative importance of intra- versus interspecific variation in the research question, and consequently, whether it is important to design a study which maximises variation within species (e.g. replicate individuals or populations) or across species and communities (e.g. more replicate species). While these are also critical questions for seedling studies, there are additional considerations unique to the seedling stage that will determine which seeds to use in an experiment. The seeds chosen and how they are handled and stored before the start of an experiment can influence the seedling trait variation observed—sometimes in unintentional ways that may obscure the intra- or interspecific patterns a study seeks to evaluate. Because of this, an understanding of the tight connection between seeds to seedlings is an important place to start.

Seed-plant diversification has multiplied exponentially since the evolution of seeds ca. 370 million years ago (Linkies et al., 2010; Onstein, 2020) and includes many strategies that can be explored in a seedling trait study. Seeds are influenced by maternal effects, whereby the genotype, phenotype and environment of the maternal plant influences the phenotype of offspring (i.e. individual seeds; Boyd et al., 2007). Maternal effects are often studied as a type of plasticity influencing trait expression in seeds and seedlings, and they can facilitate adaptation to a new environment or

protect offspring against unfavourable conditions (Sultan, 2000). Accounting for maternal effects is especially important in seedling studies, as trait variation may be attributed or explained by maternal effects rather than experimental treatments or questions being asked (e.g. Alexander & Wulff, 1985; Boyd et al., 2007).

Potential maternal effects should be considered when selecting seed sources for seedling studies—especially when research questions are related to adaptation and variation within populations or species. The broader origin of seeds (i.e. source population), the source of seeds (e.g. wild collected vs. commercially produced), the provenance of seeds (i.e. location collected) and seed storage methods, among other factors, can each affect traits typically measured in ecology studies (De Vitis et al., 2020; Erickson & Halford, 2020; Pedrini et al., 2020) and are important considerations for seedling research and reported metadata. For example, population genetics can affect study outcomes from both wild collected seeds and from those commercially multiplied (Conrady et al., 2023; Durka et al., 2017). Seed storage methods and durations can also alter viability, emergence timing and overall seedling vigour (De Vitis et al., 2020).

It is also important to recognise that seed germination is often naturally low and highly variable within and across wildland species, populations and functional groups (Fenner, 2000). Unknown germination characteristics can often be a barrier to studying or using particular species in research (Ladouceur et al., 2018), and for practical and pragmatic use (Jiménez-Alfaro et al., 2020; Segar et al., 2022). Thus, establishing an experimental design and monitoring approach that allows for low, slow and variable germination, and accounting for multiple sources of variation, is important for any study utilising native seeds (e.g. Kildisheva et al., 2020). Knowing the germination characteristics of study species is essential for successful seedling experiments, but also for interpretation of experimental results. As a starting point, it is helpful to test seed viability (via X-ray or tetrazolium viability test) to anticipate the potential rate of germination success, and to know species dormancy type and dormancy-breaking cues to encourage germination (Marin et al., 2017). Stratification, scarification or different seed treatments are crucial to break dormancy for many species, and these treatments can be applied before experiments to increase germination success (Baskin & Baskin, 1998). Additionally, accounting for unpredictable and variable germination rates, and causally diagnosing low germination rates (e.g. potentially low viability) is key to making substantive inferences about the seedling stage of plant life (Frischie et al., 2020). We recommend screening seed germination characteristics in advance of seedling trait trials to address these challenges, particularly when coordinated timing is critical for the experiment (e.g. having a standard number of seedlings germinating on the same day, across species).

Given the potential influence of seed origin, condition and germination characteristics for seed success and seedling trait expression, future seedling research should provide as much metadata as possible regarding seed source, age, storage, location, viability and germination treatments (see Appendix 2). Careful reporting of study

metadata will facilitate cross-study comparisons and may be critical to explain variation in future research and synthesis questions (e.g. species seeds collected from wild vs. domestic populations; stored seeds vs. freshly collected seeds, etc.).

5 | WHAT?—SEEDLING TRAITS TO MEASURE?

What traits are measured will critically influence the ability to draw inferences in relation to the research question (the “what?” in Figure 3). Plant functional traits include morphological, physiological or phenological characteristics that influence fitness through their links to performance (Violle et al., 2007). In seedlings, traits are only as explanatory as their functional ties to the local environmental and biotic drivers that mediate seedling emergence and establishment. Reviews and methodological handbooks that describe key traits in adult plants provide a starting point, as many of these traits have functional analogs in seedlings (e.g. Freschet et al., 2021; Funk et al., 2017; Perez-Harguindeguy et al., 2016). For example, similar to leaves, seedling cotyledons that are thin and have high photosynthetic area per mass (analogous to high specific leaf area) may be linked to photosynthetic activity and shade tolerance (e.g. Wang & Zhou, 2022). We provide a non-exhaustive list of seedling traits that have been explored in some capacity (Table 1) but note that there are relatively few tests of trait functionality in seedlings. From our experience, morphological characteristics are the functional traits commonly measured. In many cases, correlationally assigned function of seedling morphological traits is based on the functional significance of that trait in adult plants. New research in this area will continue to expand and reshape this list of traits and expected functions moving forward.

When refining research questions and deciding what traits to measure, researchers should start by considering which environmental or biotic filters are most likely to influence seedling performance in the study system of interest. Different traits may alter exposure to-, tolerance of-, or escape from different drivers (e.g. Fernández-Pascual et al., 2021; Gardarin et al., 2016; Larson et al., 2015; Wang et al., 2018). For example, timing of germination or emergence may impact whether seedlings are exposed to (or avoid) freezing, drought, or competition (Moles & Westoby, 2004), while early leaf (or cotyledon) and root traits may impact how seedlings tolerate hazards, resource limitation, or biotic factors like soil pathogens or herbivory (e.g. Wagner & Mitschunas, 2008). Since seedling emergence and establishment success may depend on access to above- or below-ground resources over relatively small spatial scales, seedling growth metrics like rooting depth, height, or root- and shoot elongation rates may also be informative (Garbowski et al., 2020; but see Harrison & LaForgia, 2019).

Several challenges must be acknowledged in selecting, measuring and utilising seedling traits. Given the relative lack of testing around seedling trait functionality, evidence to help differentiate between related traits (e.g. root depth, length or elongation rate)

TABLE 1 A non-exhaustive list of seedling functional traits extracted from previously published work, their purported functional significance in seedlings, and the critical gaps or questions to address with these example traits. We highlight these traits because of their frequency of use and their association (even if correlational) with a described ecological function. However, we do not include caveats or cases where trait associations were evaluated but not found. More research is needed to explicitly evaluate the function of seedling morphological traits. Additionally, seedling physiological traits are rarely included in seedling studies. For the most part, this is because the methods for measuring the same traits in adult plants may not be transferable to seedlings. References cited in this table are available in [Appendix 1b](#).

Trait	Functional significance in seedlings	Critical gaps or questions to address with seedling traits
<i>Morphological traits</i>		
Specific leaf area ^{2,3,10,12-14}	Productivity; competitive strategies; drought strategies; shade strategies	Is specific cotyledon area functionally similar to specific leaf area? At the earliest ontogenetic stages (e.g. <1 week), is it appropriate to compare the first true leaf in grasses to cotyledons in forbs? How do we account for ontogenetic shifts in this trait?
Leaf or root nitrogen content ^{23,24}	Establishment, herbivore interactions, plant-soil interactions	Is there a correlation with stored N in seeds, and how long does it persist through ontogeny? Is tissue N correlated to seedling growth rate (as suggested in mature plants)? Traits are rarely included in seedling trait studies despite evidence of functional significance in adults; alternative methods may be required to obtain enough material for chemical analysis (e.g. pooling of samples)
Rooting depth ^{5,20}	Establishment; drought strategies	Is seedling root length or root elongation rate a reliable proxy for seedling root depth? Up to what ontogenetic stage?
Specific root length ^{1,7}	Drought strategies	Is this trait negatively correlated with microbial colonisation and symbiosis in seedling roots (as demonstrated in the 'collaboration gradient' among mature plants)? Unlike mature plants, it is not feasible to separate 'fine' and 'coarse' root fractions (trait summarised across the entire root system)
Root diameter ^{6,9,24,25}	Drought strategies; soil penetration; competitive strategies, plant-soil interactions	Is this trait positively correlated with microbial colonisation and symbiosis in seedling roots (as demonstrated in the 'collaboration gradient' among mature plants)? Unlike mature plants, it is not feasible to separate 'fine' and 'coarse' root fractions (trait summarised across the entire root system)
No. of root tips or forks ^{3,6,17}	Competitive strategies; establishment	Are architectural traits better predictors than root morphological traits? Given that most evidence comes from within-species comparisons, are traits also predictive across species?
Tissue dry matter content (total, root, or leaf) ^{1,2,3,7,12}	Competitive strategies; drought strategies	Given evidence of ontogenetic shifts in these traits, how should we account for age-variation in experiments and analyses? Are these traits correlated to tissue longevity and herbivory (as suggested in mature plants)? Is seedling dry matter content (g dry mass:g fresh mass) functionally interchangeable with tissue density (g dry mass cm ⁻³)?
Biomass allocation (root: shoot) ^{6-8,10,21,23}	Drought strategies; competitive strategies; compensatory growth; shade strategies	With mixed evidence across studies, what contextual factors (e.g. ontogeny, environment) mediate the influence of biomass allocation on seedling performance?
<i>Physiological traits</i>		
Photosynthetic rate ^{4,8}	Resource use efficiency; herbivore interactions	Seedlings are often too small to measure photosynthetic rates of individual leaves; Can available methods of placing entire seedlings in photosynthesis chambers also provide insight into stomatal conductance and other physiological traits?
Photosynthetic nitrogen use efficiency ¹⁴	Competitive strategies	How should resource use and water use efficiency be assessed? Can nutrient availability explain seedling function during establishment?
Respiration rate ⁴	Resource use efficiency	Can physiological drought tolerance be approximated in seedlings as in mature plants (e.g. by tissue osmotic potential or stable isotope ratios)?
<i>Phenological traits</i>		
Germination or emergence timing in natural settings (e.g. day of year) ^{3,16,18}	Establishment; drought strategies	How much variation exists within and among species, seed lots (seeds collected from a population of plants) and environments? Are germination and emergence phenology correlated with each other, and with later stages of development (e.g. timing of first true leaf)?

TABLE 1 (Continued)

Trait	Functional significance in seedlings	Critical gaps or questions to address with seedling traits
Germination or emergence speed in controlled conditions (e.g. days to germination, thermal times) ^{19,22}	Establishment; drought strategies; germination patterns in field settings	Are germination timing metrics measured in the lab reliable indicators of seedling emergence dynamics in the field?
<i>Performance/Growth traits</i>		
Plant height ^{5,13,19}	Competitive strategies; drought strategies	Is plant height functionally similar to relative growth rate in seedlings? Does seedling height relate to maximum adult height?
Relative growth rate (RGR) ^{1,10-13}	Competitive strategies; shade strategies	Are growth metrics like seedling relative growth rate more informative than easier-to-measure metrics like seedling biomass?

is often limited, and it is unclear whether inferences of trait functionality in adult plants translate to those in seedlings. Researchers will need to address these critical knowledge gaps around trait functionality (along with other questions identified in Table 1) to build a body of evidence that will streamline trait selection moving forward. Once traits are selected, the small size of seedlings could still limit what traits are possible to measure—from photosynthetic activity (requiring specialised chambers) to biomass (requiring ultra-sensitive microbalances). We recommend that researchers draw on methodologies from example references (including from model- and agricultural systems; Table 1) and continue developing, testing and publishing new approaches that make seedling traits accessible.

Finally, when selecting traits, researchers should simultaneously consider how they will test the functionality of traits, and select those that are relevant at the same spatiotemporal scale as the environmental and biotic factors being studied. For example, if the aim of a seedling study is to test traits with hypothesised links to light or water limitation, we recommend manipulating or measuring these environmental factors on a scale that is relevant to seedling structures (e.g. root length over cm rather than m of soil; seedling response over hours or days rather than months)—otherwise, the seedling traits selected are unlikely to explain variation in environmental response.

6 | WHEN?—ONTOGENETIC CONSIDERATIONS IN SEEDLING TRAIT STUDIES

In plant biology, ontogeny refers to the process of plant development through a series of discrete life stages: seeds, seedlings, juveniles and adults (Barton & Boege, 2017). Identifying the beginning and end of each stage can be difficult since changes associated with specific stages are often gradual and influenced by plant size or age. Further, functional traits may differ substantially within (e.g. Garbowski et al., 2021; Havrilla et al., 2021) and between stages (e.g. Mason et al., 2013). For example, most evidence suggests that seedlings shift trait values from acquisitive to more conservative

as they grow and develop (e.g. Garbowski et al., 2021; Havrilla et al., 2021; Mason et al., 2013). This further complicates our ability to make ontogenetically informed comparisons within and among species.

To explicitly account for ontogenetically driven changes in seedling traits, researchers can adopt two distinct but complementary approaches. The first is to measure traits and compare species, populations, or varieties at a specific time point such as *n*-days post emergence (the “when?” in Figure 3; e.g. Larson et al., 2020; Garbowski et al., 2021). This approach allows researchers to compare groups based on time, with the understanding that variation in trait values may be influenced by differences in phenology or growth rate. Alternatively, to reduce variation resulting from differences in growth rate or phenology, traits can be measured and groups can be compared at specific developmental stages (e.g. emergence time of the first true leaf; Mason et al., 2013).

7 | WHERE AND HOW—SEEDLING STUDIES IN THE LAB OR FIELD

Environmental conditions and their influence on seedling performance and trait expression may differ substantially depending on how experiments are set up. This makes it critical to consider and describe environmental drivers and experimental set-up when designing research (the “where and how?” in Figure 3; reviewed in Poorter et al., 2016). Different set-ups have pros and cons, which can be viewed as trade-offs in experimental control and reproducibility versus making measurements in more realistic settings that may have clearer implications for management actions and expectations (Table 2). Here, Table 2 is meant to complement Figure 3 above by providing examples of experimental set-ups in multiple settings, treatments and methodological approaches to studying seedlings. For example, in the context of seedling studies, experimental control and reproducibility are most easily achieved in growth chamber and greenhouse settings, and are more difficult to achieve in common garden and field settings. In contrast, common gardens and field experiments provide conditions that are closer to what seedlings would experience in nature (Huxman et al., 2022).

TABLE 2 A non-exhaustive review of experimental set-ups used to study seedlings in growth chambers, greenhouses, common gardens and in the field. We separate treatments from methods as there are numerous studies that are strictly observational and might not include treatments in their design but still describe methods for germinating and growing seedlings. Check marks represent advantages and limitations of each experimental set-up. Three check marks indicate the set-up is advantageous in relation to a specific research consideration (e.g. experimental control) whereas one check mark indicates the set-up has limitations that should be carefully considered. A question mark indicates the set-up is too case-dependent to estimate potential advantages. References cited in this table are available in Appendix 1c.

Experimental set-up	Growth chamber	Greenhouse	Common garden	Field
Experimental treatments (non-exhaustive)	Water availability ^{1-4,8,24}	Water availability ^{2,8,17-19,21-23,27}	Water availability ^{25,26}	Water availability ²⁸
	Temperature ⁹	Temperature ²⁷	Competition ^{31,32,34,37}	Grassland management ³³
	Nutrients ¹⁴	Competition ⁵	Litter ^{30,32}	Pollutants ³⁹
	Pollutants ^{11,12}	Simulated herbivory ¹⁸	Fertilisation ^{34,37,43}	—
	Light ¹⁵	Soil types ²⁰	Flooding ³⁵	—
Allelopathic extracts ^{3,13,30}	Herbicide ²⁹	—	—	
Experimental methods (non-exhaustive)	Petri dish with filter paper ^{1,2,3,7}	Pots with soil ^{2,10,16-23,27,29}	Pots with soil ^{25,26,30-32,34,37}	Experimental plots ^{28,33}
	Petri dish with Agar medium ^{2,24}	Mist bench ¹⁰	Shade cloth ⁴¹	Nylon mesh ³⁸
	Well Plates with filter paper ⁶	Fertilisation ³⁶	—	Open-top chambers ^{39,40}
	Rockwool cube ¹⁵	Shade cloth ⁴²	—	—
	Pots with soil ^{4,11,14}	—	—	—
<i>Trade-offs associated with experimental set-ups</i>				
Experimental control	✓✓✓	✓✓	✓✓	✓
Reproducibility	✓✓✓	✓✓	✓✓	✓
Realistic setting	✓	✓	✓✓	✓✓✓

Time and monetary resource investments can be highly variable for each experimental set-up and depend on many factors including whether equipment (e.g. growth chambers) is already available, distance to field sites (e.g. fuel costs) and infrastructure needed to implement research (e.g. irrigation in field settings). There may be advantages and added value to combining multiple methods to ensure measurements meet a maximum standard in reproducibility, for example, by combining a growth chamber seedling emergence study with a field trial (e.g. Blossey et al., 2017). There are also novel facilities such as ecotrons, or controlled environment facilities (Roy et al., 2021), that aim to cover both ends of the reproducibility-realism gradient (e.g. Vanderkelen et al., 2020), but are limited by space and experimental cost.

Based on a non-exhaustive literature search, seedling trait measurement methods with a high level of reproducibility (i.e. growth chamber or greenhouse experiments) are most prevalent in quantifying seedling traits and their responses to multiple abiotic and biotic drivers (Table 2). Experimental set-ups at the other end of the reproducibility-realism gradient (i.e. field-based or common garden experiments) are exceptionally rare (Table 2; e.g. Orrock et al., 2023). Further, water resources are oftentimes the only driver manipulated across experimental set-ups, whereas other drivers (e.g. those related to biotic interactions) are rarely included and are usually applied in greenhouse and outdoor common garden studies (Table 2).

8 | CONCLUSIONS

Seedlings are the earliest and shortest stage of the plant life cycle and their successful transition to later life stages plays a pivotal role in population and community level processes, ecosystem development, plant conservation and ecological restoration. An understanding of seedling function and its environmental and biotic controls will not only expand ecological concepts like community assembly theory but also create pathways to strategically improve conservation and restoration outcomes. As an emerging field of research, the many considerations and limitations outlined above represent incredible opportunities for engagement. Here, we distill what we see as the most critical next steps to advance seedling functional trait research.

For future research in this area, we stress the importance of understanding the degree of intra- versus interspecific variation for different traits. Such insight will identify which aspects of seedling function should be prioritised to source seeds in a way that accounts for local adaptation and potential maternal effects (“who”; Figure 3). Next, seedling physiology and phenology are much less-studied than morphology, even though we know that seeds and seedlings respond to environmental variation on very short timescales; germination timing and growth can have cascading impacts on later life stages (“what”). Methodological development and comparisons of new and

existing approaches will be essential for identifying which methods should be applied to garner the physiological and phenological information researchers are seeking (Table 1). Working within the parameters of a common set of approaches (described below), more research is needed on seedling functional traits at different timepoints of seedling development ("when"). A broader grasp on which traits vary through seedling development across species and systems, and what traits are good indicators of changing seedling vulnerabilities (i.e. freezing, herbivory, drought, etc.) will clarify when traits ought to be measured across research agendas. Finally, for the purpose of broader syntheses across systems and species, accumulating large amounts of trait data from controlled, reproducible conditions is obviously helpful ("where and how"; Appendix 2). Yet, as a starting point, seedling functional trait values need to be compared across basic experimental approaches (i.e. filter paper, agar, or soil as growth media) to better understand the pros and cons of each method and to understand the variation in traits arising from these different approaches (Table 2). Along with trait data, recording robust metadata is also important to allow future assessments of study comparability (Appendix 2).

Ultimately, the field of seedling functional ecology is growing rapidly and includes multiple closely-related ecological disciplines. The value of this research is sure to promote scientific advancement and ecological understanding of the processes that build populations and communities, confer ecosystem services and, together with an open synthesis community, have the potential to rapidly apply concepts to meet management needs and global challenges in an age of change.

AUTHOR CONTRIBUTIONS

Lotte Korell and Christoph Rosche organised the workshop that led to this paper. All authors conceived ideas for the paper, outlined and drafted early versions of the text. Daniel Winkler led the writing and manuscript development and all authors contributed to the final manuscript. Magda Garbowski illustrated Figures 2 and 3.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

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ORCID

Daniel E. Winkler  <https://orcid.org/0000-0003-4825-9073>

Magda Garbowski  <https://orcid.org/0000-0002-5427-7916>

Kevin Kožić  <https://orcid.org/0000-0003-4738-0242>

Emma Ladouceur  <https://orcid.org/0000-0002-4943-4358>

Julie Larson  <https://orcid.org/0000-0001-7968-916X>

Christoph Rosche  <https://orcid.org/0000-0002-4257-3072>

Christiane Roscher  <https://orcid.org/0000-0001-9301-7909>

Mandy L. Slate  <https://orcid.org/0000-0002-4026-7952>

Lotte Korell  <https://orcid.org/0000-0001-7051-8903>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix 1. Facilitating comparable research in seedling functional ecology.

Appendix 2. Example metadata for seedling functional studies.

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