

Using herbaria to study global environmental change

Patricia L.M. Lang¹, Franziska M. Willems², J.F. Scheepens², Hernán A. Burbano^{1*} and Oliver Bossdorf^{2*}

¹ Research Group for Ancient Genomics and Evolution, Max Planck Institute for Developmental Biology, Tübingen, Germany

² Institute of Evolution and Ecology, University of Tübingen, Tübingen, Germany

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ORCID IDs: 0000-0001-6648-8721 (PLML), 0000-0002-5481-3686 (FMW), 0000-0003-1650-2008 (JFS), 0000-0003-3433-719X (HAB), 0000-0001-7504-6511 (OB).

***Corresponding authors (shared):**

Oliver Bossdorf (oliver.bossdorf@uni-tuebingen.de)

Hernán A. Burbano (hernan.burbano@tuebingen.mpg.de)

20

Abstract

21 During the last centuries, humans have transformed global ecosystems. With their temporal
22 dimension, herbaria provide the otherwise scarce long-term data crucial to track ecological and
23 evolutionary changes over these centuries of global change. The sheer size of herbaria,
24 together with their increasing digitization and the possibility of sequencing DNA from the
25 preserved plant material, makes them invaluable resources to understand ecological and
26 evolutionary species responses to global environmental change. Following the chronology of
27 global change, we highlight how herbaria can inform about long-term effects on plants of at
28 least four of the main drivers of global change: pollution, habitat change, climate change, and
29 invasive species. We summarize how herbarium specimens so far have been used in global
30 change research, discuss future opportunities and challenges posed by the nature of these
31 data, and advocate for an intensified use of these 'windows into the past' for global change
32 research and beyond.

Introduction

Global environmental change is one of the major challenges of the 20th and 21st century. It is evident since the age of industrialization in the late 18th century – sometimes also referred to as the advent of the anthropocene – and has continuously gained momentum (**Fig 1a**; ref^{1,2}). Biologists study global change for its broad ecological impact, and its negative effects on biodiversity. Also, as it represents an unplanned, long-term and large-scale experiment, studying global change can promote understanding of fundamental processes such as rapid adaptation. Experimental approaches to study these topics are usually locally focused, and limited to a duration of a few decades³. While observational methods are often more large-scale and long-term, they are with few exceptions still restricted to a time frame of 50 to 80 years^{4,5}. To understand both the extent of global change as a long-term process, and its full ecological and evolutionary impact, global data that goes back to around the onset of industrialization is crucial.

In this context, natural history collections are an underused treasure of temporally and geographically broad samples that we have just begun to dust off⁶. Especially rich is the botany section of this vault – plants collected, pressed and preserved, in most cases together with meta-information on species, collection site, date and collector (**Fig 2**): In terms of extent, with > 350 million specimens in almost 3,000 herbaria worldwide (**Fig 1b**, ref⁷, <http://sweetgum.nybg.org/science/ih/>), sampled from the 16th century until today⁸, and in terms of the collections' potential uses that range from classical taxonomy and systematics, to archaeobotany, -ecology — and climate change research⁹. Since plants are sessile, they are particularly exposed to environmental change. The time courses of many of their responses to environmental change are preserved in herbarium specimens, which therefore provide unique spatio-temporal data for studying global change^{10–13}.

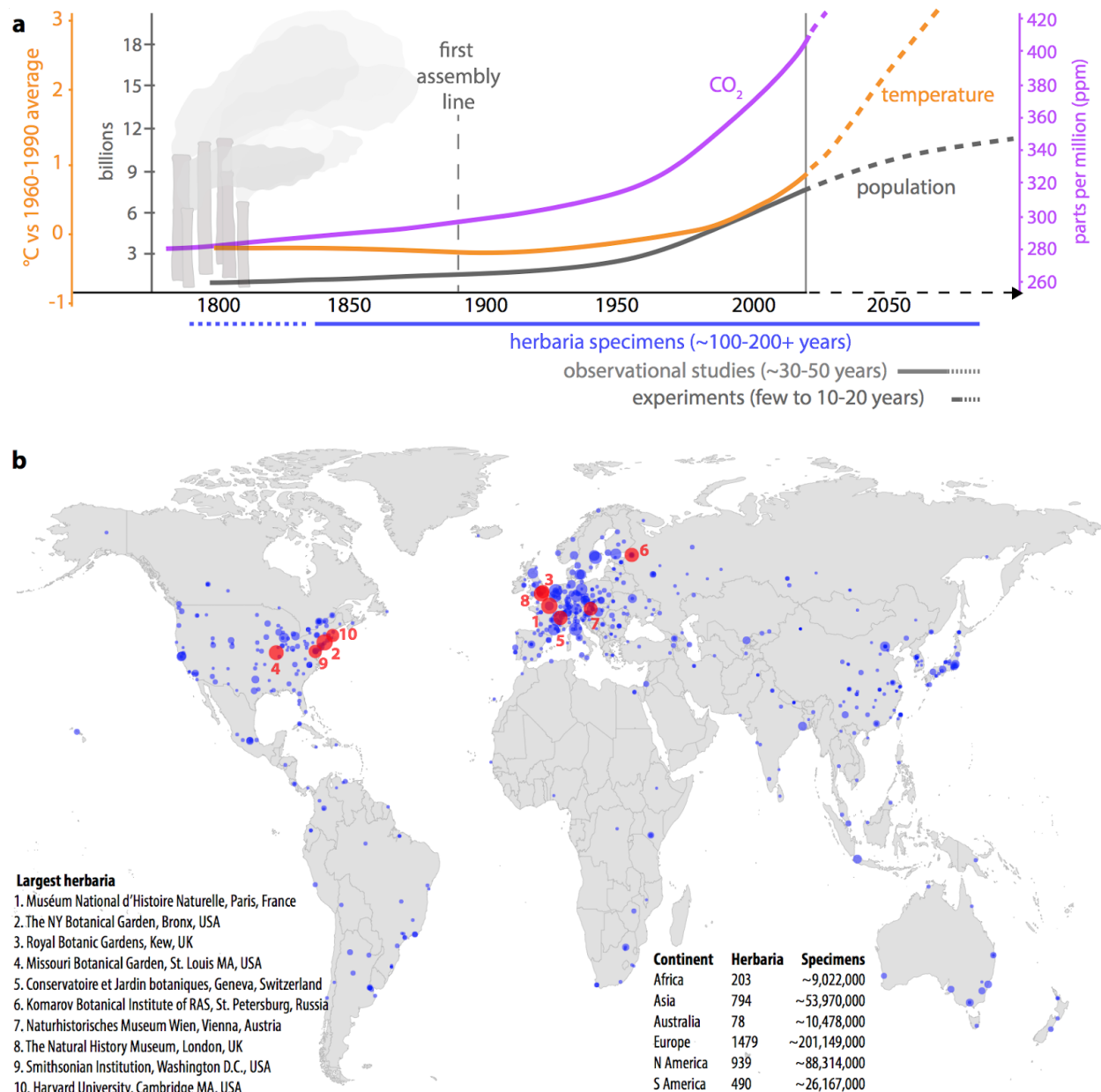


Figure 1. Herbaria as global change witnesses. **(a)** Timeline of global change, with lines tracking changes in world population, air temperature and atmospheric CO₂ during the last ~200 years. Dashed line ends indicate future projections. Bars below plot indicate the typical temporal extent of herbarium samples versus observational studies and experiments. (Population growth: United Nations, Department of Economic and Social Affairs, Population Division (2017). World Population Prospects: The 2017 Revision. <http://esa.un.org/unpd/wpp/>; temperature: representative concentration pathway 8.5, Intergovernmental Panel on Climate Change, www.ipcc.ch; ref¹⁴; CO₂: ref¹⁵). **(b)** Map with global distribution of all herbaria with >100,000 specimens, names of the largest ten herbaria, and number of herbaria and herbarium specimens per continent (Herbarium data from Index Herbariorum, <http://sweetgum.nybg.org/science/api/v1/institutions/>. Accessed in April 2018.)

Recent studies have emphasized the scientific value of herbaria for a broad range of global change-related topics (**Fig 2**), including phenology shifts^{16,17}, morphological effects of increasing CO₂ levels¹⁸, pollution¹⁹, or changing species distributions²⁰. Dense time series of herbarium specimens even permit studying long-term processes such as recent invasions and their genetic population history²¹.

Even though herbaria were used as early as in the 1960s to study global change (e.g. ref^{22,23}), and are in the process of being made available online via digitization (>45,800,000 specimens in the Integrated Digitized Biocollections portal alone; as of 5 April 2018, <https://www.idigbio.org/portal/>) the community has not fully adopted herbaria as valuable 'time machines' to the past^{10,11}. Especially with the advent of high-throughput methods and recent technical developments, the value of these collections is now more apparent than ever. Worldwide collection digitization efforts in combination with increasingly fast and sophisticated methods for image analysis enable researchers to analyze large numbers of samples, even if they are scattered all across the globe (e.g. ref²⁴).

Simultaneously, next generation sequencing (NGS) techniques now allow for in-depth genetic analysis of century old specimen up to whole genome sequencing of plants and even of their equally preserved pathogens (e.g. ref^{21,25-27}). This extends the spectrum of available long-term data far beyond morphology or phenology. Dense sampling of such full genetic information across time – and geography – for instance enables population genetics studies, to follow speciation processes through time, or to quantify changes in genetic diversity in historical contexts. Working with these small samples of degraded DNA, so-called ancient DNA (aDNA), retrieved from historic collections, is technically challenging and has recently boomed in the animal field (e.g. ref²⁸⁻³⁰), while in the plant field the approach is still rarely used³¹.

Here, we present an overview of the different types of herbaria analyses possible in global change research (**Fig 2**). Following a timeline from industrialization onwards, we divide herbarium-related approaches into four main areas related to four main drivers of global change: industrialization causing increased **pollution**, which coincides with increasing loss of habitat and **changes in land use** as well as **climate change**, and finally global trade and transport resulting in an increasing number of **invasive species** world-wide. In addition, in excursions dedicated to molecular methods (**Box 1**), collection biases (**Box 2**), and the digitization challenge (**Box 3**), we provide insight into three key methodological issues herbaria research is currently dealing with, and inspire with ideas for extended utilization of botanical

100 collections. Our aim is to advocate broader use of herbaria as 'witnesses' of global change. We
101 believe that they have the potential to fast-forward our understanding of the impacts of this
102 unplanned biological experiment, to substantiate our predictions of its long-term outcomes,
103 and to inform conservation measures.

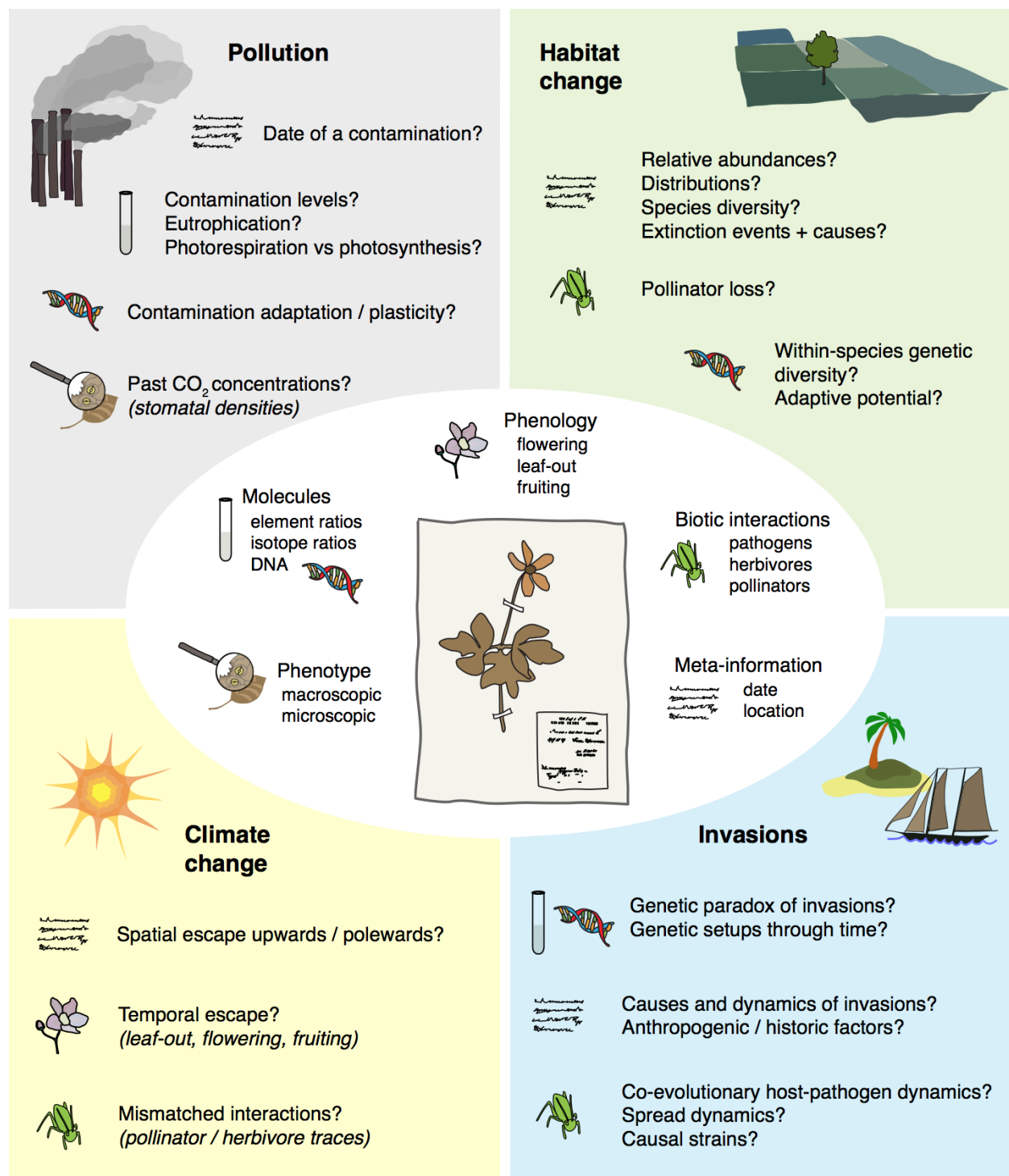


Figure 2. Diversity of herbarium data and their applications. Herbarium sheet in the centre surrounded by types of data that can be obtained from a specimen, with the questions this data can help to answer around, ordered by respective global change driver. Symbols indicate the type of data used to address each question.

Main text

Pollution

Technological developments and the mechanization of work in the second half of the 18th century, known as industrialization, changed the landscape worldwide. Key contributors were improved efficiency of steam engines, the replacement of biofuels with coal and the emergence of a chemical industry. A larger average income, increasing population sizes and accelerated urbanization led to the production of before unseen quantities of waste and exhausts (**Fig 1a**). Herbarium specimens can be used to track historical pollution levels and to connect waste production with species' reactions – even on the genetic level in the context of local adaptation.

Heavy metals

As metals from the atmosphere, soils and groundwater are deposited on or taken up by plants, and remain present in herbarium specimens, the latter can be used as indicators of pollution, and due to their meta-information facilitate the dating of contamination^{32–34}. Depending on species, their morphology, physiology, and proximity to a pollution source, plants are exposed to and take up more or less pollutants^{32,35}. Studying lead pollution levels, for example, the isotopic lead composition in moss or lichen samples collected at roadsides reflects fluctuations in local motor vehicle traffic, efforts to reduce lead emissions and changes in petrol origin or composition over time³⁶. Contrarily, overall-, or background lead levels can be assessed with samples from remote sites away from pollution sources^{33,35}. In addition to lead, herbarium samples also track levels of other metals like cadmium, copper and zinc to follow their temporal and spatial trends in relation to anthropogenic activities^{19,32,34}. Combining pollution records and genetic information from historical and contemporary samples from contaminated sites even can enable studies of plants' adaptation to pollution on the genetic, heritable level, e.g. by studying the association between pollution levels and specific alleles, and thus give indications about long-term adaptation to changing conditions. Such approaches are already well established for contemporary data alone^{37–39}.

Anthropogenic nitrogen

Similarly, herbaria document human influences on global nitrogen (N) cycling, that started with the rise of the chemical industry and the production of fertilizers, and has peaked since around 1960⁴⁰. Moss leaf N-contents (as well as levels of phosphate and sulfur) determined from stable isotope ratios enable inferences about realized N sources and further cycling processes⁴¹. Such analyses show a retention of additional, anthropogenic nitrogen within terrestrial ecosystems⁴¹. They can also point to variation in pollution levels among local plant communities, such as the increased exposure of mountainous bryophytes to nitrogen due to polluted snow-melt causing 'acid flushes'⁴². Improved knowledge of these nutrient dynamics within different ecosystems helps to understand eutrophication. Additional detail on the biotic effects of nitrogen fluctuations could be retrieved via shotgun-sequencing of historical plant-roots, given that *bona fide* microbiomes could be recovered, as it has been shown that the bacterial species composition of roots (and soils) is heavily influenced by overabundance of nitrogen⁴³.

Increased carbon dioxide

Pollutants such as nitrogen or carbon dioxide (CO₂) can influence overall organismal morphology, making their effects partially measurable without destructive sampling. Increased fossil fuel combustion and the concurrent increase in CO₂ concentrations since the industrial revolution, for example, correlate with a reduction of stomatal densities (SDs) on the leaves of herbarium specimens. This trend was already observed in 1987 in a 200 years spanning study of woody angiosperm herbaria samples. Further analyses under controlled experimental conditions^{44,45} and of conifer needle stomatal densities from herbaria, living trees and even subfossil peat cores⁴⁶, confirm historic samples as proxies to reconstruct past carbon dioxide concentrations.

In addition to morphological studies, herbarium specimens enable complementary measurement of global change effects on plant carbon metabolism. Using mass spectrometry to estimate the relative abundances of different carbon isotopes, studies indicate increased water-use efficiency – the ratio of photosynthesis to water loss – with rising CO₂ levels^{47,48}. A more direct way to assess intra-plant carbon cycling, e.g. the ratio between photorespiration and photosynthesis, is deuterium Nuclear Magnetic Resonance. The distribution of intramolecular deuterium can even provide quantitative data on long-term adjustment of plants

to anthropogenic emissions⁴⁹. With time-series of genetic variation from herbaria, it is now further possible to determine what part long-term adaptive changes or phenotypic plasticity play in such physiological or chemical responses.

There is however one *caveat* for measurements of any type of chemical compounds in long-term stored historical samples: Do chemicals suffer degradation processes similar to hydrolytic damages occurring in DNA over time (see **Box 1**)? If so, to which extent and at what rate do compounds degrade, and what influence do factors like species, specimen mounting or general storage conditions have on such a decay? Systematic studies of chemical degradation through time will permit to assess whether absolute or relative values should be used in historic specimens-based long-term comparisons.

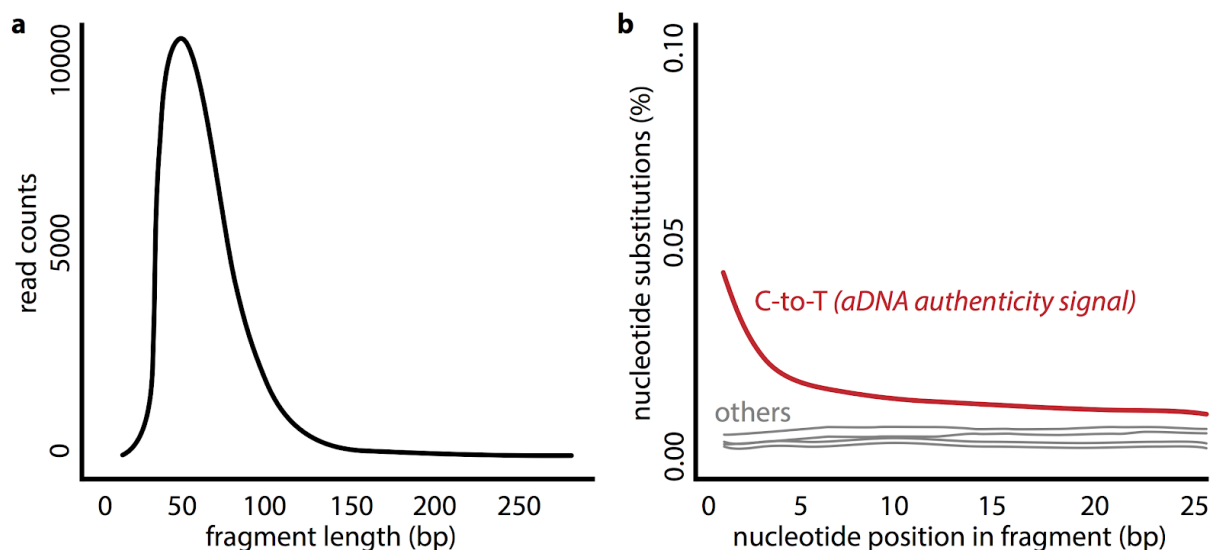
Box 1: Molecular analyses and degradation

The age of herbarium specimens is both their strength and their weakness, as aging is a corrosive process. Changes in the appearance of samples, loss of colors and brittleness, hint at degradation also at the molecular level. For most chemicals, the extent, rate and end-results of this process are not defined in herbarium samples. Still, it is clear that age, but also preservation practices or storage conditions can alter tissue chemical contents. This is evident for example when nitrogen concentrations measured in stored tissues diverge from the results of previous methods and studies – in this case likely due to post-collection contamination⁵⁰. Hence, in-depth analyses of correlations between the age and chemical compound quantities in old samples are necessary in order to make claims about historical absolute abundance values⁵⁰.

For DNA from historical samples, referred to as ancient DNA (aDNA), age-related degradation-dynamics are fairly well-characterized^{51,52}. Due to chemical modifications, DNA in dead tissue gets increasingly fragmented over time (**Figure a**), and particularly in fragment ends, aDNA-characteristic deamination drives nucleotide-substitutions of cytosine with thymine (ref⁵¹, **Figure b**). This *per se* does not lessen the potential of aDNA-studies³¹ – specialized protocols even allow extraction of ultra-short fragments of <50 base pairs⁵³, and the correlation of nucleotide mis-incorporations with time enables its use as authenticity criterion of ancient DNA^{51,54}. Still, these particular characteristics call for categorical rules for herbarium genetics to minimize contamination risks, verify authenticity and maximize the

information gained from precious old plants: Samples have to be processed in clean room facilities to avoid contaminations with fresh DNA, and sequenced to a certain depth to yield useful information. Pure PCR analyses on the contrary are inappropriate for aDNA studies, as they do not allow the necessary authenticity verification and, due to the fragmentation of aDNA, are unlikely to yield consistent results.

Such quality requirements are particularly important due to the limitation of available material. Unlike traditional approaches that rely on metadata or morphology of historical samples, molecular analyses require tissue probes and hence destructive sampling of specimens. Therefore, it is the duty of any herbarium scientist to optimize their methods, minimize the amount of sample needed, and employ state-of-the-art analyses to retrieve maximum information from their samples.



Typical molecular characteristics of herbarium DNA. **(a)** Fragment size distribution and **(b)** damage pattern found in ancient DNA (adapted from ref³¹).

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Habitat loss and land use changes

209 Apart from pollution, increasing human population densities, urbanization, and in particular
 210 modern agriculture have caused extensive losses, fragmentation or changes of natural
 211 habitats. This affects plants' geographic distribution and densities, for example causing range
 212 reductions to more pristine environments⁵⁵. Information about such habitat alterations in
 213 response to global change are documented in herbaria. Herbarium sheets normally contain

information not only about the presented species, but also about the time and place of collection. Hence, comparison between past and present localities serves to infer a species' distribution through time⁵⁵.

Distribution changes

Many factors have contributed to converting the landscape into a patchwork of agricultural fields, interspersed with cities and roads: industrialization-associated population growth, urbanization, increasing agricultural acreages due to mechanization of work, or expansion of railroads and other transport systems. Overall, species abundances tend to decrease with habitat and land use changes, for instance for American ginseng (*Panax quinquefolius*), both as a result of deforestation and of heavy harvesting of wild populations⁵⁶. In light of an area's geography, such data can also give implications about species' conservation and future trends⁵⁶. However, retrospective studies of species' abundance in a certain location based on historical collections are sensitive both to the quality of available georeferencing data, and to fluctuating collection efforts and similar biases (see **Box 2**). A reference set of specimens picked from the herbarium randomly and independent of species identity can be used to establish a general 'expected collecting frequency', which can balance these biases, and for example reveal that trends of relative abundance changes are species-dependent, even within one plant division (see e.g. ref⁵⁷ for mosses).

When herbarium records are used to relocate historical populations, current data complements herbarium-inferred distributions and abundances^{58,59}. Revisiting surveys can detect local extinction events, and, in correlation with current land use practices or site protection status, be used to study their causes⁵⁸. They can further document changes in overall plant diversity, which, too, is affected by habitat fragmentation⁵⁹. Such approaches are particularly useful to evaluate changes in the local flora and motivate biodiversity monitoring campaigns, and can inform large-scale diversity surveys.

Box 2: Collection biases

Imbalanced sampling is a well-acknowledged issue for the use of herbaria e.g. to map species distributions or assess diversity (e.g. ref^{60,61}). Temporal biases are caused by intense

collection periods, and seasonal preferences⁶². Also, collections often concentrate on easily accessible or much-frequented sites (geographic bias, e.g. ref⁶³), and on common or particularly interesting species which – depending on the collectors – can change over time (taxonomic bias, e.g. ref⁶⁴). When working with herbarium data, it is necessary to explicitly test for these biases, for example to avoid a few dominant species generating trends in a dataset⁶⁵. Depending on the type of question or analysis, biases may need to be corrected for by different means: normalizing collection efforts with different types of reference sets (e.g. ref^{56,57,66}), measuring invader distributions in relation to native species⁶⁷, or verifying trends with additional, non-herbarium datasets (e.g. ref^{46,58} or even citizen science⁶⁸). In particular when models are based on historical records, comparisons with modern data can support extrapolations or generalizations – but only if biases have been dealt with: Models, for example in the context of invader dynamics and spread, have to take species persistence into account, as historic occurrence does not equal contemporary presence and may cause overestimation of plants' distribution and abundance⁶⁹. This is particularly the case for species targeted by eradication measures, like the human health hazard *Heracleum mantegazzianum*, where herbarium specimens can indicate suitable habitats, but not current occurrence or general invasion dynamics⁶⁹. Further, there is often no data on early invasion stages, as herbarium records indicate only the presence of a species, while its absence is not reliably documented by a lack of records. Conclusions based on modeling and statistical analysis particularly of early invasion stages should hence be used as indications, but not be over-relied on⁷⁰. Finally, a currently rising bias are low collection efforts, a well-known problem for tropical areas²⁰ that is, with overall declining collections, threatening to become global⁷¹. While this particularly jeopardizes studies of new or recent invasions⁷², it strongly affects all herbarium-based research.

Indirect effects of habitat fragmentation

Similar to farming-related landscape changes, urbanization is a prominent driver of biotic interaction changes. One of the most crucial, commercially important types of plant-animal interaction jeopardized, among others, by urbanization and diversity loss, is pollination. Depending on a plant's anatomy, herbaria also house documentation of such interactions, and can illustrate pollinator species decrease or loss. Presence or absence of pollinaria in

rehydrated orchid herbarium specimens of the oil-secreting orchid *Pterygodium catholicum*, for example, reflects the historical pollination rate that depends strictly on activity of a specific oil-collecting bee (*Rediviva peringueyi*)⁷³. The bee's observed decrease with increasing urbanization is consistent with a simultaneous shift in local orchid communities towards selfing species, equally established from herbarium and contemporary data⁷³. Impairment of interactions between plants and their pollinators, caused for instance by such abundance decreases or temporal mismatches, likely also leaves genetic signatures. Given that affected biotic interactions could be identified using historical plant and insect collections, these signatures could be traced through time and inform the potential of other species-pairs to overcome future mismatches.

Besides the apparent decrease of species diversity, losses of within-species genetic diversity are a less conspicuous consequence of habitat loss, and are, like phenology mismatches, a result of shrinking and increasingly isolated populations^{74,75}. Improved high-throughput sequencing techniques and novel molecular approaches have recently made within-species genetic diversity — as preserved in herbaria — accessible. Retrieval of such ancient DNA (aDNA) requires specialized clean-room facilities, and demands strict measures of quality and authenticity (see **Box 1**, ref⁵³). Once obtained however, this ancient genetic information extends the information on habitat loss and decreasing relative abundances to the genetic level^{76,77}. This is crucial knowledge for conservation measures, as genetic diversity, especially in times of increasingly fluctuating environmental conditions, is an indispensable resource for heritable phenotypic variation and rapid adaptation^{78,79}. Reduction of genetic diversity via abrupt decimation of a population, referred to as a bottleneck, can hamper the population's persistence, as selection is less efficient in small populations, where there is more stochasticity and less standing variation to act upon^{74,75,80}. Comparison of contemporary versus historical genetic diversity can serve to prioritize the conservation of specific populations over others, and to identify genetically diverse source populations for potential reintroductions to balance bottlenecks⁷⁶.

Climate change

Some factors on the rise since the start of industrialization, potentially even prior to that, have less direct, but long-term effects on ecosystems: The so-called greenhouse gases such as methane (CH₄) and carbon dioxide (CO₂; **Fig 1**). Their atmospheric increase, for CO₂ a result of

enhanced fossil fuel burning in factories, power plants and for transportation, causes global climate change⁴⁰. Thus, in addition to the earlier mentioned direct effects of the pollutant CO₂ on plant morphology and physiology (see **Pollution**), progressive CO₂-related global warming influences plant life cycles, as is observed for instance already in shifts of plant 'phenology', that is timing of life cycle events such as flowering and fruiting, to earlier dates. However, herbaria not only directly track these climate-related plant responses, but also give insights into their ripple-effects on pollinators, herbivores, and even nutrient cycling.

Range shifts as spatial escape

One possible response of plants to global warming can be distributional shifts when plants escape from unfavorable conditions, which is traceable using herbarium time-series. Comparison of field with herbarium data verifies predictions that with progressive global warming, species will move both upslope and poleward, following their original climatic niches. For instance, historic time-series have monitored movements and consecutive diversity shifts in California, Costa Rica, and South America as a whole^{64,81,82}, and hence can differentiate successfully moving species from those that may not persist under continuously changing conditions⁸².

Phenology timing

Instead of spatial movements, plants can also escape global warming 'in time' by shifting phenological events like flowering or fruiting towards more favorable conditions. To track such changes in the past, flowering timing, for example, can be approximated from collection dates of flowering herbarium specimens. Using a combination of contemporary flowering time observations with a herbarium specimen series across >100 years and 37 genera, Primack and colleagues⁸³ were first to connect meteorological data with earlier flowering, which was to a great part explained by increasing spring temperatures. This trend has been confirmed by multiple analogous studies (e.g. ref⁸⁴) and also broader approaches that integrated herbarium data with phenology records obtained from field notes and photographs to cover recent years of herbarium record scarcity⁸⁵. While spring temperature is not in all plant species the main cue for induction of phenological events, this does not diminish the value of historic records for those plants, on the contrary: they can point out different sensitivities of spring- versus

summer- or autumn-flowering species to the ongoing temperature fluctuations, and identify diverse cueing mechanisms like photoperiod or winter chilling⁸⁴.

Spatial scale and statistical power are important factors for this type of studies. Since phenology also depends on latitude, altitude and other environmental factors, broad sampling is necessary to separate climate change effects from other influences. Moreover, as phenology is partly species- or plant functional type-specific, it is useful to study contrasting flowering seasons, native status, pollination syndrome or growth form⁸⁶. All of this is facilitated by large-scale digitization and hence improved accessibility of specimens worldwide (ref¹⁰, e.g. in ref²⁴, see **Box 3**). Such studies for example showed that annual plants are generally more responsive to climate change than perennials, and indicated contrasting responses of different grass functional types to increasing temperature — C3 grasses presenting accelerated, C4 delayed phenology^{24,86}. Compilation of large cross-species datasets furthermore allows to search for phylogenetic signals and thus to identify evolutionary processes involved in shaping the observed responses⁸⁷. Apart from inter-species or -family variation, plant responses also vary across geographic regions. Combined, herbaria worldwide enable approaches that include remote localities across the globe, for example monitoring *Rhododendron* in the Himalayas⁸⁸, or demonstrating premature flowering in diverse species across the arctic⁸⁹.

Flowering is not the only phenological event heavily influenced by climate change that can be tracked from herbarium specimens. Depending on a plant's reproductive structures, also seed dispersal timing can be evaluated. At least for the Arctic, dispersal timing, too, seems to advance with increasing temperatures, in correspondence with associated flowering data⁸⁹. Contrariwise, autumnal mushroom fruiting, also estimated from collection meta-information, especially of early fruiting species is delayed in Norway⁹⁰, possibly reflecting a prolonged growth period due to warm autumn and winter temperatures.

Another parameter that affects entire communities and ecosystem processes is the leaf-out timing of deciduous trees, as it impacts trophic interactions as well as nutrient and water cycling⁹¹. In addition, leaf-out should be taken into account for quantification of the 'buffer' plants provide against climate change: an early leaf-out potentially prolongs the growing season, and thereby may boost forests' carbon uptake⁹². Such data can be collected from herbarium records, and for example confirms large-scale patterns of earlier leaf-out inferred with satellite data⁹³.

Box 3: Digitization challenge

Large-scale digitization is crucial to make biodiversity data more accessible, balance the unequal distribution of collections worldwide (ref⁹⁴, see also locations of all herbaria worldwide with >100,000 herbarium specimens, **Fig 1b**), increase use of herbaria in general, and the number of specimens included per study specifically¹⁰. Various online databases already offer access to vast amounts of data (e.g. <https://www.idigbio.org/>, www.gbif.org, <http://vh.gbif.de/vh/> or <http://avh.chah.org.au/>). But the digitization task is enormous, with over 350 million specimens to process, and expensive. To optimize and speed up the process, various institutions have developed affordable digitization workflows, for example the Royal Botanic Garden Edinburgh⁹⁵, New York Botanical Garden⁹⁶, or smaller collections such as the Arkansas State University Herbarium⁹⁷. As digitization nevertheless is time consuming, it can, depending on data needs, be done in a prioritized way. In conservation biology for instance, a fraction of available specimens appears to be enough to reliably detect threatened species and trigger conservation efforts⁹⁸.

Apart from cost and speed, the transcription of meta-information, and particularly geo-referencing information, is another digitization bottleneck. Optical character recognition may help sorting entries by collector or country⁹⁹, as can the development of semi-automated imaging pipelines¹⁰⁰. Other projects use citizen science approaches to transcribe specimen labels (ref¹⁰¹; <https://www.notesfromnature.org/active-expeditions/Herbarium>), and computer vision or machine learning (re-)classify specimens that are unidentified, or whose identification was based on an old taxonomy^{102,103}. Still, imprecise or wrong geo-referencing is common in herbarium data (ref¹⁰⁴), an issue that is particularly problematic in conservation, for species distribution assessments, or prediction approaches¹⁰⁵. While care with location-data from herbaria is hence necessary, digital field notebook apps like ColectoR may at least help guarantee complete and correct meta-information for novel collections¹⁰⁶.

389 Mismatching biotic interactions

390 Naturally, these climate change-related phenomena also affect biotic relationships beyond
391 plants, hence cannot be seen only as isolated processes. Changes of their timing are likely to

affect evolutionarily synchronized relationships, and even their breaking-up over time is, together with flowering change, partially recorded in herbaria. Combined with entomological museum specimens, herbaria for example document disruption of the plant-pollinator relationship between the bee *Andrena nigroaenea* and the orchid *Ophrys sphegodes*¹⁰⁷. In herbivory relationships, herbarium specimens can actually directly reflect insect reactions to warming. Increased traces left by the scale insect *Melanaspis tenebricosa* on maple tree leaves collected in warmer years evidence a higher insect density, perfectly in accordance with observations in the field¹⁰⁸. Herbaria can thus help overcome the lack of historical insect abundance records and facilitate evaluation of climate change effects beyond plants alone.

The greatest challenge of most aforementioned approaches investigating species' responses to pollution, habitat- and climate change, is their inability to distinguish between plastic responses and evolutionary adaptation^{24,109}, i.e. whether observed differences among herbarium specimens reflect genetic changes or just environmentally-induced phenotypic changes caused for instance by physiological processes^{110,111}. Quantitative genetics methods using herbarium time-series could help disentangling these two alternative hypotheses^{112,113}. Once the genetic basis of phenotypic differences is identified, local adaptation can be further tested using traditional approaches such as common garden experiments and reciprocal transplant studies¹¹⁴.

Biological invasions

Natural long-distance dispersal of plants is rare¹¹⁵, but as a side effect of global change, plants increasingly move long distances¹¹⁶ anywhere mass transportation carries them — either as purposely packed passengers, or as stowaways in crop seed bags and shoe soles. This movement massively increased with human migration waves towards the New World in the 16th century, and further accelerated with growing trade and faster transportation – coinciding with the core time range of herbarium collections. Today, jet-setting plant stowaways establish as 'neophytes', 'aliens' or 'invaders' wherever conditions are favorable enough. With this growing alien species richness, the global species distribution is getting more homogenous¹¹⁷. Local plants lose habitats and thus genetic diversity to the invaders, which are therefore considered a threat to biodiversity⁴⁰.

Understanding invasion dynamics

Understanding the causes and spatio-temporal dynamics of invasions is indispensable to prevent further damage, preserve natural ecosystems, and prioritize management actions^{118,119}. While contemporary surveys depict the current status of invasive species, herbaria track invasions from the first recorded colonizer onwards — which can serve as a proxy, even if it is not the actual first colonizer. With native plants as baseline for collection efforts and abundance, herbaria illustrate geographical and temporal spreads that may — in search for invasion causes — be connected with historic events. For instance, a map of Chilean alien expansions uncovers two spread peaks, one connected to the spread of agriculture, the other to its increased mechanization¹²⁰. Understanding such causalities can feed early preventive measures: Retrospectively mapped invasions identify geographic invasion hotspots, and the environmental and anthropogenic factors crucial for their creation. In this way, herbaria can contribute to understanding the general invasibility of particular habitats^{121,122}. Further, combined with contemporary data they can help to identify characteristics of successful invaders, and to quantitatively connect an established naturalization risk with external factors, and rank potential new invaders¹²³.

Herbaria also provide a means of assessing the continued success of invasive species after establishment in a new environment. Previous studies have used it both to predict and to verify predictions of the climatic niche plants can potentially occupy. For example, the size of the native range of an invasive species has been found to be highly correlated with its abundance in the new range, as documented for many highly invasive Eurasian species around Québec¹²⁴. Herbaria can also enable estimation of a weediness index, i.e. how much a plant associates with human-caused disturbance, which often also overlaps with plant invasiveness¹²⁵. Such estimates hold well in comparison with field surveys, and for example accurately predicted relative weediness of *Melampodium* in the Mexican state of Nayarit¹²⁶. More precise forecasts of a species' spread can further include its native climate range, again extrapolated from herbarium records. Mapping such records, a study predicted a much wider range for invaders compared to native species in North America, which once more reflects the relationship between range size and invasiveness¹²⁷. Moreover, such data enables inferences of realized invasion potential, as it roughly visualizes occupation of a possible climatic niche¹²⁷. Much as modeling the dynamics and spread of invaders is crucial to inform containment

measures, it is very sensitive to biases in historical collections, and increasingly at risk from decreasing collection efforts (see **Box 2**).

Genetic changes of invaders

Irrespective of whether invasive species stay within their native climatic range or move beyond, they face challenges when establishing in new environments. Successful invasive species often adjust to the novel conditions, and it is therefore important to understand such changes in the invasive range.

Adjustment of morphological traits to novel environments is often well-captured in herbaria, as demonstrated with Australian invasives where 70% of surveyed species showed at least one phenotypic trait changing over time¹²⁸. With next generation sequencing, it is now possible to define whether this trait variation is associated with genomic changes - caused either by drift, or potentially adaptive - or more likely the result of phenotypic plasticity. In addition, these methods can potentially solve the 'genetic paradox of invasion' – the surprising success and spread of colonizers in spite of their reduced genetic diversity¹²⁹: Do these species adapt based on their (reduced) standing genetic variation, do they borrow pre-adapted standing variation from native species (adaptive introgression, ref^{37,130}), or do they rely on *de novo* mutations and hence novel variation²¹?

Comparison of historic native and invasive populations with contemporary genetic diversity can also point to diversification or hybridization events prior to species expansion. A recent herbarium genetics study has for example shown strong divergences of flowering time genes particularly during the establishment phase of the invader *Sisymbrium austriacum* ssp. *chrysanthum*, possibly enabling a subsequent spread¹³¹. Such patterns change over the course of invasion. In the Eurasian *Alliaria petiolata* invading North America, invasive success declines along with population age and reduced phytotoxin production in late stages of invasion¹³². Contrary to that, chemical analyses of herbarium specimens of the phototoxic *Pastinaca sativa*, a European weed also invading North America, displays increased levels of phytochemicals over time since invasion, which coincide with the emergence of the herbivore *Depressaria pastinacella*¹³³. Studies using ancient DNA also have pointed to anthropogenic landscape disturbances causing genetic admixture in *Ambrosia artemisiifolia*'s native populations prior to its introduction to new habitats, potentially a prerequisite for later invasive success⁷⁷. In this sense, herbarium material allows us to compare genetic composition through time, and to

identify so-called 'cryptic', i.e. hidden, invasions, where native genotypes are dispelled by phenotypically indistinguishable but more successful and aggressively spreading non-native relatives¹³⁴.

Hitchhiking invaders: pathogens and herbivores

Moving beyond plant invasions, herbaria even harbor information about hitchhikers traveling with the original plant stowaways — pathogens, purposely or unknowingly sampled together with their hosts¹³⁵. Thereby, they track the invasion (success) stories of plant pathogens like *Phytophthora infestans*, the microbe at the root of potato late blight and the Irish potato famine^{25,26,136}. Other preserved pathogens of particular interest for agriculture include rust fungi, or downy-mildew causing oomycetes. Likely, even pests like the sap-sucking aphids have left sequenceable genetic traces in preserved host plants. Herbaria allow identification of causal strains, their genetic characteristics, and their tracking to contemporary pathogen diversity. Coupled with host plant analyses, they provide a (genetic) timeline of host-pathogen dynamics to study and illustrate co-evolutionary principles such as the arms-race between hosts and their pathogens. Genetic analysis of such systems can hence provide crucial insight into spread dynamics of pathogens that could have devastating consequences on crop monocultures worldwide.

Even for invasive herbivores, historic samples may contain a genetic record. The horse-chestnut leaf-mining moth *Cameraria ohridella* for example is preserved pressed and dried in leaves of its host plant¹³⁷. Genetics can backtrack the moth's spread from its native Balkan, and in conjunction with host plant analyses may identify resistant cultivars and biocontrol agents for the invasive pest¹³⁷.

Conclusions and outlook

Plants preserved in herbaria offer unique perspectives on global change and its consequences, as they are directly affected victims (**Fig 2**). In that, they represent an invaluable temporal, geographical and taxonomic extension of currently available data employed to understand global environmental change, predict its course and inform conservation measures. To fully take advantage of this potential, and to increase and sustain the value of herbaria for the future,

three core areas demand continued attention: digitization, collection and genomics (see also **Boxes 1-3**).

Even though many herbaria are already investing in digitization, only a fraction of the about 350 million specimens worldwide have been digitized so far. Large-scale digitization would both encourage the use of herbaria for research, and strengthen projects (e.g. ref²⁴), as studies including digitized material are able to use large sample sizes¹⁰. Fast processing of specimens at consistently high data quality is crucial to make digital herbaria truly useful¹⁰⁴. Yet, even with increased digitization, the actual power of herbaria – for climate change- and other types of research – lies in their continuity through time. Despite growing recognition of the value of herbaria, this characteristic is currently threatened by declining collection efforts (i.e. ref^{60,71}). Consequences of lacking data for modeling and other analyses can already be seen in the tropics, where collections are generally sparse²⁰. To maintain herbaria as the treasure they are today, continued and consistent collection worldwide is essential, especially since they recently revealed themselves as a browsable memory of genetic variation and diversity. This drastically increases the value of herbaria for climate change research, and for understanding principles of adaptation and evolution in this context. To date, herbaria are still underused in this aspect¹⁰, and particularly high-quality sequencing data is scarce. With firm guidelines for protocols and quality standards, pointing also to the necessity of DNA preservation-informed sequencing efforts, this neglect is likely to change in the coming years.

Hence, being aware of all the answers herbaria can give if we use the right methods to ask, it is up to us to keep them alive and well, define what we need to know, and start the questioning.

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Competing interests

The authors declare no competing or financial interests.

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