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Interaction between plants and their microbiota is a central theme to understand adaptation of plants to their environment. Considering herbaria as repositories of holobionts that preserved traces of ancient plant-associated microbial communities, we propose to explore these historical collections to evaluate the impact of long lasting global changes on plant– microbiota interactions.

Glossary

Ancient DNA (aDNA): DNA that remains for a certain period of time (up to several 10 000s of years) after the death of an organism. aDNA is subject to time-dependent degradation that includes fragmentation, single-strand breaks, and frequent cytosine deamination, especially at single-strand extremities of the fragments. aDNA extraction and sequencing need specific protocols and equipment and contamination-proof laboratories.

Anthropocene: a new geological era where the geological and environmental processes of planet earth are dominated by human activities.

Extended phenotype: in the case of a plant holobiont, a specific phenotype that results not from the plant genome but from the interaction between the plant and one or several of its associated microorganisms. Induced resistance to a pathogen resulting from activation of resistance pathways by another microorganism can be regarded as an extended phenotype, since it would not be observed if the plant was not already interacting with other microorganisms. Modulation of other processes such as flowering time or even expression of heterosis has also been reported.

Holobiont: a unit of biological organization composed of a multicellular host (herein a plant) and its microbiota. Plant microbiota: the set of microorganisms (bacteria, archaea, fungi, protists, and viruses) associated with a given plant at a given time. Microorganisms can be tissue- or organ-specific, living at the surface and/or in the tissues of plant organs. Rhizospheric microorganisms often selected by a plant's secreted molecules are part of the plant microbiota.

Rhizospheric soil: portion of soil influenced, physico-chemically and in terms of microbial community, by the presence and physiology of the root; a concept dating back to 1904s pioneer works by Lorenz Hiltner [15].

Plants are permanently exposed to numerous microorganisms, which colonize their surface and tissues and constitute the **plant microbiota** (see **Glossary**). What we observe in the field are not just plants, but **holobionts**, whose traits partly result from lifelong interactions between the plant itself and its microbiota. Most components of the plant microbiota are acquired by plants horizontally from the environment, even if a small fraction is transmitted vertically through seeds [1]. Environmental changes that affect abundance and distribution of microorganisms in the environment are therefore likely to affect the holobiont. While the impacts of short-term disturbance

can be evaluated in comparative studies, long-term global environmental changes that characterize the **Anthropocene** are difficult to tackle in the absence of knowledge regarding historical microbial diversity. To unlock this limitation, we discuss the emerging concept that considers herbaria not as mere repositories of plant specimens but of entire plant holobionts. Herbarias have unintentionally preserved microbial components that represent direct legacies of past microbial diversity that can be brought to light through their DNA signatures.

Plant–microbe interactions, once typically studied using one plant/one microbial species research models, are now increasingly addressed taking into account the whole community of microbes interacting with the studied plant. This microbial community, largely selected from the pool of microorganisms present in the plant local environment, confers to the plant several **extended phenotypes** and contributes to its health and fitness [2]. Manipulation of the plant microbiota is thus considered as a way in agriculture to reduce the use of inputs, either chemical fertilizers or pesticides [2]. However, short-term synchronic studies show that environment disturbance, such as agricultural practices, but also any other global changes that affect unmanaged ('natural') plant communities, can have a significant impact on plant interacting microbial communities. These impacts not only regard their taxonomic diversity, but also their functional one and the interactions between members of these communities (illustrated in [3] for fungal guilds).

A new field of research is therefore required to evaluate how profoundly long-lasting environmental changes, that characterize the Anthropocene, have altered the plant– microbiota interaction and if these changes have been detrimental to plant health. Alterations encompass different facets of microbial diversity. It can be the gain of new symbionts or pathogens, extinction of previous ones, or changes in their abundances. This is documented for microbial pathogens (e.g., [4]) or in the deliberate introduction of beneficial mutualists or biocontrol agents. However, in the absence of comprehensive historical records of microorganism distribution, we cannot evaluate this for the overwhelming majority of microbial species. The terms invasion or extinction can be extended to discrete characters of relevance to plant–microbe interactions, as exemplified by avirulence genes [4] or those controlling pesticide resistance. Besides single microbial species, environmental changes also lead to more global taxonomical and/or functional shifts of entire microbial communities, altering their overall metabolic and thus functional potentials.

Where can we find traces of the past diversity of plant–interacting microorganisms? The answer is: simply in herbaria. Many of these collections of plant specimens have been built up from the 19th century if not earlier, before the simultaneous, often exponential, increase in different human driven global changes. In this respect, plants in herbaria are already used to trace historical shifts in plant distribution, time-dependent phenotypic (e.g., flowering time), or physiological (e.g., stomatal density, C:N ratio, $\delta^{15}N$, and $\delta^{13}C$ values) alterations reflecting adaptation to changing environmental conditions [5].

A large number of plant-associated microbiotas have indeed been hitchhiked on plants present in herbaria. Evidence for the presence of such microorganisms is obvious in the case of pathogens [6] or nodule-forming rhizobia (Figure 1A–C), but microscopic observations also reveal the presence of endophytic and symbiotic fungi (Figure 1E–G). Dried **rhizospheric soils** that sometimes sheath roots of herbarium plants may also conserve a (plant-selected) rhizospheric microflora (Figure 1D). While visual inspection of plants in herbaria can be used to record the presence and spread of individual pathogens [6], other tools are needed to access the diversity of inconspicuous plant-associated microorganisms (e.g., endophytes), and to probe entire communities and not only single microbial species.

Plants in herbaria are known to contain traces of DNA. Although in older herbarium plant specimens this **ancient DNA (aDNA)** is highly fragmented (often not exceeding 50–100 bp in length [7]) and chemically modified, it has been used for barcoding, to capture specific sequences by hybridization (HybSeq; [7]) or to obtain entire plant genomes [8]. High-throughput sequencing

platforms that produce short sequencing reads, and downstream bioinformatics analyses are indeed compatible with degraded DNA. The field of 'archaeomicrobiology' is currently gaining momentum in the case of animal-associated microbiota, as illustrated by the systematic sequencing of paleofeces [9] or dental calculus [10] aDNA that captures the long-term taxonomic and functional (as exemplified by the lower abundance of antibiotic resistance genes in ancient specimens) evolution of animal-associated microbiota [9,10]. Regarding plant–microbe interactions, in their seminal paper, Yoshida et al. [4] used systematic sequencing of DNA extracted from historical infected potato specimens to illustrate the time dependent spread from the Americas into Europe of different lineages of the destructive late blight pathogen *Phytophthora infestans*. The current challenge is to extend studies targeting a specific microbial species, whose presence in preserved plants can be ascertained from specific symptoms, to entire microbial communities. This challenge has been addressed using different approaches. Daru et al. [11] and Heberling and Burke [12] succeeded at amplifying and sequencing fungal barcode sequences from the leaves and roots, respectively, of plants collected up to circa 120 years before. Most noticeably, Heberling and Burke [12] obtained sequences from Glomeromycotina, a group of strictly biotrophic root-associated fungi, unlikely to represent airborne contaminants.

External contaminations are indeed an insidious problem in aDNA research that must be limited by strict laboratory procedures, including dedicated equipment and laboratories. They can also be prevented by protocols that specifically enrich in chemically-modified (uracil-containing) aDNA molecules as presented by Weiß et al. [13], who recovered sequences from epiphytic and pathogenic bacteria from herbarium plants. While the PCR based metabarcoding approach is limited and biased by aDNA fragmentation, microbial sequences can also be identified among shotgun sequences generated from plant-extracted DNA, as reported for *Arabidopsis thaliana* and *Ambrosia artemisiifolia* herbarium specimens [14]. These sequences (circa 0.8–1% of the reads) were attributed to plant-associated microbes, several of which may have, however, colonized the plant specimens after their collection, as in the case of *Alternaria alternata* sequences systematically present in herbarium specimens of both species while almost systematically absent from freshly collected conspecific plants. Thanks to the very high throughput dimension of short-read systematic sequencing of plant-extracted DNA, this technology compensates for the low percentage of sequences attributable to plant-associated microorganisms among the majority sequences of plant origin. It can thus be extended not only to new herbarium plant specimens, but also to mine the ever increasing number of existing sequence datasets deposited in public databases.

Concluding remarks To summarize, these initial scientific reports demonstrate that all of the conceptual and technical ingredients (e.g., high-throughput systematic sequencing and sequence capture by hybridization) are now available to explore the microbiota of plants preserved in herbaria and their responses to past environmental changes (see **Outstanding questions**). Unravelling the past diversity of plant-associated microorganisms may have direct implications in restoration ecology and to develop a more sustainable agriculture, in order to make better use of microbial biodiversity in a changing world.

We would like to dedicate this manuscript to the renowned botanists but also the numerous, more humble but passionate naturalists that have unintentionally contributed during the past centuries to the establishment of large collections of plant microbiota commonly known as herbaria.

Outstanding questions

Establishment of experimental and analytical guidelines to filter out DNA sequences that may represent environmental contaminants or originate from microorganisms that colonized already collected plants.

Do global changes contribute to a standardization, homogenization, 'McDonaldization of plants' microbiota? At which spatial scale?

Are some plants or (agro)ecosystems more affected than others?

Did global changes favor specific functional traits among plant-interacting microbes?

Did intensive agricultural practices disrupt the coevolution between crop plants and their microbiotas? or, did intensive agriculture result in new co-evolved relationships between crops and their microbiota?

Do we assist to unsuspected invasions by plant interacting microbes? To what extent?

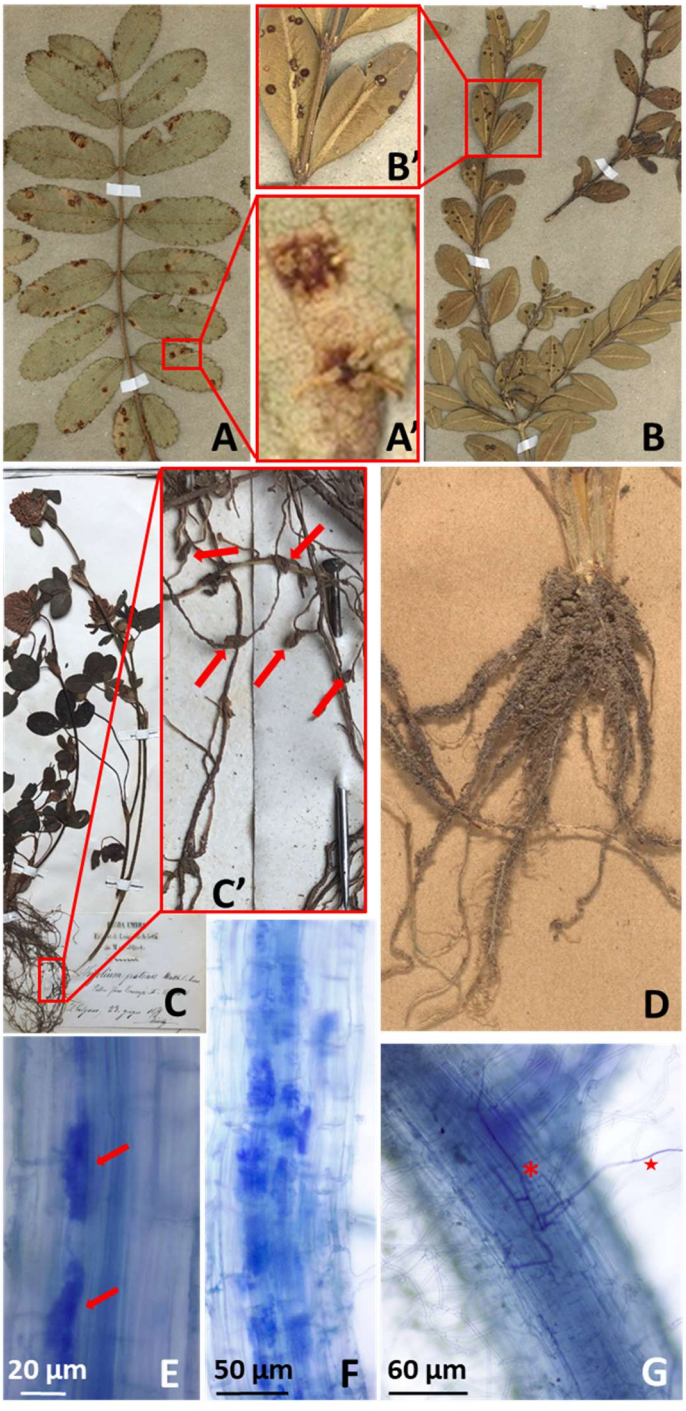

Figure 1. Examples of evidence for the presence of microorganisms associated with dried plant herbarium specimens. (A–B) symptoms induced by fungal rust pathogens on leaves of (A, A') *Sorbus aucuparia* (fungus: *Gymnosporangium cornutum*) and (B, B') *Buxus sempervirens* (fungus: *Puccinia buxi*); (C–C') nodules induced by N2-fixing rhizobia on the roots of *Trifolium pratense* collected in 1879; (D) microorganisms-containing dried rhizospheric soil sheathing the roots of a *Secale cereale* plant collected in 1905. (E–G) Microscopic observation of cotton blue-stained arbuscular mycorrhizal (AM) fungi in the roots of *S. cereale* plants collected in 1905; arrows, collapsed arbuscules in cortical cells; star, external hypha of an AM fungus reaching and growing on the surface of root epidermal cells (asterisk). Photo credits; (A–B and D), ©Herbier LY, FR-CERESE, UCBLyon1; (C) ©TO Herbarium, department of Life Sciences and Systems Biology, University of Turin; (E–G), Valeria Bianciotto. Full pictures of plants illustrated in D and E–G are visible on the Recolnat portal (https://explore.recolnat.org/) under accessions LY0662690 (D) and LY0662689 (E–G).

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