Review

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Increasing phytoremediation efficiency and reliability using novel omics approaches

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Phytoremediation is a cost-effective green alternative to traditional soil remediation technologies, but has experienced varied success in practice. The recent omics revolution has led to leaps in our understanding of soil microbial communities and plant metabolism, and some of the conditions that promote predictable activity in contaminated soils and heterogeneous environments. Combinations of omics tools and new bioinformatics approaches will allow us to understand integrated activity patterns between plants and microbes, and determine how this metaorganism can be modified to maximize growth, appropriate assembly of microbial communities, and, ultimately, phytoremediation activity. Here we provide an overview of how new omics-mediated discoveries can potentially be translated into an effective and reliable environmental technology.

The challenge of harnessing plants for soil remediation

Phytoremediation is a cost-effective green alternative to traditional soil remediation technologies such as excavation followed by chemical processing. There are also additional benefits to this approach: biomass from plants grown in a contaminated area may be harvested for use as biofuel or, alternatively, plants may continue to grow on site, potentially acting as pioneer species for ecosystem regrowth, increasing local biodiversity, and contributing to atmospheric CO_2 fixation and the restructuring of disturbed soils. Phytoremediation efficiency is the result of synergistic interactions between plants and the surrounding environment, particularly microorganisms. For instance, plants may translocate and sequester compounds such as heavy metals, whereas it is primarily microorganisms that degrade organic contaminants (Box 1).

Despite the enormous promise of phytoremediation, it has yet to gain traction as a viable remediation alternative,

mainly because of its variable effectiveness. The uncertainty of full and rapid site remediation is cited as a major impediment to investment in phytoremediation technologies [1]. For example, organic phytoremediation efficiency has varied depending on the concentration and composition of contaminants [2,3], and initial planting advantages have disappeared after a year or less [3,4]. Tradeoffs can also exist between promoting plant growth (which increases the volume of plant-influenced soil) and microbial remediation efficiency following co-treatments such as nutrient application [5].

Traditional characteristics sought for the ideal phytoremediation plant are exceptional contaminant tolerance, quick growth on degraded land, and rapid biomass production, and work has focused on developing transgenic plants

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Glossary

Endophyte: microorganism, generally a bacterium or fungus, that grows nonpathogenically inside a plant. Endophytes may assist in plant tolerance to contaminants that are taken up into the body of the plant.
Endosphere: term describing the plant interior as a microbial habitat; environment in which endophytes reside. May refer to either the aerial or root components of a plant, or both.
Metabolome: complement of metabolites associated with an individual or mixed-species community (when performed on mixed communities, sometimes termed meta-metabolome). Frequently studied in plants and may be used to assess differences in activity between plant tissues.
Metagenome: as opposed to a single isolated genome, the metagenome represents DNA elements from (ideally) all members of a mixed-species community. This term has often been used in conjunction with mixed microbial communities from which total DNA is extracted from lysed cells.
Metatranscriptome: identical to the definition of metagenome, but represents total extracted RNA. RNA can degrade quickly and expression generally varies on a timescale much shorter than total DNA, so the time of sampling and the preservation method are critical for meaningful comparisons between samples.
Microbiome: all microorganisms inhabiting a specific environment. For instance, the gut microbiome may refer to all microorganisms inhabiting a particular intestinal tract, but the composition of this microbiome can vary substantially between individuals.
Phyllosphere: surface area of the aerial portions of plants. Owing to limited resource availability (e.g., compounds released by plants, airborne compounds, sunlight), microbial diversity in these habitats is generally low relative to the rhizosphere.
Quantitative trait loci: loci that contribute to a trait that is dependent on the combined influence of multiple genes and the environment (also known as polygenic traits). Many plant traits cannot be related directly to single genes, so large data approaches will help in identifying clusters of important genes across populations and different environments.
Rhizosphere: zone in soil that is influenced directly by plant roots. This includes the surface of the roots (rhizoplane) and any external region that is affected by root exudation.

Box 1. Plant and microbial contributions to phytoremediation

The possible fates of soil pollutants following plant introduction are described clearly in a review by Pilon-Smits [69]. Here we show the major contributions of both introduced plants and partner microorganisms in the phytoremediation of mixed contaminant soils (Figure I). Soil microorganisms are the primary agents of organic mineralization in soil, and may also convert contaminants such as heavy metals to stable and/or less toxic forms. Although such microbial activity can occur in bulk soil, introduced plants have the potential to augment microbial contributions to bioremediation in the soil via stimulation of microbial biomass and/or activity in the rhizosphere, although this activity is dependent on which specific microorganisms and activities are promoted. Plant–microbe interactions are complex, and plants may favor microorganisms that promote their growth or provide protection from pathogens if pollutant stress is not sufficiently elevated, whereas opportunistic microorganisms that do not contribute to phytoremediation may also capitalize on plant-produced compounds.

Microorganisms can also facilitate the uptake of pollutants such as heavy metals by plant roots, which are then translocated (absorbed and relocated) to other components of the plant. Plants can store many contaminants in biomass that can later be harvested, but some compounds are volatilized from the aerial portions of the plant. However, volatilization without prior transformation may simply release toxic compounds into the air. Microorganisms that reside on or within aerial plant tissue can help to stabilize and/or transform contaminants that have been translocated, which may limit the extent of volatilization. Microorganisms that form direct associations with either the above-ground or below-ground portions of plants can positively (for a review on plant-growth-promoting bacteria, see [70]) or negatively influence plant growth and fitness, which alters the ability of a plant to directly remediate and/or stimulate associated organisms.
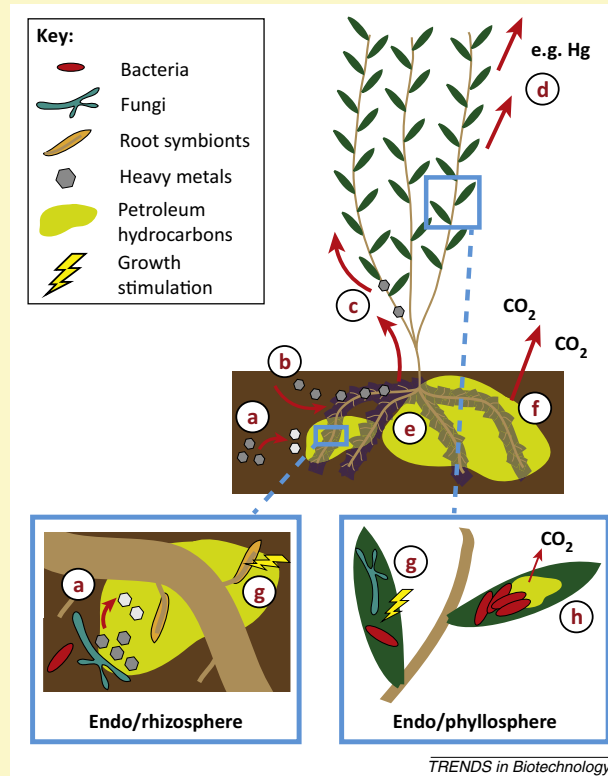

Figure I. Primary contributions of plants and associated microorganisms to phytoremediation in mixed contaminant soils. Metals may be (a) transformed by microorganisms in the rhizosphere, (b) taken up by the plant, (c) translocated to plant tissue, and/or (d) volatilized. Microbial activity can be stimulated directly by plants through (e) root exudate release, which may be particularly important for promoting (f) microbial degradation of hydrocarbons. Through a variety of mechanisms, microorganisms also influence (g) plant growth and can be involved in (h) pollutant transformation in the aerial parts of plants.

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with increased tolerance to contaminants and/or enhanced contaminant uptake abilities [6,7]. It was recently shown that the composition of plant-recruited microbial communities can be independent of these characteristics and should also be considered in plant selection [8]. Microbial contributions to phytoremediation have been challenging to target, because they depend on the interactive activities of the plant and potentially thousands of microbial taxa with varying capacities for pollutant degradation and/or transformation.

Next-generation sequencing technologies appeared on the market in 2005 and have led to an explosion in our understanding of plants, microbes, and plant–microbe interactions. Large omics data sets are already being translated into usable technologies in the health sector (Box 2), and the ever-decreasing cost of sequencing now makes it possible to apply omics to environmental issues such as soil contamination. In this review, we discuss how new high-throughput molecular approaches have advanced our understanding of plant and microbial responses to pollutants and of plant–microbe interactions, and how phytoremediation strategies can be directed by omics data sets to harness the functional potential of introduced plants and their associated microbes. Although we focus

on phytoremediation, omics probing of plant–microbe assemblages will likely guide the next generation of strategies for managing invasive species, restoring disturbed sites, and optimizing crop production.

Microbial omics: enhancing predictability and community modification

A frequently cited roadblock in the study of natural microbial communities is that over 99% of microbial taxa are yet to be cultured and can only be characterized using culture-independent methods [9]. Omics technologies now permit analysis of microbial community composition and activity while preserving the fingerprint of biotic and abiotic factors that shaped these communities *in situ*. These omics approaches can be applied at several scales that are relevant to phytoremediation (Box 3).

Metagenomics

Metagenome analysis of plant-dependent environments such as the rhizosphere, endosphere (see Glossary), or phyllosphere, whether through shotgun sequencing or amplicon-targeting of specific genes, can be used to probe plant-associated microbial communities. High-throughput sequencing of bacterial 16S rDNA has shown that

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Box 2. Manipulating complex microbial communities

Hamilton *et al.* recently used Illumina sequencing of amplified 16S rDNA from gut metagenome extracts to show that fecal transplantation from healthy donors into recipients suffering from *Clostridium difficile* infection led to major shifts in the composition of the recipient's intestinal microbiome [71]. Treated patients were quickly alleviated of symptoms related to their infection. Interestingly, it appears that the antibiotic administration was responsible for shifting the gut microbial community to a stable and infectious state that could not revert to a healthy state without intervention. A similar approach has been used extensively and successfully to reduce the incidence of chytridiomycosis in tropical amphibians [72].

The addition of efficient bioremediating microorganisms to existing soil microbial communities has not seen wide success in enhancing bioremediation, presumably because of competitive exclusion by well-adapted indigenous communities [73], but many of the microbial genes and taxa required in remediation are already present in the environment. It remains to be shown whether true alternative stable states (distinct communities that persist under identical environmental conditions) can exist for microbial communities [74], but community composition can be shifted using treatments that alter the environment. Although not an efficient or feasible approach at large scales, administration of antibiotics to diesel-contaminated soils modified microbial community composition and increased bioremediation in some cases [75]. However, a metagenomic analysis of 16S rDNA amplicons from diesel-contaminated soils showed that nutrient addition predictably shifted bacterial communities and the capacity of the community to degrade diesel compounds [24]. This shift depended strongly on soil properties, but when using plant hosts that target key microorganisms, plant stimulation may supersede the influence of other environmental factors. In addition, plant stimulation can be modified; a recent study showed that treatment of *Arabidopsis* with methyl jasmonate led to substantial shifts in rhizosphere 16S rDNA composition [76].

Another promising approach to community manipulation is the transformation of resident microorganisms through plasmid introduction. This has been successfully used to provide contaminant-degrading abilities to endophytes in the aerial portions of plants [19,20]. Plasmid-bearing microbes have also been added to seeds before germination and have transferred their abilities to surrounding microorganisms [77]. Various stresses encourage cross-species plasmid transfer [78], so additional treatments may facilitate more extensive exchange of introduced mobile elements, whereas the rhizosphere is already known as a hotspot for horizontal gene transfer [16].

Microbial manipulation in the environment has not been widely explored, but other approaches such as plant selection and development, fungal partnering, and modification of plant physiological states warrant further investigation for their potential in modifying plant-associated microbial communities.

microbial communities in bulk soil, the rhizosphere, and the root endosphere are distinct [10,11]. Assembly of root-inhabiting bacterial communities depends partly on the plant and soil type and partly on generic plant structural features [10]. Sequencing of 16S rDNA amplicons from leaf surfaces showed that environmental factors, not plant type, shape bacterial community composition [12]. The degree to which plants used in phytoremediation exert control over microorganisms also depends on soil contaminant concentrations. A recent study conducted as part of a phytoremediation field trial used targeted amplicon sequencing to show that fungal communities are shaped by both plant phylogeny and soil contaminant concentrations [8]. Similarly, molecular analysis showed that

arbuscular mycorrhizal composition in the rhizosphere depends on heavy metal concentrations [13]. This suggests that plant stress may actually determine stimulation of microbial bioremediation. Although root exudates can repress organic biodegradation [14,15], exudates produced by hydrocarbon-exposed wild rye were less repressive than exudates from control wild rye [14], demonstrating that plants can alter the extent to which they promote rhizosphere biodegradation.

Mobile DNA, specifically plasmid DNA, is also of interest in phytoremediation, because the plant rhizosphere and contaminated soils are hotspots for plasmid exchange [16,17]. In toxic environments, plasmids are considered a source of genomic innovation and allow microorganisms to adapt to novel stress [18]. Metagenomic sequencing of the 'mobilome' (preferentially extracted plasmid DNA) from wastewater treatment plants showed that heavy metal resistance genes are disproportionately abundant in plasmids [18] and that various genes involved in organic degradation are plasmid-borne [17]. As mentioned in Box 2, plasmids can play an important role in microbial community manipulation. For instance, plasmids bearing toluene degradation genes were successfully introduced to aerial plant endophytes that apparently lacked such genes, and facilitated degradation of contaminants that would have been volatilized [19,20]. Metagenomics could be used to search for novel plasmids that allow gene distribution to a wider range of microorganisms to quantify transformation efficiency within the microbial community, and combined with (meta)transcriptomics to determine how plants and indigenous microbial communities are affected by plasmid introduction. Analysis of plant-associated viromes has revealed that many nonpathogenic viruses with unknown functions inhabit plants [21]. As isolation of specific metagenome components such as the virome becomes routine, less-studied forms of genetic exchange, including viruses and phage-like gene transfer agents [22,23], should be examined for potential in modifying microbial community function.

Metagenomic analyses can also assign functional attributes to microorganisms and microbial assemblages. Metagenomic surveys performed across environmental gradients showed that the genetic and taxonomic composition of a microbial community can be related to its carbon/hydrocarbon-metabolizing activity [24,25]. A PhyloChip analysis of metagenomic DNA identified specific taxa associated with plant disease suppression in soils [26]. This demonstrates the power of metagenomics for environmental applications, because microbial gene and taxonomic abundance can be directly related to functions such as hydrocarbon biodegradation and plant growth promotion in determining precise environmental parameters that favor target microorganisms.

To better characterize the potential of microorganisms that have yet to be cultured in contaminant remediation or plant growth promotion, new single-cell isolation and sequencing techniques can determine the genome content of uncultured strains and may potentially identify targets for isolation or manipulation in the field [27]. Diffusion chambers that allow signals to flow from the original environment can aid in microbial cultivation [9], and integration of

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Box 3. Applying omics at multiple scales

Omics technologies can be applied at multiple scales to assay biological responses to soil contaminants. In the context of environmental biotechnology, plants and microbes are the main targets of studies aiming to link genetic structure to function using omics-based approaches.

Single-organism omics

(i) Cultured microorganisms

Culturable microorganisms form the basis of our understanding of microbial physiology. Comprehensive sequencing of the genome of cultured isolates is now routine, and 100X coverage of a 5-Mbp genome can be achieved with 500 Mbp of sequencing, equivalent to a full run of 454 FLX or less than 1% of a flowcell of an Illumina HiSeq 2000 run. Genome assembly from a contaminant-tolerant isolate can reveal complete biotransformation pathways or genes needed to adapt to contaminant toxicity [79], while transcriptomic or proteomic approaches can identify differentially regulated genes in the presence of contaminants or plant metabolites.

(ii) Isolated single cells of uncultivated microorganisms

Droplet-based microfluidic devices now make it possible to isolate even uncultivable single microbial cells from the environment into arrays of wells [80]. Omics approaches similar to those described for cultured microorganisms can determine the genomic content and activity of these separated cells, potentially identifying key phytoremediation roles for uncultured taxa.

(iii) Plants

Plant omic analyses can be performed on individuals, communities, or populations, but in general, omics data are related back to single plants. Most efforts to characterize gene, protein, and metabolite networks have focused on single organisms, but high-throughput comparative approaches are revealing interplant variability [51], which will assist in plant selection and cultivation efforts.

Multiple-organism omics

(iv) Mixed microbial communities

Culture-independent omics analyses of DNA (metagenomics), RNA (metatranscriptomics), and proteins (metaproteomics/metaproteogenomics) permit assays of the structure and activity of mixed microbial populations living in and around plants. The greatest advantages of this approach are that important microorganisms that have yet to be cultivated are not ignored, and the *in situ* activity of the community can be determined.

(v) Large-insert functional screening

One drawback of omics-based approaches is that functions cannot be reliably assigned to many newly discovered genes. Insertion of large genomic fragments into plasmids and then *E. coli* hosts can screen for properties such as hydrocarbon metabolism. This has traditionally been a laborious process, but high-throughput sequencing of clones yielding positive hits for a given function will assist in this characterization.

(vi) Metaorganism omics

Rather than separating plant and microbial components, metaorganism omics would rely on high-depth analysis to cover the integrated responses of plants and microorganisms to contaminants. Because many plant and microbial activities are intimately interdependent, this may be the most rapid way to assess the suitability of particular plant–microbe assemblages for phytoremediation applications.

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plant-produced compounds or soil contaminants may help in isolating target microbes. In addition, clone libraries with large metagenomic inserts can be screened to assign functional properties to genes [28] and assess functions for organisms that remain resistant to isolation.

Metatranscriptomics

Although the metagenome reveals microbial genetic potential, the metatranscriptome more accurately represents activity and provides some indication of which taxonomic groups contribute to contaminant transformation or reduction. This is an important link because abundant microorganisms could occupy limiting resources without actively contributing to bioremediation or plant survival. A recent study showed a strong link between relative mRNA abundance and atrazine-degrading activity, and demonstrates that, in at least some cases, metatranscriptomics can directly predict microbial community function [29]. Although mRNA enrichment is challenging [30], reduced sequencing costs mean that metatranscriptomes can now be sequenced with sufficient depth to accurately assess active taxa and genes without PCR bias. In the context of phytoremediation, a metatranscriptomic comparison was used to investigate shifts in rhizosphere microbial expression following willow introduction in both uncontaminated and contaminated soils [31]. Interestingly, willow presence only marginally altered the structure of the active microbial community, but dramatically shifted expression patterns. Many genes involved in hydrocarbon biodegradation were most abundantly expressed in planted contaminated soils, but so were antibiotic resistance and biofilm formation genes; this suggests that interspecies interactions had an increased importance in these soils. The probable composition of willow root exudates was also indirectly indicated through shifts in gene expression for carbon and amino acid uptake. Although the identity of many transcripts remains elusive, continued efforts in culturing, functional metagenomics, and predictive bioinformatics will refine metatranscriptomic assessments of microbial activity.

Eukaryotes generally represent a minute proportion of metagenomic reads, but a metatranscriptomic comparison of rhizosphere communities showed that eukaryotes represented over 25% of (non-plant-related) reads in some cases, but less than 10% of reads in the bulk soil control [30]. In the same study, a *sad1* oat mutant (no antifungal avenacin production) had substantial effects on eukaryotic expression, which demonstrates the ability of plants to contribute to selection of their surrounding microbiota. In the future, combination metagenomic–metatranscriptomic approaches will indicate how effectively plant-associated microbial communities contribute to phytoremediation relative to their phytoremediation potential. A paired study showed that although genes involved in hydrocarbon biodegradation were abundant in discharges from the Deepwater Horizon spill, the expression of many of these genes was limited [32]. Identification of such gaps between actual and potential activity will indicate whether reducing environmental limitations can enhance microbial function or if community modification is essential. In addition, temporal metatranscriptomic studies can be exploited to observe how key microbial activities shift over time (Box 4).

Metaproteomics

Metaproteomics has been used less extensively to investigate microbial activities associated with plants and

Box 4. Observing rapid microbiome shifts using metatranscriptomics

Although the microbiome metagenome may include genes and organisms that do not contribute substantially to community function, the metatranscriptome may overlook some that do. A comparative transcriptomic study of *Lactococcus laccis* showed that mRNA half-lives ranged from seconds to roughly half an hour, but were shorter on average in faster-growing cultures [81], which in a mixed community may represent the organisms that are most active. This not only highlights the importance of appropriate RNA preservation techniques during sample collection, but also indicates that activities not occurring at the exact time of sampling will not be observed. These concerns can be partly alleviated by using sufficient replication, which reduces the bias associated with skewed mRNA proportions in single samples. Replication has allowed robust comparison of rhizosphere metatranscriptomes [30,31], revealing huge numbers of active organisms and genes.

Rapid mRNA turnover also means that metatranscriptomics are particularly practical for temporal studies. Klatt *et al.* found that different bacterial taxa in a geothermal mat dominated expression of phototrophic genes throughout the day [82]. Similarly, metatranscriptomic activity from mouse intestinal tracts was compared over the course of colitis flare-up and recovery [83], and a comparative metatranscriptome–metagenome approach was able to specifically identify two microbial groups, as well as a core set of genes, that were activated during extracellular electron transfer by sampling the metatranscriptome shortly after modifying redox conditions in a microbial fuel cell [84].

In the context of phytoremediation, temporal studies would clearly be advantageous for relating activity to contaminant composition during biodegradation, but could also be used to examine the strength of plant–microbe interactions throughout the course of plant development or with respect to changes in plant health. In addition, controlled studies could identify shifts not only in plant response but also within the rhizosphere microbiome during the invasion of pathogens or bioaugmented microbial strains, as was done in a study of the oral microbiome [85].

contaminants. A comparative metaproteomics/metagenomics approach revealed disproportionate dominance of methylotrophic proteins in the phyllosphere when compared to methylotroph abundance in the metagenome, although the authors noted that this might be due to curated database biases that favored identification of these proteins [33]. Metaproteomics is especially powerful in low-diversity environments such as the phyllosphere because proteins can be linked to one of only a few microorganisms to directly indicate which taxa exploit which carbon substrates [34]. Metaproteomics has also been combined with stable isotope probing of fluorene and naphthalene to reveal proteins synthesized by bacteria that exploit these organic pollutants [35]. Such an approach could also distinguish microorganisms that primarily metabolize plant exudates from those that degrade organic contaminants to identify microbial targets for promotion in phytoremediation.

Plant omics: cultivating the ideal host

Many plant species can naturally contribute to bioremediation through several routes, primarily pollutant accumulation, degradation (directly or indirectly via microbial stimulation), stabilization, and volatilization. Although plants were more visible to science than microbial communities before the advent of omics technologies, new omics approaches are revealing the genetic mechanisms behind phytoremediating activity and showing the incredible molecular variability between seemingly similar individuals.

Genomics

Genomic approaches have identified genes implicated in phytoremediation and/or plant tolerance to various soil contaminants, and high-throughput molecular techniques are especially important for identifying plant traits that depend on the combined activity of multiple genes. For instance, mapping of quantitative trait loci (QTL) of the progeny of a backcross between F1 hybrids of the zinc-tolerant *Arabidopsis halleri* and the zinc-intolerant *Arabidopsis lyrata* with *A. lyrata* identified three genomic regions that, when combined, explained 42% of plant zinc tolerance variation [36]. Similarly, QTLs for arsenic accumulation were identified in the leaves, stems, bracts, and kernels of recombinant inbred maize lines [37]. Interestingly, this study found that only one QTL was shared between tissues, suggesting that different genes are responsible for arsenic accumulation in different plant tissues. The identification of potential genes involved in phytoremediation does not necessarily have to pass through controlled crosses. For example, an association mapping study of 349 wild-collected *Arabidopsis thaliana* accessions helped to identify the heavy metal ATPase 3 gene as the sole locus responsible for cadmium accumulation in leaves [38]. Given the complexity within many plant genomes, sometimes including extreme polyploidy, much more extensive genomic characterization across phytoremediating plants will be needed to identify the mechanisms involved in rapid remediation across varied environments.

Transcriptomics, proteomics, and metabolomics

Activity-focused approaches such as transcriptomics, proteomics, and metabolomics allow examination of the function of candidate phytoremediators under different environmental and contaminant scenarios, and have been particularly useful in identifying plant activity in metal-contaminated environments. Transcriptomics has been used to identify genes actively involved in phytoremediation in *Brassica juncea* in sulfur- and chromium-contaminated environments [39] and to characterize the cadmium stress response of the hyperaccumulator *Sedum alfredii* [40]. Comparative transcriptomic analyses between species with contrasting phytoremediation abilities can also reveal genes responsible for these differences. For example, a study comparing the transcriptomes of *Solanum nigrum* (Cd hyperaccumulator) and *Solanum torvum* (low accumulator of Cd) revealed higher expression of certain genes in *S. nigrum*, including metal transporters, in response to Cd addition [41]. Although most plant omics analyses in the context of phytoremediation have been performed on plants exposed to metal contaminants, plants may play important direct roles in degrading, accumulating, or volatilizing organic molecules, in addition to stimulating organic-degrading microorganisms. Some plants accumulate polychlorinated biphenyls (PCBs) [42], 1,3,5-trinitro-1,3,5-triazine (RDX) [43], and 2,4,6-trinitrotoluene [43]. Even more interesting is that certain plants can directly hydroxylate PCBs [44]. Although few omics analyses have targeted plant responses to organic

contaminants, a gene expression study in poplar showed that five major genes involved in the detoxification of xenobiotic pollutants were differentially expressed for growth in the presence and absence of RDX [45].

Proteomics and metabolomics have also been used to identify candidate genes and reveal plant responses to pollutant-induced stress [46,47]. For instance, proteomics was used to characterize changes induced by cadmium in *Populus*, and showed that plant responses varied between leaves and cambium tissues and that the plant proteome is affected both directly by cadmium in tissues and indirectly through reduced metabolic rates caused by systemic toxicity [48]. As with microorganisms, future studies combining multiple omics techniques will provide a more global view of gene, protein, and metabolite networks, their interaction at the plant level, and variation at the environmental level [49].

A future for high-throughput plant screening?
To date, most plant omics analyses, particularly in the context of phytoremediation, have ignored patterns of natural variation, whether between closely related species or among individuals of the same species within a population. Yet analyses such as the QTL mapping studies mentioned above demonstrate that phytoremediation properties are often polygenic, and that natural variation exists and can be exploited for plant selection. It was previously suggested that population-level screening using omics technologies could be used to increase plant breeding efficiency [50]. Screening of natural variation in populations has shown that variation can be very important for bioremediation traits [38] and plant–microbe interactions [8]. Currently, large-scale omics comparisons are being used to determine genetic variability in model species such as *Arabidopsis* [51], but plant biotechnological applications including phytoremediation will depend on inexpensive comparative approaches to examine plants, especially non-model species, across environmental gradients. The traditional study of single clones, cultivars, or ecotypes may not be representative of particular species, and likely gives a biased view of phytoremediation potential.

Towards an integrated approach: the plant–microbe metaorganism
In both root and aerial parts of plants, plant–microbe relationships range from obligate endosymbioses to free-living microorganisms feeding on plant exudates [52]. Interactions in one part of a plant can even impact activity elsewhere; metagenomic analyses showed that shifting the soil microbiome can alter the leaf metabolome and ultimately leaf herbivory [53]. Reciprocal transplants demonstrated that soil microbiome adaptation to a particular stress can increase plant fitness in the presence of that stress [54], and fungal symbiont identity has altered plant metal uptake and distribution in tissues [55,56]. It is perhaps characterization of the plant–microbe metaorganism that is the most powerful application of omics technologies in phytoremediation, because this will create a (nearly) complete picture of how integrated biological communities interact to adapt to contaminant stress and enhance soil remediation. The metaorganism concept is now recognized as essential to

Table 1. Properties of the phytoremediation metaorganism that can be targeted by integrating; omics with traditional methods

Metaorganism subcomponent	Target properties
Microbiome	Optimal *in situ* contaminant degraders and/or stabilizers
	Intermicrobial interactions
	Relationships between gene content, expression, translation, and activity
	Role of plasmidome and virome as functional gene vectors
Interactome	Suppression and promotion of microbes by plants
	Suppression and promotion of plant growth by microbes
	Plant–microbe co-treatment of contaminants (e.g., contaminant translocation)
	Interkingdom gene transfer
	Integrated metabolic responses
	Relative plant:microbe investment in phytoremediation activity
	Nature of plant–microbe relationships (e.g., pathogenic, mutualistic, etc.)
Plantome	Root exudation patterns
	Physiological responses across contaminant gradients
	Physiological responses across heterogeneous environments
	Variations in expression between tissues
	Interspecies variability in gene content, expression, and activity

many areas of biology, because distantly related organisms are sometimes entirely interdependent in their activities [57]. Such integrated omics approaches may reveal information that remains undetected when organisms are examined in isolation (Table 1).

Combining omics with metaorganism-targeted isolation
Undoubtedly one of the greatest impediments to isolating microorganisms that have yet to be cultured is that most do not exist in isolation. Although the production of completely sterile plant hosts is likely not feasible, drastic reductions in the number of associated microorganisms would create a unique model for non-axenic isolation of microbes that are not captured using traditional *in vitro* culturing techniques. Plant-based culturing of arbuscular mycorrhizal fungi is established practice because this often represents an obligate symbiosis [58], but such techniques may be extended to the isolation of non-mycorrhizal microorganisms and plant-associated consortia, such as root-associated biofilms [59]. In addition, plant–microbe and microbe–microbe interactions within the plant environment may be tested by combining such models with omics approaches. Jonkers *et al.* examined the transcriptome and metabolome of competitor maize-infecting fungi both in isolation and in combination *in vitro* [60]. Interestingly, profiles from each were drastically altered in combination, and there was strong evidence of direct competition. The absence of the plant partner limits our understanding of how this interaction takes place *in situ* and whether the plant mediates this competition, demonstrating the utility of a metaorganism-based 'culture' system.

Co-expression patterns (interactomics)

The concept of coupled plant–microbe metatranscriptomic sequencing was proposed previously [61] and rapid decreases in sequencing costs have made this feasible, providing insight into an important functional interactome. For example, plant roots and adherent soil could be retrieved and ground, and the combined RNA extracted and sequenced without prior separation of plant and microbial RNA. Sequence discrimination would occur only *in silico* based on homology. Even following several cleaning and sterilization steps, identifiable bacterial sequences can represent over 25–30% of all transcripts in plant transcriptomic studies [62,63], and nearly 10% of assigned reads in some rhizosphere metatranscriptome data sets were classified as Viridiplantae [30]. Separated roots and adherent soil could also be sequenced separately to determine activity distributions inside and outside of the plant host, although separation and washing may discriminate against microorganisms that interact intimately with outer plant surfaces. Key discoveries for phytoremediation and other plant-based technologies will be (i) how plants trigger the assembly of beneficial microbial activity, (ii) how microorganisms respond to plant cues, and (iii) how undesirable microorganisms exploit plant hosts and potentially suppress the activity of co-occurring taxa. These points can be addressed using multi-organism omics across well-defined contaminant and environmental gradients, and such approaches will rapidly pinpoint how phytoremediation benefits of this metaorganism can be maximized, leading to quicker translation of omics discoveries into usable technologies.

The metaorganism in practice

Certain tightly connected symbioses have already been treated as metaorganisms using omics analyses. The first lichen transcriptome was recently sequenced for the reindeer lichen *Cladonia rangiferina* without separating the intimately connected algal and fungal partners [64]. Relative expression between partners was not representative of relative biomass, and certain pathways were shared between the two organisms. Similarly, a legume–rhizobium transcriptome was extracted from plant–rhizobium pairings, and the proportion of variation in expression across treatments due to each partner was determined using microarrays [65]. Whole-genome sequencing of *Hydra* inadvertently produced a complete genome of a novel bacterial endosymbiont, a *Curvibacter* species, that contains a much higher diversity of sugar transporters than known related bacteria, suggesting a unique adaptation to its symbiotic lifestyle [66]. Although the phytoremediation metaorganism consists of a considerably larger array of interacting members, remediation efficiency will depend on the combined activities of these participants. Extension of the metaorganism approach will reveal which organisms are most affected by treatments or most involved in activity, and will yield the possibility of discovering interaction-dependent processes that cross species- and kingdom-level boundaries.

Concluding remarks and future perspectives

Phytoremediation is a promising technology that can become a reliable, efficient alternative for remediation of contaminated soils with direction from new omics

Table 2. Practical approaches for producing useful omics data that can direct improvements in phytoremediation

Application	Approach	Example	Refs
Isolate uncultivable microorganisms	Mine omics data to identify unknown factors needed for microbial survival in culture	Metatranscriptomics identified growth substrates needed by uncultivated bacterium in medicinal leech gut and a new medium led to successful cultivation	[86]
Select optimal plant hosts	Identify differential shifts in associated microbial communities	Amplicon-targeted metagenomic sequencing showed differential promotion of fungal communities in the rhizosphere by willow cultivars	[8]
	Assess the effect of plant presence on microbial activity	Comparative metatranscriptomics showed that the expression of hydrocarbon biodegradation genes was higher in contaminated soils when willows were present	[31]
	Select plants with highest bioremediation activity for breeding	Genetic mapping of genes involved in accumulation of Zn, As, and Cd	[36–38]
Improve genetic engineering of plants	Compare plant genomes with bioremediation efficiency to identify optimal/novel targets for transgenes	Identified genes potentially involved in the detoxification of xenobiotic (RDX) pollutants via transcriptomics	[45]
Efficient isolation of remediation-related genes	High-throughput sequencing of functional metagenomic libraries	Review of function-based metagenomic screening	[28]
Determine efficacy of soil amendments	Apply amendments over a wide range of environmental conditions and compare omics and functional responses to determine parameters of efficiency	Amplicon-targeted metagenomic sequencing showed that diesel degradation efficiency following mono-ammonium phosphate addition was correlated to organic matter content and promotion of Betaproteobacteria	[24]
Identify cryptic improvements in bioremediating microbial communities	Screen for desired microbial communities/genes. Use comparative omics to determine whether communities are optimal, even if limited expression or translation prevents increases in bioremediation activity	Metagenomic–metatranscriptomic comparison showed that many important biodegradation genes were not being expressed in the plume of the Deepwater Horizon spill, despite gene presence in the metagenome	[32]
Identify microorganisms lacking from target soils	Screen soil metagenomes for microorganisms that are limited by dispersal. Determine whether desired plant symbionts are present/abundant	Clone libraries and GeoChip analyses on metagenomic DNA showed dispersal limitations of fungal structure and function across a chronosequence following glacier recession	[87]

Box 5. Outstanding questions

- To date, genetic engineering of plants for phytoremediation has focused on modifying traits such as contaminant tolerance or uptake by the plant. Plants are capable of promoting microbial communities that are beneficial to phytoremediation, but this is also dependent on additional plant stimuli. Will paired microbe–plant omics reveal modifiable genetic targets for plant promotion of desirable microbial communities across heterogeneous environments?
- Degradation of organic contaminants in soils often occurs much more slowly than in culture, likely as a result of both abiotic limitations and intermicrobial competition. Although omics analyses are revealing the abiotic factors that limit the growth of microorganisms in soil communities, assessment of competition is more challenging. How can interspecies competition be assessed using large culture-independent data sets, and can the expression of specific genes be correlated to the intensity of competition in the soil environment?
- Plant activity in response to contaminants varies between species and across individuals of the same population. Can high-throughput approaches be developed to allow plant analyses to keep pace with meta-omic analyses of microbial communities?
- Separate plant and microbial omics analyses can reveal pathways that are activated by each in response to soil contamination. Would metaorganism omics enable the identification of cross-species or cross-kingdom functions that can be targeted to increase soil remediation?

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approaches. Already, large omics data sets have defined many environmental parameters that shape microbial communities and plant activity following contaminant introduction, and have helped in characterizing interactions between plants and microorganisms. The potential of natural systems can be harnessed by modifying microbial communities through plant introduction, or plant activity through microbial community manipulation, which has applications not only for phytoremediation but also for fields such as biofuel production, invasive species management, and agriculture.

A huge advantage of the omics revolution is the enormous, ever-growing bank of freely available data. As more omics studies focus on microorganisms associated with plants and contaminated soils, meta-analytic approaches will extract new insights from these data. Of particular interest will be the ability to predict shifting microbial activities over time [67] to optimize phytoremediation activity as contaminant composition changes. The advent of sequencing platforms yielding reads in the kilobase pair range will also greatly improve such studies by facilitating the identification of large fragments, yielding complete operons and minimizing tedious genomic assembly.

Translation of knowledge to usable technologies will depend on studies that employ omics approaches with clear applied goals (Table 2). In addition, some efforts to optimize phytoremediation systems should occur at the metaorganism level rather than specifically targeting plants or microorganisms. Although omics analyses are increasingly inexpensive, these data must eventually be combined with high-throughput isolation and screening of key microbial characteristics such as growth rate [68]. This will help in determining the potential activity of microbes that are perhaps not naturally dominant, but that should be targeted in phytoremediation treatments. Questions

remain regarding the best use of omics to yield practical results (Box 5), but the enormous diversity of genes and organisms identified from contaminated soils to date suggests that innovation in this field is just beginning.

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