Postprint (doi: 10.1016/j.tibtech.2014.02.008)

Increasing phytoremediation efficiency and reliability using novel 'omics approaches

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Abstract

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 also allow us to understand integrated activity patterns between plants and microbes, and determine how this *metaorganism* can be modified to maximize growth, appropriate microbial community assembly, 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.

Keywords: phytoremediation; metagenomics; metatranscriptomics; metaorganism; bioremediation; next-generation sequencing

Glossary

Endophyte: a microorganism, generally a bacterium or fungus, which grows non-pathogenically inside a plant. Endophytes may assist plant tolerance to contaminants that are taken up into the body of the plant.

Endosphere: used to describe the plant interior as a microbial habitat, and is the environment in which endophytes reside. May refer to either the aerial or root components of a plant, or both.

Metabolome: the complement of metabolites associated with an individual or a 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. Often this term has been used in conjunction with mixed microbial communities, from which total DNA is extracted from lysed cells.

Metatranscriptome: identical to the definition of the metagenome, but represents total extracted RNA. As RNA can degrade quickly and expression generally varies on a timescale much shorter than total DNA, the time of sampling and preservation method is critical to making meaningful comparisons between samples.

Microbiome: refers to 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: generally refers to the surface area of the aerial portions of plants. Due 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). Since many plant traits cannot be related directly to single genes, big data approaches will help to identify clusters of important genes across populations and different environments.

Rhizosphere: the 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.

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, while 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 due to its variable effectiveness. Uncertainty of full and rapid site remediation is cited as a major impediment to investment in phytoremediation

technologies [3]. For example, organic phytoremediation efficiency has varied depending on contaminant concentration and composition [4, 5], and initial planting advantages have disappeared after a year or less [5, 6]. 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 [7].

Traditional characteristics sought for the ideal phytoremediation plant are exceptional contaminant tolerance, quick growth on degraded land, and rapid biomass production, while work has focused on developing transgenic plants with increased tolerance to contaminants and/or enhanced contaminant uptake abilities [8, 9]. Recently, however, it was shown that the composition of plant-recruited microbial communities can be independent of these characteristics, and should also be considered in plant selection [10]. Microbial contributions to phytoremediation have been challenging to target, as 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 datasets 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 plant-microbe interactions, and how phytoremediation strategies can be directed by 'omics datasets 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 [23]. 'Omics technologies now permit microbial community

composition and activity analysis 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 (e.g. rhizosphere, endosphere (see Glossary), phyllosphere (see Glossary)), 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 microbial communities in bulk soil, the rhizosphere and root endosphere are distinct [27, 28]. Assembly of root-inhabiting bacterial communities depends partly on plant and soil type and partly on generic plant structural features [27]. Sequencing of 16S rDNA amplicons from leaf surfaces showed that environmental factors, not plant type, shaped bacterial community composition [29]. 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 were shaped by both plant phylogeny and soil contaminant concentrations [10]. Similarly, molecular analysis showed that arbuscular mycorrhizal composition in the rhizosphere depends on heavy metal concentrations [30]. This suggests plant stress may actually determine stimulation of microbial bioremediation. While root exudates can repress organic biodegradation [31, 32], exudates produced by hydrocarbon-exposed wildrye were less repressive than control wildrye exudates [31], demonstrating that plants can alter the extent to which they promote rhizosphere biodegradation**.**

Mobile DNA, specifically plasmid DNA, is also of interest in phytoremediation, as the plant rhizosphere and contaminated soils are hotspots for plasmid exchange [22, 33]. In toxic environments, plasmids are considered a source of genomic innovation, allowing microorganisms to adapt to novel stress [34]. Metagenomic sequencing of the 'mobilome' (preferentially extracted plasmid DNA) from wastewater treatment plants showed that heavy metal resistance genes were disproportionately abundant on plasmids [34], and various genes involved in organic degradation are also plasmid-borne [33]. 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, facilitating degradation of contaminants that would have been volatilized [18, 19]. 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 non-pathogenic viruses with unknown functions inhabit plants [35], and as it becomes routine to isolate specific metagenome components like the virome, less-studied forms of genetic exchange, including viruses and phage-like gene transfer agents [36, 37], 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 productivity [16, 38], while a PhyloChip analysis of metagenomic DNA identified specific taxa associated with plant disease suppression in soils [39]. This demonstrates the power of metagenomics for environmental applications, as microbial gene and taxonomic abundance can be directly related to functions like hydrocarbon biodegradation and plant growth promotion, while determining precise environmental parameters that favour target microorganisms.

To better characterize as-yet-to-be-cultured microorganisms with potential in contaminant remediation or plant growth promotion, new single-cell isolation and sequencing techniques can determine the genome content of uncultured strains, potentially identifying targets for isolation or manipulation in the field [40]. Diffusion chambers that allow signals to flow from the original environment can aid in microbial cultivation [23], while integration of plant-produced compounds or soil contaminants may help isolate target microbes. Additionally, clone libraries with large metagenomic inserts can be screened to assign functional properties to genes [41] and assess functions from organisms that remain resistant to isolation.

Metatranscriptomics

While the metagenome reveals microbial genetic potential, the metatranscriptome (see Glossary) more accurately represents activity, and provides some indication of which taxonomic groups contribute to contaminant transformation or reduction. This is an important link, since 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, demonstrating that, in at least some cases, metatranscriptomics can directly predict microbial community function [42]. Although mRNA enrichment is challenging [43], 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 [44]. Interestingly, willow presence only marginally altered active microbial community structure, 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, suggesting an increased importance of interspecies interactions. The probable composition of willow root exudates was also indirectly indicated through shifts in carbon and amino acid uptake gene expression. 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 [43]. In this same study, a *sad1* oat mutant (no anti-fungal avenacin production) had substantial effects on eukaryotic expression, demonstrating the ability of plants to contribute to selection of their surrounding microbiota. Moving forward, 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 while genes involved in hydrocarbon biodegradation were abundant in the Deepwater Horizon spill discharge, expression of many

of these genes was limited [45]. Identifying 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 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 note that this may be due to curated database biases that favoured identification of these proteins [51]. Metaproteomics is especially powerful in low-diversity environments such as the phyllosphere, as proteins can be linked to one of only a few microorganisms to directly indicate which taxa exploit which carbon substrates [52]. Metaproteomics has also been combined with stable isotope probing of fluorene and naphthalene to reveal proteins synthesized by bacteria exploiting these organic pollutants [53]. 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; see Glossary) of a backcross progeny between the F1 hybrid of the zinc-tolerant *Arabidopsis halleri* and the zinc-intolerant *A. lyrata* with *A. lyrata* identified three genomic regions that, when combined, explained 42% of plant zinc tolerance variation [54]. Similarly, QTLs for arsenic accumulation were identified in the leaves, stems, bracts and kernels of maize using recombinant inbred lines [55]. 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, however. 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 [56]. Given the complexity within many plant genomes, including sometimes 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 the function of candidate phytoremediators to be examined 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 sulphur- and chromium-contaminated environments [57] and to characterize the cadmium stress response of the hyperaccumulator *Sedum alfredii* [58]. 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* and *S. torvum* (a hyperaccumulator and low-accumulator of Cd, respectively) showed higher expression of certain genes in *S. nigrum*, including metal transporters, in response to Cd addition [59]. 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 have been shown to accumulate PCBs [60], 1,3,5-trinitro-1,3,5-triazine (RDX) [61], and 2,4,6-trinitrotoluene (TNT) [61]. Even more interesting is that certain plants can directly hydroxylate PCBs [62]. 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 when grown in the presence or absence of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) [63].

Proteomics and metabolomics have also been used to identify candidate genes and reveal plant responses to pollutant-induced stress [64, 65]. For instance, proteomics was used to characterise changes induced by cadmium in *Populus*, showing that plant response 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 [66]. 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 [67].

A future of 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 [68]. Screening of natural variation in populations has shown that variation can be very important for bioremediation traits [56] and plant-microbe interactions [10]. Currently, large-scale 'omics comparisons are being used to determine genetic variability in model species such as *Arabidopsis* [26], but plant biotechnological applications including phytoremediation will depend upon inexpensive comparative approaches to examine plants, especially non-model species, across environmental gradients. The study of single clones, cultivars or ecotypes, as has generally been done, 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 the root and aerial portions of the plant, plant-microbe relationships range from obligate endosymbioses to free-living microorganisms feeding on plant exudates [69]. Interactions in one part of the plant can even impact activity elsewhere, as metagenomic analyses showed that shifting the soil microbiome can alter the leaf metabolome (see Glossary) and ultimately leaf herbivory [70]. Reciprocal transplants demonstrated that soil microbiome adaptation to a particular stress can increase plant fitness in the presence of that stress [71], while fungal symbiont identity has altered plant metal uptake and distribution in tissues [72, 73]. It is perhaps characterization of the plant-microbe *metaorganism* that is the most powerful application of 'omics technologies in phytoremediation, as it 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 many areas of biology, as sometimes distantly related organisms are entirely interdependent in their activities [74]. 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 as-yet-to-be-cultured microorganisms is that most do not exist in isolation. Although production of completely sterile plant hosts is likely not feasible, drastically reducing 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 since this is often an obligate symbiosis [75], but such techniques may be extended to isolating non-mycorrhizal microorganisms and plant-associated consortia, such as root-associated biofilms [76]. Additionally, plant-microbe and microbe-microbe interactions within the plant environment may be tested by combining such models with 'omics approaches. Jonkers et al. [77] recently examined the transcriptome and metabolome of competitor maize-infecting fungi both in isolation and in combination *in vitro*. 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 has been proposed previously [78], and rapid drops in sequencing costs now make this feasible, providing insight into an important functional interactome. For example, plant roots and adhering soil could be retrieved and ground, and the combined RNA extracted and sequenced without pre-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 [79, 80], while nearly 10% of assigned reads in some rhizosphere metatranscriptome datasets classified as *Viridiplantae* [43]. Separated roots and adherent soil could also be sequenced separately to determine activity distribution 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 1) how plants trigger assembly of beneficial microbial activity, 2) how microorganisms respond to plant cues, and 3) 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 [81]. 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 [82]. Whole genome sequencing of hydra inadvertently produced a complete genome of a novel bacterial endosymbiont, *Curvibacter* sp., which contained a much higher diversity of sugar transporters than known related bacteria, suggesting a unique adaptation to its symbiotic lifestyle [83]. Although the phytoremediation *metaorganism* consists of a considerably larger array of interacting members, remediation efficiency will depend on the combined activities of these participants. Extending the *metaorganism* approach will reveal which organisms are most affected by treatments or most involved in activity, and yields the possibility of discovering interaction-dependent processes that cross species- and kingdom-level boundaries.

Concluding remarks and future directions

Phytoremediation is a promising technology that can become a reliable, efficient alternative for remediating contaminated soils with direction from new 'omics approaches. Already, large 'omics datasets have defined many environmental parameters that shape microbial communities and plant activity following contaminant introduction, and have helped characterize 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 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 [84] 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). Additionally, some efforts to optimize

phytoremediation systems should occur at the *metaorganism* level, rather than specifically targeting plants or microorganisms. While '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 [87]. This will help determine 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 (see Outstanding Questions), but the enormous diversity of genes and organisms identified from contaminated soils to date suggests that innovation in this field is just beginning.

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 stimuli to the plant. 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, assessing competition is more challenging. How can interspecies competition be assessed using large culture-independent datasets, 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?

Acknowledgements

The authors acknowledge funding for this work by the GenoRem Project, which is primarily financed by Genome Canada and Genome Quebec. TH Bell was also supported by a grant from the Fonds québécois de la recherche sur la nature et les technologies.

Disclaimer statement

The GenoRem project is funded in part by industrial partners, but these partners in no way influenced the content or concepts of this manuscript.

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Element Descriptions

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 [1]. 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 through stimulation of microbial biomass and/or activity in the rhizosphere (see Glossary), although this activity is dependent on which specific microorganisms and activities are promoted. Plant-microbe interactions are complex, and plants may favour microorganisms that promote their growth or provide protection from pathogens if pollutant stress is not sufficiently elevated, while 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. Volatilization without prior transformation, however, 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 aboveground or belowground portions of plants can positively (for a review on plant growth promoting bacteria, see [2]) or negatively influence plant growth and fitness, which alters the plant's ability to directly remediate and/or stimulate associated organisms.

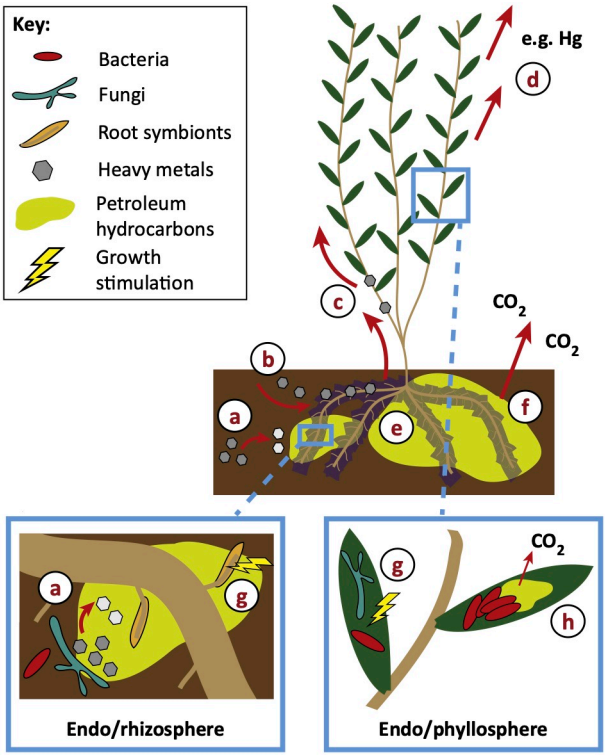

Figure I. The primary contributions of plants and associated microorganisms in phytoremediation in mixed contaminant soils. Metals may be a) transformed by microorganisms in the rhizosphere, b) taken up by the plant, c) translocated to plant tissue, and/or d) volatilized. Microbial activity can be stimulated 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 portions of plants.

Box 2. Manipulating complex microbial communities.

Hamilton et al. [11] recently used Illumina sequencing of amplified 16S rDNA from gut metagenome (see Glossary) 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 (see Glossary). Treated patients were quickly alleviated of symptoms related to their infection. Interestingly, it appears that the administration of antibiotics 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 [12].

The addition of efficient bioremediating microorganisms to existing soil microbial communities has not seen wide success in enhancing bioremediation, presumably due to competitive exclusion by well-adapted indigenous communities [13], 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 [14], but community composition can be shifted using treatments that alter the environment. While 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 [15]. A metagenomic analysis of 16S rDNA amplicons from diesel-contaminated soils, however, showed that nutrient addition predictably shifted bacterial communities, as well as the capacity of the community to degrade diesel compounds [16]. This shift depended strongly on soil properties, but 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 [17].

Another promising approach to community manipulation is transforming resident microorganisms through plasmid introduction. This has been used successfully to provide contaminant-degrading abilities to endophytes (see Glossary) in the aerial portions of plants [18, 19]. Plasmid-bearing microbes have also been added to seeds pre-germination and have

transferred their abilities to surrounding microorganisms [20]. Since various stresses encourage cross-species plasmid transfer [21], additional treatments may facilitate more extensive exchange of introduced mobile elements, while the rhizosphere is already known to be a hotspot for horizontal gene transfer [22].

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 state warrant further investigation for their potential in modifying plant-associated microbial communities.

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

a) Cultured microorganisms

Culturable microorganisms form the basis of our understanding of microbial physiology. Comprehensive sequencing of cultured isolate genomes is now routine, as 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 [e.g. 24], while transcriptomic/proteomic approaches can identify differentially regulated genes in the presence of contaminants or plant metabolites.

b) 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 [25]. '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.

c) Plants

Plant 'omic analyses can be performed on individuals, communities, or populations, but in general, 'omic data will be related back to single plants. Most efforts for characterizing gene, protein and metabolite networks have focused on single organisms, but high-throughput comparative approaches are revealing inter-plant variability [26], which will assist in plant selection and cultivation efforts.

Multiple organism 'omics

d) 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 as-yet-to-be-cultivated microorganisms are not ignored, and the *in situ* activity of the community can be determined.

e) Large-insert functional screening

One drawback of 'omics-based approaches is that functions cannot be reliably assigned to many newly discovered genes. Inserting 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 this characterization.

f) Metaorganism 'omics

Rather than separate plant and microbial components, *metaorganism* 'omics would rely on high-depth analysis to cover the integrated responses of plants and microorganisms to contaminants. As 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.

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 [46], which in a mixed community may represent the organisms that are most active. Not only does this highlight the importance of appropriate RNA preservation techniques during sample collection, but activities that are not occurring at the exact time of sampling will be 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 [43, 44], revealing huge numbers of active organisms and genes.

Rapid mRNA turnover also means that metatranscriptomics are particularly practical for temporal studies. Klatt et al. [47] found that different bacterial taxa in a geothermal mat dominated expression of phototrophic genes throughout the day. Similarly, metatranscriptomic activity from mouse intestinal tracts has been compared over the course of colitis flare-up and recovery [48], 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 [49].

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. Additionally, controlled studies could identify shifts not only in plant response, but within the rhizosphere microbiome, during the invasion of pathogens or bioaugmented microbial strains, as was done in a study of the oral microbiome [50].

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

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

Application	Approach	Example	Reference
Isolate uncultivable microorganisms	Mine 'omics data to identify unknown factors needed for microbial survival in culture.	Used metatranscriptomics to identify growth substrates needed by uncultivated bacterium in medicinal leech gut, and produced new medium that led to successful cultivation.	[85]
Select optimal plant hosts	Identify differential shifts in associated microbial communities	Used amplicon-targeted metagenomic sequencing to show differential promotion of fungal communities in the rhizosphere by willow cultivars.	[10]
	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.	[44]
	Select plants with highest bioremediation efficiency for breeding	Genetic mapping of genes involved in accumulation of Zn, As and Cd.	[54, 55, 56]

Improve genetic engineering of plants	Compare plant with bioremediation efficiency to identify optimal/novel targets for transgenes	Identify genes potentially involved in the detoxification of xenobiotic (RDX) pollutants via transcriptomics.	[63]
Efficient isolation of remediation-related genes	High-throughput sequencing of functional metagenomic libraries	Review of function-based metagenomic screening.	[41]
Determine efficacy of soil amendments	Apply amendments over a wide range of environmental conditions and compare 'omic and functional responses to determine parameters of efficiency.	Using amplicon-targeted metagenomic sequencing, found that diesel degradation efficiency following mono-ammonium phosphate addition was correlated to organic matter content and promotion of *Betaproteobacteria*.	[16]
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.	Through metagenomic-metatranscriptomic comparison, found that many important biodegradation genes were not being expressed in plume of Deepwater Horizon spill, despite gene presence in metagenome.	[45]

Identify microorganisms lacking from target soils	Screen soil metagenomes for microorganisms that are limited by dispersal. Determine whether desired plant symbionts are present/abundant.	Used clone libraries and GeoChip analyses on metagenomic DNA to show dispersal limitations of fungal structure and function across a chronosequence following glacier recession.	[86]