Horizontal Gene Flow in Managed Ecosystems

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Abstract

Mobilization of DNA by horizontal gene transfer (HGT) is an important process in the evolution of many organisms because it allows the recipient lineage to rapidly acquire metabolic innovations and adapt to new ecological niches. However, the significance of HGT in specific ecosystems remains poorly understood. In this review, we present major findings that illustrate how HGT affects managed ecosystems, such as farmlands, orchards, pastures, and managed grasslands. First, acquisition of functions via HGT can lead to the emergence of novel or more virulent pathogens and parasites of crops by allowing them to circumvent host defenses and currently used pest management approaches. Second, HGT of antibiotic resistance genes from the application of wastewater effluent for irrigation or manure as fertilizer can facilitate the emergence of highly resistant microbial lineages. Lastly, HGT can enhance the functional diversity of microbial communities and potentially influence biogeochemical processes. Characterization of lineages possessing horizontally acquired genetic material and their ecology will aid in enhancing the productivity and sustainability of managed ecosystems. We conclude with recommendations for key research directions that will advance our understanding of the causes and consequences of HGT in managed ecosystems.

INTRODUCTION

Horizontal gene transfer (HGT) is a fundamental biological process that allows a recipient organism to acquire genetic material from a donor, without the two having a direct ancestor-descendant relationship. This process occurs in all three domains of life, with genetic material transferred even across taxonomically recognizable species boundaries. Through HGT, many organisms can rapidly acquire pre-existing adaptive traits instead of gradually accumulating mutations and undergoing successive selection events, which could take millions of years.

Numerous studies have demonstrated the crucial role of HGT in the evolution and diversification of microorganisms. Many have focused on the early evolution of life on earth (Fournier et al. 2011, Szöllosi et al. 2013) and the biology of niche-specific organisms, such as extremophiles (DeMaere et al. 2013, Le Fourn et al. 2011, Nelson-Sathi et al. 2012), obligate symbionts (Alves et al. 2013, Leclercq et al. 2011), and clinical pathogens (Chewapreecha et al. 2014, McCarthy et al. 2014). In some instances, the acquisition of exogenous genes has radically modified the existing ecological conditions of a lineage. For example, a recent study reported the transfer of genes from a cellulolytic bacterium to a methanogenic archaeon of the genus *Methanosarcina* that occurred roughly 250 million years ago (Rothman et al. 2014). The transfer allowed the recipient organism to efficiently convert marine organic carbon, a rich but inaccessible source of energy, to methane. This new phenotypic acquisition instigated a sudden, explosive growth of these organisms, spewing prodigious amounts of methane into the atmosphere and causing significant perturbation in ocean and atmospheric levels of CO_2 and O_2 during the end-Permian era (Rothman et al. 2014). Rothman and colleagues (2014) hypothesized that such large-scale biogeochemical disruption likely contributed to the Earth's greatest mass extinction during this period. In another example, genes involved in plastid functions were acquired from *Chlamydia*-like bacteria early in the evolution of algae and plants. The acquired genes permitted export of the products of photosynthesis (or photosynthates) from the plastid and their polymerization into a storage polysaccharide in the cytosol (Price et al. 2012), an integral function in photosynthetic eukaryotes. These examples highlight the importance of HGT in generating ecological modifications that have widespread and lasting consequences on many lineages and the ecosystem as a whole.

The impact of HGT goes beyond the individual or species level and is therefore a vital component of the dynamics of any ecosystem. This is primarily because the acquisition of novel biological functions and traits can significantly alter the roles that an organism plays in its environment and the interactions it has with the rest of the community. Although many studies have generated crucial insights into the nature and mechanisms of HGT, a complete understanding of this process at the ecological level remains lacking. In this review, we present major findings that illustrate how HGT affects managed ecosystems at the ecological level, including structure, dynamics, and interactions between community members, with potential consequences for ecosystem productivity and sustainability. We define managed ecosystems as lands influenced by human actions aimed at maintaining or enhancing one or more ecosystem functions; examples include agro-ecosystems, farmlands, orchards, pastures, and managed grasslands. Several studies have already reported the occurrence of HGT between agricultural and clinical settings, but these have focused primarily on livestock (Spoor et al. 2013, Valentin et al. 2014, Worobey et al. 2014). In this review, we discuss the sources and impacts of horizontally transferred genes other than those originating from livestock, to highlight components of managed ecosystems that contribute to HGT and are often overlooked. Our review aims to bring these concepts to microbial ecologists, agronomists, plant and animal breeders, and others concerned with the sustainable management of ecosystems.

MECHANISMS OF HORIZONTAL GENE TRANSFER

Three primary mechanisms exist by which genetic material is horizontally acquired by microorganisms. Natural transformation occurs through the direct uptake of naked DNA from the environment. This DNA can come from cells and viral particles that are disrupted or decomposing, or it can be excreted by living cells (Thomas & Nielsen 2005). Natural transformation is considered the primary process by which DNA from distantly related lineages is obtained (Thomas & Nielsen 2005).

HGT can also occur through transduction, wherein viruses (phages) act as DNA vectors, or shuttles, between cells. The origin of DNA obtained through transduction largely depends on the host range of the phage. Some viruses are restricted to specific taxonomic groups for their hosts, as in the case of cyanophages in photosynthetic Cyanobacteria (Ma et al. 2014); actinophages in soil-dwelling, antibiotic-producing *Streptomyces* (Smith et al. 2013); and haloarchaeoviruses in the salt-requiring Haloarchaea (Luk et al. 2014).

Conjugation refers to the transfer of DNA through direct cell-to-cell contact. This process is mediated through the formation of (*a*) junctions that directly link cells to each other and (*b*) pores through which DNA, most commonly plasmids, can pass (Thomas & Nielsen 2005). Because conjugation requires compatible mating machineries between donor and recipient cells, the transfer of DNA usually occurs between close relatives (e.g., organisms of the same species or genus), as in the case of conjugative transfer within the genus *Streptomyces* (Thoma & Muth 2015). In rare cases, interphylum conjugation occurs, which can further expand the gene pool of the recipient lineage (Encinas et al. 2014, Shintani et al. 2014).

Plasmid: a small, double-stranded DNA molecule that is distinct from the chromosomal DNA and can replicate independently

Mobile genetic elements (MGEs): DNA segments that encode proteins that allow movement within the genome or between bacterial cells

Transposition: the relocation of a chromosomal segment to a different location in the genome

The repertoire of HGT mechanisms is further amplified by a variety of mobile genetic elements (MGEs) that facilitate the rapid dissemination of DNA between lineages irrespective of phylogenetic distance. Among the most common are transposons, gene transfer agents (GTAs), inteins, and integrons. Transposable elements or transposons are able to move around within a single cell through transposition and consist of retrotransposons, DNA transposons, insertion sequences (ISs), and helitrons (Ivancevic et al. 2013, Siefert 2009, Thomas et al. 2010). GTAs are tailed, phage-like particles that contain random segments of DNA from the GTA-producing cell and are able to transfer it to recipient cells (Lang et al. 2012, McDaniel et al. 2010). Unlike a virus, which carries a whole viral genome containing structural genes required for assembly, a GTA does not necessarily contain GTA-encoding genes and may carry only DNA pieces from the host cell (Lang et al. 2012). Inteins are self-splicing genetic elements that are able to precisely self-excise from a precursor protein (Swithers et al. 2013) and usually carry a homing endonuclease that permits horizontal transfer between highly divergent microbial lineages and even across domain boundaries (Clerissi et al. 2013, Soucy et al. 2014, Swithers et al. 2013). HGT is also facilitated by genetic elements called integrons, which can acquire exogenous DNA and convert it into functional genes through the direction of a promoter located upstream of the integration site that directs the transcription of gene cassette units (Labbate et al. 2012, Mazel 2006).

Horizontal chromosome transfer provides yet another important and rapid mechanism for conferring novel pathogenic attributes to recipient strains. Conditionally dispensable chromosomes (CDCs) carry host-specific toxin genes in a variety of fungal species, as in the case of CDC transfer in the ascomycete fungus *Alternaria arborescens*, the causal agent of stem canker in tomato (Hu et al. 2012). CDCs are extra chromosomes of $\leq$2.0 Mb in size and are not required for the fungus to survive, but they may carry genes required for specialized functions, such as infection of a host plant (van der Does & Rep 2012).

Whichever mechanism of transfer is employed, once an organism successfully acquires a new trait through HGT, it can rapidly spread across the population through secondary transfer to the

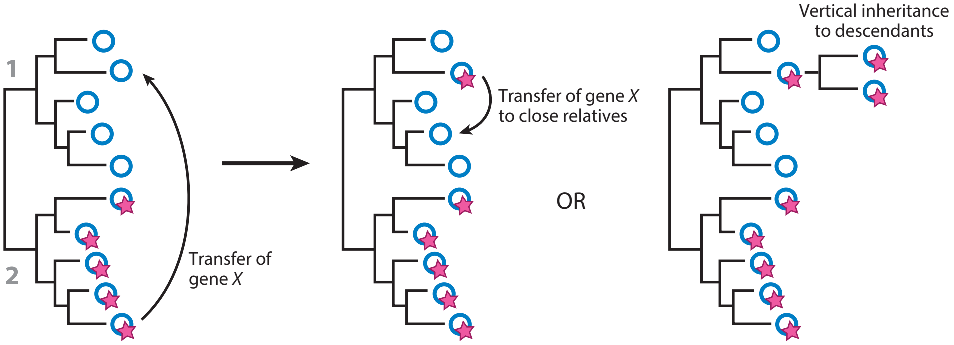

Figure 1

Schematic diagram showing the spread of a transferred gene within a population. Shown are microbial lineages one and two. Lineage two carries a trait, represented by a pink star, not present in one. A transfer from a donor strain in lineage two to a recipient strain in lineage one occurs. This is followed by secondary transfer from the original recipient strain in lineage one to a close relative and/or vertical inheritance to its descendants.

organism's close relatives, vertical inheritance from parent to progeny, or both (**Figure 1**). If the acquired trait persists through many generations, as in the case of the methanogens discussed above, it can eventually become a defining characteristic of that particular lineage (Huang & Gogarten 2009). Horizontally acquired traits can therefore be used as important molecular markers that can provide insight into the evolutionary history of major taxonomic lineages.

MANAGED ECOSYSTEMS AND THE ECOLOGY OF HORIZONTAL GENE TRANSFER

Occupying nearly 40% of the land in the world (Wirsenius et al. 2010), managed ecosystems are deliberately altered by humans to obtain specific goods and services. These systems experience pressures distinct from those found in most natural ecosystems because tremendous amounts of energy and material constantly enter and leave every year (e.g., fertilizer and seeds in, crops and livestock out). These pressures are brought about by intensive application of chemicals and other inputs as well as constant modifications of crop, soil, and water characteristics on relatively short time scales (e.g., seasonal, annual). Natural ecosystems can approach a steady state with less material input; more material is retained and recycled within the system, and larger disturbances occur less frequently (Cox 1984). Alternatively, natural systems can have large inputs and outputs owing to changes in plant community, disturbance, or human activity, but these types of alterations are unlikely to be as consistent as the pressures seen in agro-ecosystems. Thus, for managed systems to remain sustainable and productive in the long term, it is imperative that we understand how the movement of energy and materials can influence the generation of genetic and functional diversity (e.g., via HGT) present in the system.

In natural ecosystems, the characteristics and ecological relationships of different lineages have been established through millions of years of selective pressures from the environment. Acquisition of new traits through HGT undergoes the same selection process. By contrast, the frequent environmental modifications faced by managed ecosystems can significantly alter the structure and functions of the biological community relatively quickly. Thus, different lineages need to rapidly acquire novel traits to persist in the face of rapid perturbations in their environment. In addition, genotypes with novel traits can quickly emerge, which may have unknown and lasting

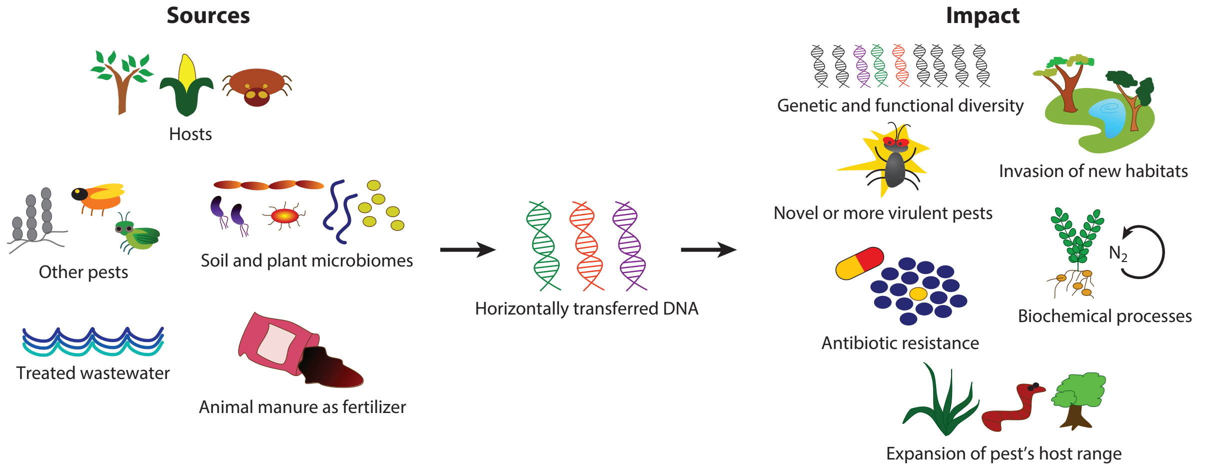

Figure 2

Sources and impacts of horizontal gene transfer in managed ecosystems. An overview of the sources of horizontally acquired genetic material in managed ecosystems is depicted, showing the contributions of plant and animal hosts, pests, microbial communities in soil, wastewater effluent, and animal manure used as fertilizer to the widespread availability and spread of exogenous DNA. When incorporated into the genomes of recipient lineages, horizontally acquired genes can potentially affect the genetic and functional diversity of the recipient population. The recipient can also acquire novel virulence properties, invade new hosts and ecological niches, and acquire antibiotic resistance. Biogeochemical cycles involving microbial transformation processes can also be affected through changes in the rates of biochemical reactions and the diversity of the microbial communities involved.

impacts on the rest of the ecosystem. Indeed, modeling the influence of conjugation-mediated HGT on the mutation-selection balance in bacteria has shown that HGT confers an advantage to populations in highly dynamic environments (Raz & Tannenbaum 2010). Using the presence of antibiotics as an environmental stressor, the authors of this model proposed that environmental stress on a population can speed up rates of HGT-mediated adaptation, as, for example, through the rescue of nonresistant strains by conjugation with resistant bacteria (Raz & Tannenbaum 2010). Although experimental work must be done to confirm these results, it is safe to assume that HGT is a rapid adaptive strategy utilized by many organisms and is particularly important for lineages in managed ecosystems, where environmental perturbations are constantly occurring.

The ecology of any organism includes the multitude of species it interacts with, which means that horizontally mobilized DNA can come from a variety of sources (**Figure 2**). If the recipient organism is associated with a plant or animal host through symbiosis, HGT can move genetic material between the host and the microbe. The totality of the microorganisms (microbiome) of the soil, rhizosphere, and plants also offers a rich source of transferrable DNA. Genetic material can be shared between the pathogens, commensals, and mutualists that comprise the microbial community. Finally, DNA from the material inputs in managed ecosystems, such as fertilizers and irrigation water, can be disseminated through viruses, plasmids, and other mobile elements. The variety of sources of horizontally transferred genes in managed ecosystems therefore provides a ready source of traits that confer a competitive advantage to the recipient lineage.

Microbome: totality of the microorganisms and their collective genetic material present in a specific environment

Rhizosphere: region of soil surrounding plant roots in which intense biological and chemical activities occur due to root-secreted compounds

Ecological relationships can either facilitate or restrict the extent and rate of transfer between donors and recipients. For example, in the gut microbiome, patterns of gene exchange are shaped principally by shared ecology, wherein microbes thriving in similar ecological conditions exchange genes more frequently with each other than with microbes inhabiting ecologically different

Pathovar: a set of bacterial strains with distinct pathogenic characteristics to one or more plant hosts

Genomic island: a group of discrete DNA segments, usually 10–200 kb, that differ between closely related strains, with probable horizontal origin

environments (Smillie et al. 2011). Obligate relationships can also greatly restrict transfers, primarily as a consequence of fewer opportunities available to encounter other organisms.

Despite the significant leap in HGT research in the past few decades, the ecological consequences of HGT remain poorly explored. Nevertheless, they are an important consideration in ecological studies. In managed ecosystems where the environment is constantly changing due to crop rotation, tillage, foraging, pesticide and herbicide use, plant breeding, and irrigation, HGT can greatly contribute to genetic diversity and the emergence of functional and metabolic traits in many lineages. Hence, incorporating aspects of HGT research in both managing pests and enhancing ecosystem services may significantly improve the overall and long-term integrity and stability of these systems.

ROLE OF HORIZONTAL GENE TRANSFER IN PATHOGENICITY AND PARASITISM

In managed ecosystems, bacterial pathogens and eukaryotic parasites thrive in close association with their plant or animal hosts, endlessly developing strategies to evade selection imposed by human intervention. HGT can lead to the rapid emergence of novel or more aggressive parasites and pathogens through acquisition of pathogenicity genes directly from the environment or from other organisms. In fact, HGT appears to have occurred in all of the most important bacterial pathogens and eukaryotic parasites of crops, and the likely donors of the transferred genes are incredibly diverse (**Figure 3**, **Table 1**). Different species of the same genus vary widely in the total number of genes they have acquired: In the case of the three species of *Xanthomonas*, the number of transferred genes ranges from 19 to 136 (**Figure 3**). These differences are likely driven by interactions with their hosts, the plant parts that they inhabit, the composition of the microbial community in their specific environments, and the management strategies used by crop growers that are specific to each plant host.

Horizontal Gene Transfer in Bacterial Pathogens: Focus on *Pseudomonas syringae*

The genus *Pseudomonas* constitutes many species with diverse lifestyles and physiological and pathological characteristics, several of which are important human, animal, and plant pathogens. Of noteworthy importance in agro-ecosystems is *P. syringae*, which consists of at least 50 pathovars, with a number of strains causing disease in many economically important host plants. It also includes strains from a variety of natural habitats (e.g., freshwater, snowpack, litter) (Berge et al. 2014, Demba Diallo et al. 2012, Hwang et al. 2005, Marcelletti & Scortichini 2014, Monteil et al. 2013). A multilocus sequencing analysis of 836 strains reveals tremendous genetic heterogeneity in this species, with multiple subgroups representing 13 clades, each of which possesses unique characteristics in terms of host range, toxins produced, and cell wall–degrading enzymes (Berge et al. 2014). This genetic diversity is largely driven by *P. syringae*'s highly dynamic genome, which is characterized by extensive HGT, gene losses, genomic rearrangements, and the presence of highly mobile plasmids, transposons, and genomic islands (Baltrus et al. 2011, Nowell et al. 2014, Silby et al. 2011).

The type III secretion system (T3SS) is a key virulence determinant in *P. syringae*. This syringe-like structure translocates secreted effector proteins into the host cell. Once inside the host cell, the effectors promote pathogenesis by disrupting the host defense system (Lee et al. 2012, Vinatzer et al. 2006). The repertoire of type III effectors in different *P. syringae* strains is highly diverse, with some that are sparsely distributed across the species (Baltrus et al. 2011). The totality and diversity

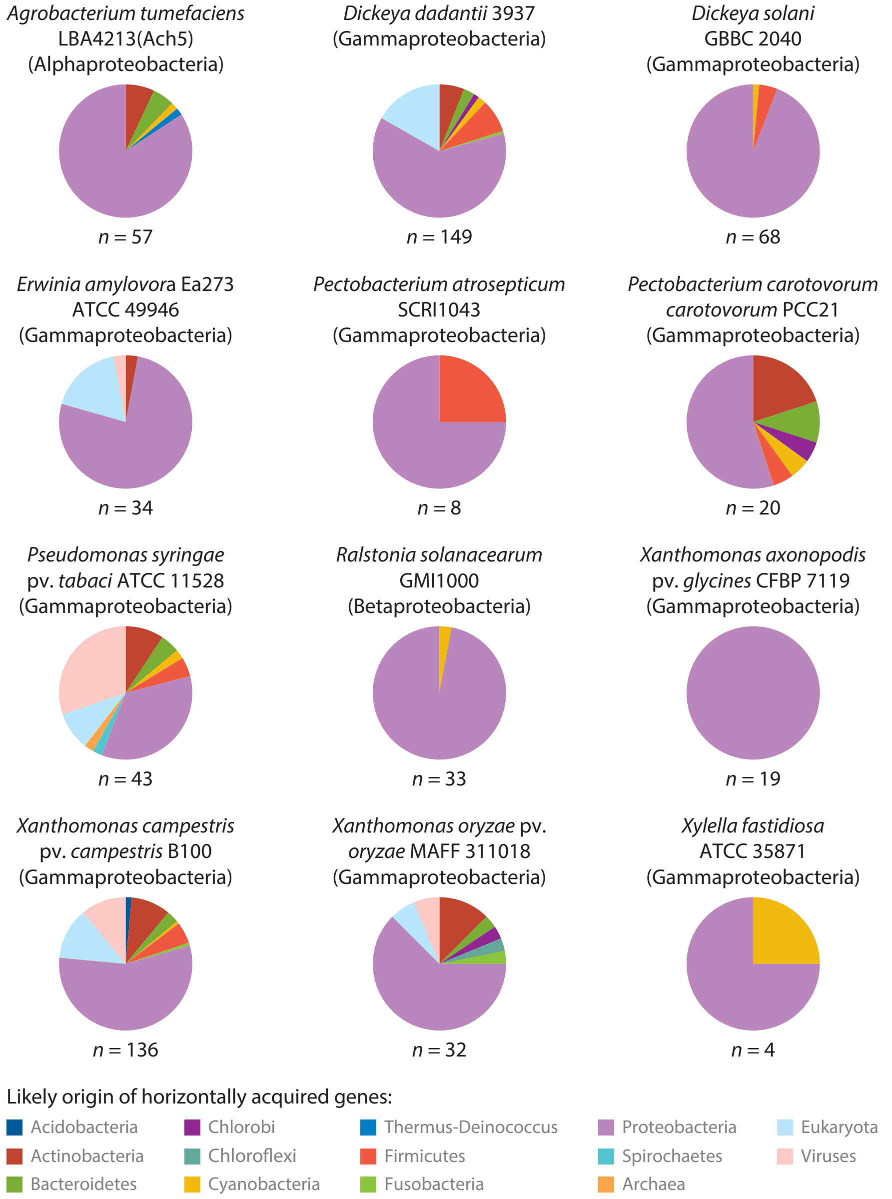

Figure 3

Likely origins of horizontally transferred genes in the most important bacterial pathogens of crops. These pathogens were identified through a survey done by Mansfield and colleagues (2012), who asked bacterial pathologists associated with the journal *Molecular Plant Pathology* to nominate bacterial pathogens based on their scientific and economic importance. All of these pathogens are members of the phylum Proteobacteria, and the class is indicated in parentheses below the species name. Only the major donor bacterial phyla are included in this figure. Proteobacterial donors include members of the Alpha-, Beta-, Delta-, Epsilon-, and Gammaproteobacteria. The number and likely origins of horizontally acquired genes in each genome were obtained from the Integrated Microbial Genomes system of the Joint Genome Institute, US Department of Energy (as of March 28, 2015) (Markowitz et al. 2014). The number at the bottom of each pie chart indicates the total number of inferred transferred genes.

Table 1 Examples of horizontal gene transfer in eukaryotic parasites of crops

Recipient	Disease caused	Most likely donor(s)	Acquired genes	Reference(s)
Plant parasitic nematodes (e.g., pine wood, root-knot, and cyst nematodes)	Large galls in the roots of infected plants	Soil- and rhizosphere-dwelling bacteria and fungi, bacterial plant pathogens, and plant-associated bacteria	Cell wall–degrading enzymes, such as cellulases, pectate lyases, and expansin-like genes; genes involved in the modification of the nematode's feeding structure, modulation of the plant defense system, and processing of nutrients absorbed from the plant	Danchin et al. 2010, Haegeman et al. 2011, Paganini et al. 2012, Shinya et al. 2013, Palomares-Rius et al. 2014
Coffee berry borer (insect)	Damage of coffee seeds as a result of larvae feeding	Bacteria	Gene that encodes for mannanase, which allows the insect to hydrolyze a major storage polysaccharide in the coffee berry plant	Acuña et al. 2012
Emerald ash borer (insect)	Canopy die-back as a result of damage in the phloem and cambium of the plant where the larvae feed and develop	Firmicutes (bacteria)	Two β-fructofuranosidases involved in breaking down dietary plant sucrose	Zhao et al. 2014
Mustard leaf beetle (insect)	Damage to leaves of cabbage, horseradish, turnip, and radish where larvae feed and develop	Gammaproteobacteria	Xylanases, a group of plant cell wall–degrading enzymes	Pauchet & Heckel 2013
Chrysomeloidea (leaf beetles, long-horn beetles) and Curculionoidea (weevils)	Damage to leaves of many flower hosts, fruit trees, berries, and ornamental plants as a result of feeding of adult beetles	Fungi	Polygalacturonases, which degrade polygalacturonan, a carbohydrate component of the pectin present in the cell walls of plants	Kirsch et al. 2014
Two-spotted spider mite	Damage through sucking of plant fluid from the foliage of most vegetables and food crops	Actinobacteria and Chloroflexi (bacteria)	Genes coding for UDP-glycosyltransferases, which are involved in xenobiotic detoxification and the biosynthesis, storage, and transport of secondary metabolites, including a variety of plant allelochemicals and insecticides Gene coding for cyanase, which is used to circumvent the production of hydrogen cyanide, a common plant defense mechanism	Grbić et al. 2011, Wybouw et al. 2012, Ahn et al. 2014

(*Continued*)

Table 1 (*Continued*)

Recipient	Disease caused	Most likely donor(s)	Acquired genes	Reference(s)
Different species of oomycetes or water molds (*Phytophthora ramorum*, *Phytophthora sojae*, *Phytophthora infestans*, *Hyaloperonospora parasitica*)	Seedling blight, damping-off, root rots, foliar blights, and downy mildews	Soil fungi and bacteria	Thirty-four genes involved in breaking down plant cell walls, nutrient acquisition, and resistance against plant defenses	Richards et al. 2011
Fusarium pseudograminearum (fungus)	Crown and root rot in barley, wheat, rice, and maize	Bacteria and other fungi	Fourteen genes involved in virulence, including amidohydrolase and dienelactone hydrolase	Gardiner et al. 2012
Moniliophthora perniciosa and *Moniliophthora roreri* (fungi)	Witches' broom disease and frosty pod rot of the cocoa tree	Bacteria and oomycetes	Genes coding for necrosis-inducing proteins from Oomycetes, mannitol phosphate dehydrogenase from Firmicutes, and metallo-dependent hydrolase from Actinobacteria	Tiburcio et al. 2010
Alternaria arborescens (Fungus)	Stem canker in tomato	Other species of fungi	Toxin genes carried in conditionally dispensable chromosomes	Hu et al. 2012

of these effectors contribute to the host range of specific strains, and the genes coding for them are often lost and regained through HGT (Baltrus et al. 2011). Even the secretion system itself can be mobilized, as was observed for a type IV secretion system (T4SS) carried on a 1-Mb megaplasmid in cucumber isolates of *P. syringae* (Baltrus et al. 2011). In addition to the secretion system, the self-transmitting megaplasmid also carries a variety of hypothetical protein genes, tRNA genes, and housekeeping genes (Romanchuk et al. 2014). Their transfer therefore expands the genomic content of the recipient strain. The T4SS found in *P. syringae* is most closely related to that found in the human pathogen *Legionella pneumophila*, the causal agent of the respiratory disease legionellosis (Baltrus et al. 2011), indicating the potential risks brought about by the HGT of virulence determinants between agricultural and clinical isolates. How this transfer was mobilized between two bacterial species of different lifestyles remains unclear, although the presence of intermediary hosts acting as shuttles is the most likely explanation.

Other virulence factors in *P. syringae* have been horizontally transferred between multiple pathovars. For example, in addition to the more common form of T3SS carried by most strains, a second T3SS has been identified in the pathovars *oryzae*, *tabaci*, and *phaseolicola* (Gazi et al. 2012). This atypical T3SS exhibits the greatest similarity to that found in the soil-dwelling, nitrogen-fixing symbiont *Rhizobium* and is likely a result of HGT mediated by an overlap in their ecological niches (Gazi et al. 2012). Another example is the 23-gene cluster coding for the chlorosis-inducing phaseolotoxin, which is carried by a pathogenicity island and is present in three pathovars (*phaseolicola*, *actinidiae*, and *syringae*); this cluster has been independently acquired via two HGT events

Megaplasmid: an extrachromosomal genetic element ranging in size from 100 kb to several megabases

Open reading frame: a span of a nucleotide sequence that can be read by the genetic machinery and translated into a protein

throughout the evolution of *P. syringae* (Murillo et al. 2011). Pathogenicity islands are large genetic elements (10–200 kb) that possess a sequence signature, such as GC content, dinucleotide frequency, or codon usage, which differs from the chromosome carrying them (Che et al. 2014). They play a pivotal role in pathogen evolution and diversification because of their ability to mobilize a variety of virulence-associated genes, phage-related genes, tRNA genes, and mobility genes (Che et al. 2014). In contrast to the phaseolotoxin gene, the mangotoxin biosynthetic operon, which causes apical necrosis in mango trees, has undergone a single transfer event to the common ancestor of the pathovars *aptata*, *avellanae*, *japonica*, *pisi*, and *syringae* (Carrión et al. 2013). The origins of these two toxin genes remain unknown, but that they can be mobilized between strains means that nonpathogenic strains of *P. syringae* have the potential to become pathogenic and expand their host range by acquiring novel virulence genes.

Strains from natural habitats can also act as a steady source of genetic diversity for pathogenic strains, which contributes to their ability to circumvent the selective pressures imposed on managed ecosystems. Indeed, environmental *P. syringae* strains in natural habitats have been reported to be an important reservoir of allelic diversity for the T3SS apparatus of pathogenic strains (Demba Diallo et al. 2012). *P. syringae* strains isolated from grasses also possess their own unique collection of virulence-associated genes, including numerous non-T3SS virulence factors (Dudnik & Dudler 2014), which crop-associated strains can potentially utilize. With this comes the risk for genotypes with more virulent characteristics to appear and strains causing disease outbreaks to re-emerge, as has been observed for the pathovar *actinidiae*, which causes kiwifruit canker disease. Each outbreak of this pathovar in different geographical regions has been caused by a distinct lineage characterized by a unique set of accessory genes, effectors, and toxins, with HGT contributing to its diversity (McCann et al. 2013).

Genomic analyses have also revealed the prominent role of MGEs in the diversification, evolution, and virulence of *P. syringae*. In fact, MGEs comprise 5% and 7% of the total open reading frames of the pathovars *phaseolicola* 1448A and *tomato* DC 3000, respectively (Buell et al. 2003, Joardar et al. 2005). ISs and miniature inverted-repeat transposable elements (MITEs), both of which drive genomic rearrangements and genetic diversity, are common within this species. ISs are short, autonomous mobile elements carrying a transposase-coding gene bounded by terminal inverted repeats (IRs) (Siguier et al. 2014). MITEs are 200- to 300-bp mobile elements that possess IRs but lack their own transposase and hence are incapable of self-transfer (Delihas 2011); however, they are often mobilized via the transposases encoded by other mobile elements within the genome (Delihas 2011). Both types of MGEs can shuttle neighboring genes, including virulence-associated and niche-adaptive genes. In the pathovar *maculicola*, a particularly large virulence-associated MITE (611 bp) has homologs in diverse bacterial genera in both clinical and environmental settings (Stavrinides et al. 2012). The prevalence of homologous MITEs in distantly related bacteria reveals the need to elucidate the nature of reservoirs of HGT that are often overlooked in plant pathology studies.

Horizontal Gene Transfer in Plant Parasitic Nematodes

Prokaryote-to-eukaryote HGT is particularly interesting because numerous cellular, physical, and physiological barriers need to be overcome for the successful genomic integration of the acquired gene. One notable example in agro-ecosystems is the horizontal acquisition of genes involved in plant parasitism in some species of nematodes, an ancient and highly diverse phylum of animals characterized by their ability to periodically molt their outer cuticle (Blaxter & Koutsovoulos 2014). Common to nematode infestation is the formation of large galls in the roots of infected plants, from which nematodes obtain nutrients (Caillaud et al. 2008, Dinh et al. 2014). Infection causes a decline in plant health and growth owing to reduced nutrient and water uptake, which can then lead to significant economic losses.

Parasitism emerged in nematodes as a result of HGT from bacterial and fungal sources. The transferred genes allow them to degrade the various polysaccharides in the plant cell wall—the first barrier that they need to overcome to establish themselves as parasites (Danchin et al. 2010, Haegeman et al. 2011, Hewezi et al. 2008). The abundant polysaccharides in the plant cell wall provide a rich source of energy; hence, organisms that possess the ability to digest them have a clear selective advantage. Many horizontally acquired genes have been identified in nematodes, including a variety of genes involved in cell wall degradation, modification of the nematode's feeding structure, secretion, modulation of the plant defense system, and processing nutrients absorbed from the plant (Danchin et al. 2010a, Haegeman et al. 2011, Hewezi et al. 2008, Kikuchi et al. 2011, Palomares-Rius et al. 2014, Shinya et al. 2013). Evidence for microbe-to-nematode HGT includes phylogenomic analyses, nematode infection assays of *Arabidopsis*, immunolabeling and functional assays of cellulose-binding proteins secreted by nematodes, and the direct interaction of these proteins with specific plant proteins. Although donor lineages cannot be precisely defined, transferred genes in plant nematodes exhibit the greatest similarity to genes found in soil- and rhizosphere-dwelling bacteria and fungi, bacterial plant pathogens, and plant-associated bacteria (Danchin et al. 2010, Haegeman et al. 2011, Paganini et al. 2012). A shared ecological niche, primarily through plant-bacteria-fungi-nematode interactions in the soil, is likely the driving factor behind the success of plant parasitism in these animals.

Horizontal Gene Transfer in Pest and Disease Management

The role of potential transfer partners and donors is pivotal in understanding how HGT can be incorporated in pest and disease management. When the structure and dynamics of microbial communities are significantly altered, as in the case of intensive application of material inputs, the composition and availability of the mobile gene pool also changes. This means that opportunities for potential donor and recipient lineages to come together in close proximity increases considerably. Introduction of chemical and other control measures may also inadvertently alter microbial community dynamics, as one population takes over the ecological niche left vacant by the elimination of specific pests. Changes in population dynamics of pathogens are also likely to occur when the boundaries of managed ecosystems are expanded to encroach into natural habitats, resulting in the exposure of microorganisms in both natural and managed ecosystems to new plant and animal hosts. Habitat conversion can therefore provide new avenues for pathogens to switch to new hosts, inhabit new environments, or exploit alternative sources of energy and nutrients. As we have seen in the case of *P. syringae*, HGT can drive the emergence of novel genetic variants carrying pathogenicity determinants acquired from multiple sources, including microorganisms from nonagricultural, industrial, and clinical sources.

In managed ecosystems where ecological conditions and community structure are constantly shifting as a result of human intervention and intensive agricultural practices, lineages that have not had the opportunity to interact or share the same environment previously may now have greater chances to do so. For example, introducing a pest's natural enemies as a biological pest control approach may have the unintended consequence of introducing novel genetic material into the system, which may become available for uptake by indigenous organisms. This is not limited to the genetic material of the pest's natural enemies, such as predators, parasitoids, parasites, pathogens, and other antagonists, but also includes the microbiomes associated with them.

Integrating HGT in pest and disease management plans starts with identifying the major sinks and sources of transferrable genes in managed ecosystems. When new approaches to pest and disease control are to be implemented, it is essential to determine how the microbial community will shift, as the identity of potential HGT donors and the availability of genetic material in the mobile gene pool will also likely change. Whole genome sequencing and phylogenomic analyses of pathogenic strains and their nonpathogenic relatives will provide profound insight into the patterns and direction of HGT, as well as specific genomic elements that facilitate it. Metagenomic studies of specific pathogenicity-associated genes in both agricultural and natural habitats will further expand our understanding of HGT donors and recipients. Although it may not be possible to accurately predict how HGT will modify existing traits in different lineages, a more complete understanding of the origins of infection and virulence mechanisms will greatly help in the development of novel plant protection strategies.

HORIZONTAL GENE TRANSFER OF ANTIBIOTIC RESISTANCE AND DISSEMINATION OF INFECTIOUS AGENTS

Treated Wastewater Effluent for Irrigation

Reuse of treated wastewater in irrigation is a vital element of water resource management in many agricultural areas, particularly those located in arid and semiarid regions. Treatment of wastewater includes physical, chemical, and biological processes that remove contaminants before it is reused or discharged back into the environment (Frimmel 2003). Reusing treated wastewater is considered a practical and attractive solution to alleviate water scarcity and address the decline in freshwater supplies. Although implementing this strategy has greatly eased the burden of water stress, questions remain about water quality, particularly in terms of the risks associated with persistent pathogens, antibiotic resistance genes (ARGs), and chemical contaminants. Although the ecological impacts of treated wastewater as an irrigation source have not yet been accurately characterized, it is imperative that they are not disregarded, given that soil types, climate, wastewater chemistry, land use, and cropping systems may significantly influence the potential risks associated with treated wastewater. The ecology and scope of the HGT of ARGs from processed wastewater warrant a more thorough investigation to ensure that the benefits and risks are well understood.

A diverse array of antibiotic-resistant bacteria, infectious agents, and mobile elements has been reported to persist in wastewater despite treatment processes. Vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, and diverse species of multiresistant bacteria have been detected in multiple wastewater treatment plants that discharge treated effluent for reuse (Czekalski et al. 2012; Rosenberg Goldstein et al. 2012, 2014). Other reported infectious microorganisms are enteroviruses and eukaryotic parasites that cause intestinal infections, such as *Ascaris*, *Giardia*, and *Cryptosporidium* (Battistone et al. 2013, Hachich et al. 2013).

MGEs present in treated wastewater also play a crucial role in facilitating the spread of ARGs. For example, plasmids with a broad host range carrying transferrable antibiotic resistance determinants have been found in discharged treated wastewater (Akiyama et al. 2010, Czekalski et al. 2012). Clinically relevant antibiotic determinants carried in plasmids from wastewater treatment plants confer macrolide, tetracycline, β-lactam, trimethoprim, chloramphenicol, and sulfonamide resistance (Rahube et al. 2014). The presence of integrons, another class of mobile element, in *Escherichia coli* collected from treated wastewater is associated with higher levels of resistance against fluoroquinolones (ciprofloxacin and levofloxacin), trimethoprim/sulfamethoxazole, amoxicillin/clavulanate, and piperacillin/tazobactam (Kotlarska et al. 2014).

Organic Manure for Fertilization

Manure from dairy farms is a common soil amendment in crop production. Although it is considered more ecologically friendly than mineral fertilizers, it is often contaminated with veterinary antibiotics, which can have adverse impacts on soil ecology. ARGs present in animal manure can dissipate into the soil microbial community, affecting the functional capacity of the community and therefore the health and efficiency of the dependent agro-ecosystem. A recent comprehensive survey of ARGs in cow manure–amended soil using metagenomic methods revealed tremendous genetic diversity within four classes of antibiotics: β-lactams, phenicols, aminoglycosides, and tetracyclines (Wichmann et al. 2014). This ARG survey also revealed novel chloramphenicol resistance determinants that are only distantly related to known genes (Wichmann et al. 2014). A similar increase in various ARGs was observed in soils treated with manure from swine (Heuer et al. 2011, Jechalke et al. 2013) and poultry (Obeng et al. 2012, Pourcher et al. 2014). The accumulation of antibiotics following manure application is further complicated by variation in the rates of transport and dissipation of different ARGs in the soil (Fahrenfeld et al. 2014). With the buildup of resistance genes in the soil, highly resistant bacterial strains are expected to emerge through HGT, although increases in antibiotic-resistant strains do not occur uniformly across all bacterial taxa in the soil community (Udikovic-Kolic et al. 2014).

Significant changes in the soil bacterial community structure are expected to occur with the use of manure amendments. Although variation in the prevalence and diversity of ARGs in bacterial communities in response to manure amendments may be transient (Islas-Espinoza et al. 2012, Riber et al. 2014), repeated application of manure or the use of different manure amendments may lead to changes that have long-term or widespread consequences. Changes in the community lead to changes in the identity of donors and recipients as well as the characteristics of free DNA available for uptake, increasing the potential for high-frequency HGT.

Horizontal Gene Transfer in Soil and Water Management

The soil is a vast repository of diverse ARGs, many of which have novel resistance mechanisms that have yet to be investigated (Donato et al. 2010, Hollowell et al. 2015, Owen et al. 2013). Hence, integrating HGT research into strategies to maintain the productivity of soil and water in managed ecosystems is important to mitigate the spread and accumulation of antibiotic resistance in agricultural settings. The first thing that needs to be accomplished is a thorough characterization of ARGs and other contaminants in sources of wastewater for treatment, including municipal, industrial, and hospital wastewater, using metagenomic profiling. Long-term and regular monitoring of soils before and after irrigation with treated wastewater is also necessary to ensure that treatment processes effectively and completely remove biological contaminants. Biogeographical investigation coupled with modeling of ARGs in managed ecosystems can provide further insight into temporal and spatial trends in ARG dissemination. Population-level analysis using whole genome sequences of hundreds and even thousands of microbial isolates will also aid in the identification of likely recipients of transferred ARGs.

More effective treatment of animal manure prior to soil application is also needed, to prevent the dissemination of pathogens and ARGs without sacrificing the essential nutrients needed for increasing crop yield. Although it has been reported that ARG levels in compost and soil eventually decline over time (Kim et al. 2012, Marti et al. 2014), the abundance and persistence of different ARGs can vary significantly. This persistence has been observed even when subtherapeutic levels of antibiotics are administered (Alexander et al. 2011). The animal source of the manure will also likely influence the diversity of ARGs that becomes part of the mobile reservoir in the soil, and this relationship must also be characterized. Next-generation sequencing of the microbiomes of manure from different animal sources will also greatly aid in decision making and implementation of fertilization strategies.

HORIZONTAL GENE TRANSFER IN MICROBIAL LINEAGES INVOLVED IN NITROGEN CYCLING

Widespread Horizontal Gene Transfer in Nitrogen-Fixing Bacteria

Many phylogenetically diverse microbial lineages play a crucial role in the transformation of nitrogen in the ecosystem, with different groups playing specific roles in nitrogen fixation, nitrification, denitrification, and ammonification. The stability of the nitrogen cycle and the availability of biologically usable forms of nitrogen are therefore greatly influenced by the genetic and functional diversity of these microbial populations, with HGT greatly contributing to their diversity and evolution. Diversity in the nitrogen-fixing community is integral in managed ecosystems because nitrogen is the mineral nutrient that most commonly limits terrestrial primary productivity, including crop yield.

The diversity and adaptive capacities of many nitrogen fixers have been greatly influenced by the acquisition of genes from multiple sources (Bolhuis et al. 2010, Carvalho et al. 2010, Menna & Hungria 2011, Sorokin et al. 2012). **Figure 4** shows the likely origins of transferred genes identified in two nitrogen-fixing bacteria: *Anabaena variabilis*, a free-living cyanobacterium, and *Methylobacterium nodulans*, a symbiotic bacterium found in the root nodules of legumes. Most

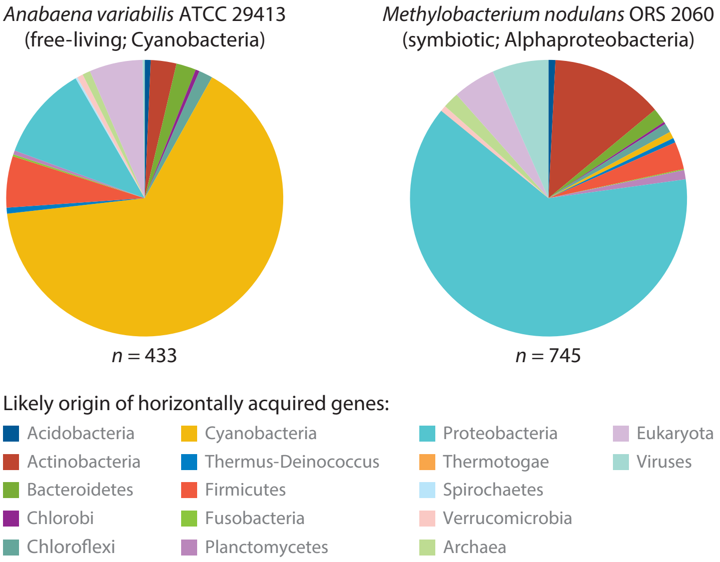

Figure 4

Likely origins of horizontally transferred genes in representative free-living and symbiotic nitrogen-fixing bacteria. Only the major donor bacterial phyla are included in this figure. Proteobacterial donors include members of the Alpha-, Beta-, Delta-, Epsilon-, and Gammaproteobacteria. The number and likely origins of horizontally acquired genes in each genome were obtained from the Integrated Microbial Genomes system of the Joint Genome Institute, US Department of Energy (as of March 28, 2015) (Markowitz et al. 2014). The number at the bottom of each pie chart indicates the total number of inferred transferred genes.

of the transferred genes are acquired from species within the recipient's own phylum, but other important donors include eukaryotes (primarily fungi), archaea, and viruses, and these transfers are likely facilitated by shared ecology. In *M. nodulans*, for instance, the second most common donors are Actinobacteria, most of which are soil dwelling and likely to encounter the root-dwelling nitrogen fixers.

A large, diverse group of bacteria called rhizobia, which form a symbiotic relationship with many leguminous plants, play an essential role in managed ecosystems. They induce the formation of nodules on plant roots; within the nodules, they fix atmospheric nitrogen in exchange for photosynthates. They frequently acquire and donate genes, including those involved in nitrogen fixation (*nif*) and nodule formation (*nod*) (Althabegoiti et al. 2014, Menna & Hungria 2011, Tian et al. 2012), thereby facilitating the spread and maintenance of these abilities within the microbial community. The evolution and HGT of the nitrogen-fixing bacterium *Burkholderia*, for example, have contributed to facilitating the diversification and establishment of their legume hosts in new habitats (Beukes et al. 2013). HGT also drives the adaptation of rhizobia to novel plant hosts, as has been observed in *Mesorhizobium*, which nodulates a wide range of plants (Laranjo et al. 2014). HGT is particularly essential in nitrogen-limited environments, where widespread mobilization of nitrogen-fixing genes within the microbial community can enhance the productivity of agricultural crops.

Ecological Considerations in Nutrient Cycling

In managed ecosystems, biogeochemical processes may be more complex, as the structure and abundance of microbial functional groups may significantly and rapidly shift as a result of different cropping and agricultural practices (Berthrong et al. 2013, Hartmann et al. 2014, Lauber et al. 2013, Lucas et al. 2013). Microbial diversity in managed systems changes rapidly across both spatial and temporal scales. Because the resulting variation may be beneficial (e.g., increased diversity and nitrogen fixation) or deleterious (e.g., increased rates of nitrate leaching and gaseous N_2O loss), future research should study pathways through which vital nitrogen cycling genes may be transferred horizontally. A more complete understanding of HGT and agricultural nitrogen-cycling genes can inform mechanistic models that better predict how soil microbial communities and biogeochemical cycles respond to management pressures and perturbations, such as tillage in annual systems and fertilizer application in annual or perennially managed systems.

Although there is not yet substantial evidence demonstrating a role for HGT in altering the rates of nutrient cycles, several studies suggest that HGT increases variation in nitrogen-cycling genes and influences process rates (Hsu & Buckley 2009, Reed et al. 2010). In a tropical rainforest, a strong increase in *nifH* abundance and diversity in free-living, nitrogen-fixing bacteria was observed with phosphorus fertilization (Reed et al. 2010). The *nifH* gene encodes dinitrogen reductase, an important component of the nitrogenase complex, which converts atmospheric nitrogen to ammonia. Reed and colleagues (2010) also found evidence that rates of nitrogen fixation were heterogeneous across the landscape and were highest in small patches where *nifH* diversity was high, suggesting resource availability and sequence diversity affect nitrogen fixation rates. Similar findings have also been observed in agro-ecosystems (Hsu & Buckley 2009, Poly et al. 2001). A long maize tillage and biomass retention experiment revealed that tillage, independent of biomass retention, increased the evenness and diversity of *nifH* and that this increased diversity was positively related to nitrogen fixation rates (Hsu & Buckley 2009). These results suggest that perturbations to soil systems, such as tillage and widespread use of fertilizers, increase instability in the microbial environment, which can promote HGT, increased genetic variation, and nitrogen fixation rates.

In soil, nitrification (ammonia oxidation) and denitrification (reduction of nitrate to N_2O and subsequently N_2) are mediated by bacteria and archaea with a complex evolutionary history involving HGT (Hallam et al. 2006, Jones et al. 2008, Leininger et al. 2006). Conversion of ammonia to nitrate was until recently considered an exclusively bacterial process; however, recent evidence shows that archaea are also important nitrifiers, with HGT reported between bacterial and archaeal ammonia oxidizers (Hallam et al. 2006, Leininger et al. 2006). Adding nitrogen fertilizer to a productive pine forest increases ammonia-oxidizing bacteria relative to ammonia-oxidizing archaea, indicating that management decisions shift community structure to the microbes that most effectively utilize the resources present (Wertz et al. 2012).

Denitrification, an anaerobic respiration process utilizing nitrate as the terminal electron acceptor, is also important in nitrogen cycling in agro-ecosystems and is mediated by bacteria from many phyla. The evolutionary history of genes associated with denitrification (*nirK*, *nirS*, *norB*, *nosZ*) is complicated and may involve multiple duplication events and HGT (Jones et al. 2008). Soil nitrogen management shifts the abundance of the different components of the microbial denitrifying community, with increased soil nitrogen leading to a community with a greater abundance of *nirK* than *nosZ* (Clark et al. 2012). The shift in denitrifiers has important consequences because it increases the amount of N_2O (an important greenhouse gas) produced versus complete reduction to N_2 (Clark et al. 2012). These results suggest that HGT events may lead to diversified nitrifier and denitrifier communities. Further investigation is needed to precisely characterize the impacts of the diversification of specific groups of microorganisms involved in the nitrogen cycle on the ecosystem.

Microbial populations also play a critical role in allowing plants to gain access to inorganic sulfate in the soil. In terrestrial ecosystems, horizontal transfer of genes involved in sulfur metabolism has not been widely investigated, although evidence from studies of marine and freshwater ecosystems indicates that HGT greatly contributes to microbial adaptation to sulfur limitation (Gregersen et al. 2011, Holkenbrink et al. 2011). Future work using metagenomic approaches can provide us with better insight into the contributions of specific sulfur-metabolizing microorganisms in managed ecosystems as well as the extent of horizontal transfer of genes associated with sulfur cycling.

Additional work should also include characterization of the most active donors and recipients of genes involved in nutrient cycling and the potential for scientists and growers to manipulate rates and patterns of HGT to enhance nutrient availability. Establishing sequence databases for specific genes involved in nitrogen and sulfur cycles is an important step, as has been initially done for the *nifH* gene (Gaby & Buckley 2011, 2014). Metagenomic characterization of nitrogen fixers and sulfur metabolizers in specific habitats will help us make better decisions about the kinds and amounts of material inputs that should be added to specific cropping systems.

CONCLUSION AND FUTURE DIRECTIONS

HGT has played an important role in the evolutionary history of many, if not all, lineages that have existed on earth. Coupled with mutation, hybridization, and other evolutionary processes, it has led to an explosion of biological diversity and adaptation. The impacts of HGT at an ecological level, however, have not been widely explored and remain to be elucidated. This is particularly crucial in managed ecosystems, where intensive chemical application and various management schemes can significantly alter the structure and dynamics of biological communities.

We propose that future research in agricultural ecology highlight the role of HGT in specific ecosystems. Three steps are necessary for integrating HGT into future research on managed ecosystems. First, long-term surveillance of potential HGT donors and recipients under different management strategies (e.g., organic farming versus conventional farming, chemical fertilizers versus animal manure, monocropping versus polyculture) will provide insight into how DNA is mobilized and disseminated. Sources and sinks of transferred genes must also be identified using metagenomic analyses of microbial communities inhabiting different habitats. Second, functional characterization of transferred genes in specific organisms is necessary to identify the physiological and ecological impacts of HGT. It is also worth determining the factors (e.g., genetic, ecological) that drive some lineages to readily acquire exogenous DNA, whereas others are more recalcitrant. This will allow us to make better predictions regarding crop disease outbreaks and multiresistant strains. Lastly, how large-scale ecological changes, such as climate change, acid rain, pollution, and desertification, affect microbial communities and the mobilization of available DNA needs to be addressed. This can be done by incorporating genomic sequence data with network and modeling analyses. The latter two approaches have been widely used in infectious disease epidemiology but are seldom applied in ecology. These methods will be particularly useful in determining how HGT relationships in biological communities are altered under different ecological conditions.

A risk-benefit assessment of implementing new agricultural practices should include the impacts of disseminated genetic material on the structure, diversity, and dynamics of biological communities that act as vehicles for gene flow. This will allow us to optimally use these genetic resources and evaluate trade-offs and risks in the pursuit of sustainable crop production.

DISCLOSURE STATEMENT

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