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Human dissemination of genes and microorganisms in Earth's Critical Zone

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Abstract

Earth's Critical Zone sustains terrestrial life, and consists of the thin planetary surface layer between unaltered rock and the atmospheric boundary. Within this zone, flows of energy and materials are mediated by physical processes and by the actions of diverse organisms. Human activities significantly influence these physical and biological processes, affecting the atmosphere, shallow lithosphere, hydrosphere and biosphere. The role of organisms includes an additional class of biogeochemical cycling, this being the flow and transformation of genetic information. This is particularly the case for the microorganisms that govern carbon and nitrogen cycling. These biological processes are mediated by expression of functional genes and their translation into enzymes that catalyze geochemical reactions. Understanding human effects on microbial activity, fitness and distribution is an important component of Critical Zone science, but is highly challenging to investigate across the enormous physical scales of impact ranging from individual organisms to the planet. One arena where this might be tractable is by studying the dynamics and dissemination of genes for antibiotic resistance and the organisms that carry such genes. Here we explore the transport and transformation of microbial genes and cells through Earth's Critical Zone. We do so by examining the origins and rise of antibiotic resistance genes, their subsequent dissemination, and the ongoing colonization of diverse ecosystems by resistant organisms.

Introduction

Earth's Critical Zone is the thin surface layer of the planet upon which terrestrial life depends. It extends from unaltered bedrock, through the land surface, to the vegetation canopy and atmospheric boundary layer. Critical Zone science is complementary to other integrative systems approaches for studying terrestrial, marine and freshwater environments. Crucially, it includes a mechanistic understanding of shallow lithosphere processes and their interactions with the above-ground ecosystems (Mobley, 2009). It addresses these interactions across wide temporal (sub-second reaction kinetics to geological time spans) and spatial scales (molecular to planetary). The Critical Zone approach recognizes Earth as a physical and geochemical substrate that supports above ground ecological functions, and extends the lower boundary of ecological function to embrace the lithosphere, and its inputs over geological time scales.

This interdisciplinary research area within geobiology links biological and geochemical processes across temporal and spatial scales. However, the distribution, transport and recruitment of functional genes has rarely been investigated via the systems perspective framed by Critical Zone science. Since investigation of Critical Zone biogeochemical processes extends the analysis of flows and transformations of material and energy to explicitly include biodiversity, a tractable approach may be to describe the geospatial dynamics of the genetic information encoded in functional genes, and the microbes that carry these genes. Above–ground human activities generate impacts that are transmitted through the vertical extent of the Critical Zone, via aquifers, and horizontally within

78 water catchments (Figure 1). Analyzing the vertical and horizontal penetration of
79 genetic material should be part of these investigations (Küsel et al., 2016).
80
81 Environmental microbes and genes were traditionally studied in one location, or in one
82 environmental compartment (such as vegetation, the water column, or soil), with little
83 attention paid to the dynamic exchange of microbes and genes across system boundaries
84 and physical scales (Zhu et al., 2017c). The advent of "omics" tools has facilitated the
85 exploration of Earth's biological 'dark matter', but there remains a substantial
86 conceptual gap between the notion of the Earth's biome and its quantitative
87 manifestation in biogeochemical fluxes. Integrating "omics" data into earth system
88 science should generate better models of biogeochemistry and improve understanding
89 of how environmental changes will impact microorganisms and vice versa. For instance,
90 incorporating environmental genomics data into biogeochemical models improves
91 predictions about nitrogen cycling (Mock et al., 2016, Reed et al., 2014).
92
93 Driven by these concepts, there is increasing attention towards system views of the
94 temporal and spatial distribution of microbes and genes in Earth's Critical Zone.
95 Metagenomics has been used to determine the influence of fluvial networks on the co-
96 occurrence of microbes, by examining biofilms in over a hundred streams (Widder et
97 al., 2014). The distribution and origins of fecal bacteria have been determined in large
98 mixed-use watersheds in Michigan, USA, also using omics technologies
99 (Verhougstraete et al., 2015). Similar ecosystem wide approaches have been used to

demonstrate how below ground microbial diversity might be a primary driver of plant diversity and productivity (Bardgett & van der Putten, 2014). Questions are also being asked about how surface activities might influence below ground biota and nutrient cycling, using combinations of omics, biogeochemical, and hydrogeological approaches (Küsel et al., 2016).

These publications are representative of recent efforts to explore the links between microbial biogeography, biogeochemistry and geological processes. In particular, they reflect a growing interest on the effects that human activities might have on the microbial world (Gillings & Paulsen, 2014). Understanding the role that humans might have in changing the distributions of microorganisms, and in generating selective forces that alter adaptive pressures, are essential if we are to predict how global change will affect microbial activity and function. However, many of the most important processes for Critical Zone function are complex, multi-gene and multi-cell interactions that are difficult to model, due to the complexity and dynamics of genetic and functional diversity within indigenous microbial communities.

There are simpler systems that we can use to understand the influences that humans have on the transport and transformation of genetic information in the Critical Zone. Antibiotic resistance, for instance, is generally a one-gene, one phenotype character, and has been the subject of considerable research over the last fifty years. Genes conferring resistance, and the cells that host these genes, could be used as a paradigm

for assessing the interactions of gene flow with the diversity of microorganisms in the Critical Zone.

Antibiotic resistance might be a good proxy that can inform more general conclusions about alterations in the distribution and activity of the microorganisms that host specific genes within the Critical Zone. Although antibiotic resistance is ancient (ref), the widespread use of antibiotics in agriculture and medicine has increased the abundance of both resistance genes and the bacteria that host them. These genes and microorganisms are then shed into environmental compartments via human and animal waste streams such as manure, sewage sludge, and wastewater (Figure 1) (Gillings, 2013). As a consequence, antibiotic resistance genes are considered to be emerging environmental contaminants (Pruden et al., 2013). On the one hand, the spread of resistance determinants within the Critical Zone is caused by human activities, and on the other hand, it also threatens human health worldwide. The research history of resistance begins in the 1950s, and is thus co-incident with the 'Great Acceleration' and the rapidly increasing impact of humans activity on the planet since this time point (Steffen et al., 2015).

Natural transport and biogeography of bacteria

We live in a world where organismal abundance and gene frequencies have been significantly shaped by human activities. Nevertheless, it is worth reflecting on the

historical dynamics of microbial organisms and ecosystems, before the rise of human influence. This allows comparisons with the modern world.

It has been known for some time that microorganisms exhibit the same taxa-area relationships and turnover in species assemblages with distance that are characteristic of larger organisms (Green et al., 2004, Horner-Devine et al., 2004). Taxa are distributed non-randomly in environments such as soil, fresh water and groundwater, at scales from meters to many thousands of kilometers (Martiny et al., 2006). These patterns are driven by a combination of factors, including: the ability to disperse over distance; selection at the destination; and stochastic processes such as drift and mutation (Hanson et al., 2012). Teasing apart the relative contributions of the processes that generate patterns of microbial biogeography is difficult, and is further complicated by the diversity and complexity of microbial communities themselves (Evans et al., 2017, Haggerty & Dinsdale, 2016). The impact of human migration as a transport vector on structuring prokaryotic communities is still poorly understood. Some authors have argued that stochastic events could be more important than deterministic factors such as competition and niche differentiation (Sloan et al., 2006).

At the largest possible temporal and spatial scales, bacteria are the best candidates to survive interplanetary transfer inside rock. Such lithopanspermia is a potential means that life could be transferred between planetary bodies within and outside our solar system (Nicholson, 2009). On Earth, but still across large spatial scales,

165 microorganisms are capable of long-distance dispersal, being ubiquitous and abundant,
166 even in the upper atmosphere (Barberán et al., 2015). Thousands of distinct bacterial
167 taxa, accompanied by other microorganisms, are carried within dust plumes in long-
168 range intercontinental transport events. For instance, Asian aerosols contribute to
169 microbial species richness in North American air (Smith et al., 2013), and dust storms
170 generated in the African Sahara-Sahel transport microorganisms that eventually
171 contribute to bacterial assemblages in European mountain lakes (Perfumo &
172 Marchant, 2010, Peter et al., 2014).
173
174 **Natural release and survival of DNA**
175 Microbial biogeography is further complicated by the ability of microorganisms to
176 acquire foreign DNA, and consequently movement of genes through the Critical Zone
177 can occur independently of organismal movement (Figure 2). DNA released from
178 organisms can transfer to unrelated species either through close contact, or at a distance,
179 when DNA can survive in the environment for extended time periods (Gillings, 2017b).
180
181 Extracellular DNA can be readily detected in environmental samples, and can originate
182 from dead bacterial, animal or plant cells. All soils contain significant quantities of
183 extracellular DNA (Frostegård et al., 1999). This DNA can persist in the environment
184 and can be transported away from cell debris. Because DNA can resist physical and
185 biological degradation under some conditions, it has even been proposed as a potential
186 signature of life during interplanetary exploration (Lyon et al., 2010).

Under natural conditions, DNA released via cell lysis is in contact with other cellular components (wall debris, proteins, lipids, RNA, etc.). The presence of both organic compounds and inorganic molecules in soil particles strongly influences the adsorption of DNA (Pietramellara et al., 2009). Consequently, DNA can be protected from enzymatic degradation in soil by adsorption onto soil minerals and humic substances (Levy-Booth et al., 2007). Protection against degradation by DNases of microbial origin is aided by the concomitant adsorption of nucleases (Demanèche et al., 2001). Many studies on survival of DNA in the environment have been conducted using plasmids and antibiotic resistance genes as markers.

The DNA persisting in soil is only a tiny fraction of the total DNA being released at any one time from decaying plants, animals and microorganisms. This DNA usually undergoes rapid degradation (Ceccherini et al., 2007, Pontiroli et al., 2007, Poté et al., 2010). Degradation is biological and enzymatic, since DNA can survive in autoclaved treatments (Zhu, 2006). Nevertheless, a proportion of extracellular DNA does persist in natural environments, either bound to soil particles, or inside biofilms, where it is an important structural component (Pietramellara et al., 2009, Whitchurch et al., 2002). In the long term, persistence eventually requires being taken up by a recipient cell, and incorporated into that cell's genome. The likelihood of this occurring improves with increasing phylogenetic and ecological similarity of donor and recipient (Beiko et al., 2005), and also improves markedly if the donor DNA can confer an adaptive phenotype.

209 This is one reason why genes that confer antibiotic resistance are a good marker for
210 these processes in natural environments.
211
212 **Movement and transport of extracellular DNA.**
213 DNA is able to be transported vertically in unsaturated soils, to eventually penetrate
214 groundwater and aquifers, where it can be immobilized through adsorption onto mineral
215 surface or be transported with groundwater flow (Poté et al., 2009). Forced pumping of
216 groundwater for drinking can thus induce rapid flow and associated transport of DNA
217 over considerable distances. DNA can also move upwards in the soil column via
218 capillary action (Ceccherini et al., 2007), potentially allowing subsequent long distance
219 movement via erosion and run-off.
220
221 The presence of extracellular DNA in environmental samples is increasingly being used
222 to perform multi-taxa surveys, or to detect rare and elusive species (Zinger et al., 2016).
223 However, the parameters that affect transport and survival of extracellular DNA are not
224 well understood, and may compromise some of these experiments (Jerde et al., 2016).
225 Given the problems of differential survival and transport of extracellular DNA,
226 guidelines for the design and interpretation of environmental DNA methods are
227 required (Goldberg et al., 2016).
228
229 Experiments to address this problem have used a variety of indicator DNAs. Antibiotic
230 resistance genes known to be associated with humans are a good choice. They have

been used to show survival and dissemination of DNA into freshwater sediments in an aquatic environment used for drinking water supply (Thevenon et al., 2012). Similarly, plasmids (Poté et al., 2003) and bacteriophages (Chetochine et al., 2006) have been used to demonstrate transport over considerable distances in water saturated soil and groundwater. However, the dynamic relationships between DNA transport, immobilization, survival, and the limits of detection are not well established (Hunter et al., 2016).

One way to track and understand dissemination of DNA through the environment, and indeed, throughout Earth's Critical Zone is to use a model system that is tractable and reflects the history of human impacts. Antibiotic resistance genes, their plasmid vectors, and the bacteria that host them are a good candidate for use as a proxy for anthropogenic influences (Gillings et al., 2015). For example, the prevalence of class 1 intregron has been verified as a molecular marker for ARGs and used in modeling in a catchment (Amos et al., 2015).

The evolutionary history of antibiotic resistance

The genes that we regard as antibiotic resistance genes are, by and large, recently descended from genes whose original functions were not to confer resistance to clinical concentrations of antibiotic compounds. Two kinds of event are responsible for the genesis of modern antibiotic resistance genes: mutation of a pre-existing gene within a cell lineage; and co-option of a gene acquired by lateral gene transfer from an unrelated

lineage (Gillings et al., 2017). In the latter case, it has been suggested that many of these laterally transferred genes originally functioned in defensive responses to small signaling molecules arising from antagonistic biota, including those molecules we now use as antimicrobial agents (Davies & Davies, 2010, Davies et al., 2006, Linares et al., 2006).

This idea is supported by the observation that natural environments and environmental bacteria contain large numbers of genes that could confer resistance to antibiotics if they were present in clinical contexts. These genes are collectively termed the resistome. The resistome is far larger and far older than the small subset of problematic resistome elements that have recently made their way into human and animal bacteria of clinical importance (Allen et al., 2010). For example, gene families that can confer resistance to particular antibiotic classes are plausibly related to defense mechanisms selected in response to naturally-occurring compounds which induce chemical stress. These gene families date back hundreds of millions of years, and can be recovered from ancient environments such as caves and permafrost (Baltz, 2008, Bhullar et al., 2012, D'Costa et al., 2011).

The widespread use of antibiotics in health care and intensive animal farming since the 1950s has exerted strong selection for rare, individual cells that had recently acquired a mutation or resistome element. As a result of continuing antibiotic use resistant organisms have rapidly increased in both abundance and distribution (Gillings, 2017b).

Under this selection pressure, resistant organisms and their genetic cargo have spread between individuals, species and continents (Bengtsson-Palme et al., 2015, Hu et al., 2016). These resistance genes are readily identifiable because their recent expansion means they have highly conserved DNA sequences. Carriage of such resistance genes is now a universal feature of gut bacteria in humans and agricultural animals (Pal et al., 2016).

As a consequence of their universal carriage, resistant bacteria are continually discharged into the environment via waste water, sewage treatment plants and animal manure, thus spreading both resistant organisms and resistance genes. These same waste streams also release antibiotics (Grenni et al., 2017, Liu et al., 2017), which have significant effects, and trigger chemical stress responses even at sub-inhibitory concentrations (Chow et al., 2015). Waste waters then become giant reactors where complex interactions occur between chemical compounds, molecular responses, cells, resistance genes, and genetic transformation driven by lateral transfer and mutation (Gillings & Stokes, 2012). However, the actual potential of resistance dissemination from waste water (and WWTPs) to the environment and humans might be less than perceived, but still be a matter for further investigations (Munck et al., 2015).

The broad-scale dissemination of bacterial genes, including resistance genes, is mediated by a number of factors. This transport and transformation is controlled at various nested levels. Firstly, DNA can be released from cells and persist in the

297 environment. From here it can be taken up and incorporated into bacteria. Secondly,
298 genes can be transported within their host bacteria. Where such bacteria are dispersed
299 by water or wind, their cargo genes are carried with them. Finally, the bacteria
300 themselves can be carried inside animal hosts via mass migration, or in the case of
301 humans, by travel and tourism. For example, Daphnia can act as a refuge for ARGs,
302 and thus may contribute to the spread of ARGs in the environment (Eckert et al., 2016)
303
304 **Tracking the movement of resistance genes in Earth's Critical Zone**
305 Interest in the dispersal of antibiotic resistance genes and their host bacteria is growing
306 rapidly as the environmental consequences of this dissemination become more apparent.
307 Partly, this is because resistance genes themselves have unique environmental
308 properties and behavior. First, they behave like pollutants which exhibit environmental
309 exposure routes, and furthermore, they can replicate, making them more akin to an
310 invasive species with multiple cellular hosts (Gillings, 2017a).
311
312 Human activities directly promote the invasion and spread of resistance determinants.
313 Waste water treatment plants occupy a position between human waste streams and the
314 aquatic environment, but do not effectively remove resistance genes, thus distributing
315 them in effluent (Aubertheau et al., 2016, Ben et al., 2017, Karkman et al., 2016).
316 Effluents also contain significant concentrations of selective agents, thus promoting the
317 survival of resistant organisms, potentially at the expense of endemic species (Borruso
318 et al., 2016, Caucci et al., 2016, Koczura et al., 2016, Lehmann et al., 2016).

Application of sewage sludge, or antibiotics alone, increases the abundance of resistance genes, and changes the microbial community in soils (Chen et al., 2016, Cleary et al., 2016).

Agricultural activities also strongly promote the environmental spread of resistance through disposal of wastes and application of manure (Heuer et al., 2011, Sandberg & LaPara, 2016). Similarly, aquaculture is increasingly being recognized as a focal point for enhancing and dispersing resistance in the environment (Muziasari et al., 2016). In both of these cases, the simultaneous release of antibiotics and other selective agents promotes selection of organisms containing resistance genes (He et al., 2016, Liu et al., 2017, Wang et al., 2016). This generates opportunities for co-selection and fixation of chemical (toxic metals) and resistance determinants in species, and within individual DNA molecules (Johnson et al., 2016, Zhou et al., 2016). An investigation by Di Cesare et al. (2016) on three WWTPs revealed that heavy metal resistance genes may play a crutial role in the spreading of ARGs via mobile genetic elements.

A combination of phenomena, including the volume of human and agricultural waste streams, and the concomitant release of selective agents, means that resistance genes and resistant organisms can become extraordinarily widespread and abundant over very short time frames. A single multidrug resistant clone of E. coli has become globally disseminated since its origin as recently as the year 2000 (Petty et al., 2014).

Antimicrobial resistance in Earth's Critical Zone is thus dependent on human activities, the action of selection in natural environments, and upon natural transport mechanisms, such as rivers, groundwater and soil movement. At landscape scale, antibiotic resistance genes can move with soil erosion and drainage from top soil to groundwater.

Modeling of the dynamics of resistance genes in the Critical Zone

Effective modelling of the spread of antimicrobial resistance is essential for making predictions that can inform policy, practice and environmental surveillance. Policy makers are interested in models for two reasons. First, they support general policies that can inform handling of antimicrobials in the environment, during production, agricultural use or waste water treatment. Second, they inform possible interventions in the face of a specific outbreak of an antibiotic resistant human or animal pathogen. Models need to be flexible, realistic, and able to be used in different contexts.

However, developing realistic and flexible models that operate on an environmental scale is a significant challenge (Sommer et al., 2017). Antimicrobial resistance (AMR) encompasses a broad range of organisms, genes and antimicrobial agents, and mobile genetic elements. Sensitive and resistant organisms live in complex, heterogeneous communities. The processes that drive fixation of resistance occur at microscopic scales. Selection and spread within the Critical Zone can involve slurry tanks (Baker et al., 2016), the animal gut (Volkova et al., 2012), wastewater treatment plants (Sharifi et al., 2014) and industrial effluents, while broader dissemination might be driven by soil

363 movement, water percolation, rivers, domestic animals and wildlife. Some initial linear
364 modeling has been tried to characterize the impact of rainfall on the spread of ARGs in
365 a subalpine river (Di Cesare et al., 2017).
366
367 Mathematical modelling of resistance spread has been applied at a range of scales.
368 Models for laboratory-scale experiments have been valuable for establishing rates of
369 mutation, selection and the spread of resistance (Bootsma et al., 2012, De Gelder et al.,
370 2004). However, while these models are useful for characterizing key processes, they
371 do not scale up to the required complexity for whole environments. Consideration of
372 the spatial structure of microbial communities, for example biofilms, gives a more
373 accurate representation of the spread resistance in a community (Lardon et al., 2011).
374 Models of farms or sewage treatment plants have shown that it is possible for resistant
375 organisms or pathogens to persist even in the absence of antibiotic treatment (Sharifi et
376 al., 2014), and can also make predictions about the duration of persistence (Volkova et
377 al., 2013). However, these models have been limited to considering a single type of
378 bacterium or antimicrobial agent. Therefore, three developments are needed to move
379 forward with environmental scale models that can be effective in understanding and
380 predicting spread or reduction in resistance in the Critical Zone: inclusion of
381 heterogeneity; multi-scaling in space and time; and effective global data sharing.
382
383 First, models will need to consider a fuller range of organisms, resistance genes, mobile
384 genetic elements and antimicrobials, that reflect the complexity of the observed system

(Chen et al., 2016, Perron et al., 2015) and the importance of co-selection of antibiotic and metal resistance genes (Gullberg et al., 2014, Pal et al., 2015). Importantly, different organisms, genes and mobile genetic elements will behave differently, leading to heterogeneity in growth, transmission and selection. However, their inclusion will be essential to determine the pace and range of spread or elimination of resistance, and the relative contributions of resistance genes to the emergence of potentially resistant pathogens. This is a considerable modeling challenge, because the number of possible genetic and resistance combinations increases exponentially with the degree of biological complexity to be included. For example, even within a mass action ordinary differential equation framework, to model populations of a single bacterial species in an environment with two different antimicrobials, two respective resistance genes, that each might be carried on one of two different mobile genetic elements, requires many differential equations, and such models are difficult to parameterize or analyze.

Second, models will need to operate on multiple scales. While the best representation of spread of AMR on a microscopic scale is through individual-based models, such models do not extend to an environmental scale. Therefore, it will be necessary to coarse-grain predictive outcomes of small-scale models into larger scale, multi-compartment models that can consider populations of humans, farm animals and wildlife in their respective geographical compartments. It may also be necessary to use models that combine deterministic with stochastic elements. Deterministic models are capable of simulating large populations of bacteria, while stochastic models can capture

rare and random events, for example the spread of a particular resistance determinant from one species to another. A further feature of such models will be the need to embed geospatial data (Pruden et al., 2012), to include factors such as topography, land use and water flows.

Third, such models will require considerable calibration against real data. Researchers carrying out environmental and field studies will need to share data in a way that is useful for embedding into predictive models. To do this, agreed standards will be required for data capture and sharing, and the development of an international database for resistance in the critical zone. Such data could include observations from a wide range of experimental techniques, and data on taxa, species, phenotypes, genomes, resistance genes, mobile genetic elements, antibiotics, heavy metals and other antimicrobials. Ideally, the data would also include geospatial coordinates so that they can be used in geospatially explicit models. While this challenge alone is considerable, there is considerable precedent for agreed data standards in other areas of high throughput biology, which this development can draw upon.

Dispersal of resistance genes in the Critical Zone – A planetary view

Understanding movement of antibiotic resistance through the Critical Zone is complex, and difficult to model. Quantifying the movement of antibiotic resistance genes (ARGs) requires the coupling between the transport of bacterial cells (and resistance genes they carry) and materials (and associated selective agents) and their interactions within the

Critical Zone (Figure 2). We can then infer more general principles about the movement and transformation of genes and microorganisms. These principles might then be tested and applied to even more complex, multi-gene phenotypes of central importance to global biogeochemistry.

Before humans had a major influence on the planet, movement of microorganisms and the genes they carry was mainly driven by natural phenomena, such as air currents and water flow. Without human influence, a relatively small number of microbial cells would be transported to any specific location, therefore chance played a large role in dispersal of bacterial cells/genes. This dispersal did not necessarily result in survival or recruitment, since locally adapted cells were already present, and filled existing niches. With the advent of the Anthropocene, human activities now have large effects on the dispersal of microorganisms and the genes they carry (Table 1). Movement of humans around the globe transports our internal microbiota to new locations at an unprecedented scale. Human migration changes the abundance of resistance genes, and successfully transports resistance genes between continents (Bengtsson-Palme et al., 2015, Sun et al., 2016).

The fact that biomass of humans and domestic animals now comprise 35 times that of wild terrestrial mammals (Smil, 2011) may have consequences for the microbial world. Firstly, humans, domestic and agricultural animals all carry resistance genes in their gut microbiota, thus vastly increasing the abundance and distribution of these genes on the

planet. Secondly, on a global scale the fecal microbiota are now mainly represented by the gut microbiota of six species: humans, cattle, sheep, goats, pigs and chickens. Thus, the overall diversity of bacteria being shed in feces has consequently declined. At the same time, the quantity of fecal microbiota has increased as the biomass of humans and their domesticates approaches five times the global carrying capacity for terrestrial vertebrates (Smil, 2011). Therefore , disposal of both human and animal manures has a significant impact on the dissemination of both microbial organisms and genes (Chen et al., 2016, Jechalke et al., 2013). These cells and genes can contaminate agricultural produce (Bengtsson-Palme, 2017, Jones-Dias et al., 2016), which is then transported between countries.

Humans disperse microorganisms by mass movement of materials (Table 1). Transport of ballast water in ships is estimated to move 10^{19} bacteria each day (Endresen et al., 2004, Ruiz et al., 2000), spreading diverse microorganisms around the globe and thus reshaping microbial biogeography (Brinkmeyer, 2016, Lohan et al., 2016). It has been suggested that anthropogenic movement of soil, sand and rock now surpasses all natural processes combined (Wilkinson & McElroy, 2007), incidentally transporting huge numbers of microbial cells. Wastewater also transports microorganisms and their cargo genes into the environment. With increasing human populations, the volume of wastewater is increasing, but global data on the treatment, reuse, or volumes of waste water is difficult to assemble (Sato et al., 2013). As an example, antibiotic resistance genes now pollute over 4,000 kilometers of the Chinese coastline at levels up to 100

473 million genes per gram of sediment (Zhu et al., 2017b). None of these genes would
474 have been present in this sediment 50 years ago.
475
476 Human activities increase the numbers of microorganisms being transported within the
477 Critical Zone and around the Earth ecosystem, thus increasing the chances for
478 successful recruitment (Table 1). Furthermore, during transport, microorganisms are
479 often exposed to pollutants, particularly during discharge of manure and waste water.
480 Exposure to antibiotics and other co-selective agents, even at low doses, can enhance
481 the rate at which bacteria generate diversity via mutation (Kohanski et al., 2010),
482 recombination (Guerin et al., 2009) and lateral gene transfer (Prudhomme et al., 2006).
483 The simultaneous dispersal of microorganisms and various selective agents increases
484 the genetic variation being generated in those microbial populations, enhancing their
485 potential to evolve (Gillings & Stokes, 2012). Consequently a subset of the cells
486 dispersed to new locations are adapted to the co-dispersed pollutants, increasing their
487 probability of recruitment at these new locations. Further, because genes for metal,
488 disinfectant and antibiotic resistance are often closely linked (Johnson et al., 2016),
489 exposure to any one selective agent drives their co-selection, and maintains mosaic
490 clusters of resistance determinants (Di Cesare et al., 2016, Gaze et al., 2005, Skurnik
491 et al., 2010). Possession of diverse resistance determinants significantly increases the
492 probability of recruitment at novel destinations by providing a selective advantage over
493 endemic microorganisms (Table 1).
494

Concluding remarks

It is becoming more and more important to understand how human activities cause systematic changes in ecosystems (Alberti et al., 2017), and especially the effects on the emergence and spread of ARGs in urbanizing Earth's Critical Zone (Zhu et al., 2017a). To better understand the dynamics of ARGs in the Critical Zone, future studies should emphasize linkages between biogeochemical cycling of nutrients and contaminants with the movement of microorganisms. Under the framework of Critical Zone science, tracking the dynamics of ARGs should give us insights into the interconnections between multiple environmental compartments within the entire Critical Zone. Due to the extreme heterogeneity of the Critical Zone, we should also focus on hot spots for ARG dissemination such as locations receiving high loads of wastewater or manure. Understanding the complex feedbacks between the dynamics of ARGs and interactions with physical, chemical and biological processes in the Critical Zone is a grand challenge. Progress can only be made by forging interdisciplinary research teams that can manage and interpret the enormous datasets of genomics and biogeochemistry, and by developing predictive models based on these datasets.

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Tables

Table 1: Dissemination of genes and microorganisms in Earth's Critical Zone.

Three phenomena, or drivers, affect microbial/gene spread. These are: opportunity for dispersal; stochastics (the number of foreign cells landing at a particular location, processes that generate local variation such as mutation and drift); and recruitment (the persistence of cells at the new location, often driven by local selection). Historically, these forces generate biogeographic patterns for microorganisms that are similar to those of animals and plants. Human impacts have changed the dynamics of these phenomena, and are altering microbial biogeography in the process.

Figures

Figure 1. **Movement of antibiotic resistance genes and bacteria in Earth's Critical Zone.** Bacterial cells and their genetic cargoes are released from human dominated ecosystems in waste water and manure. These same waste streams carry significant quantities of selective agents, promoting recruitment and survival of cells and resistance genes at all destinations. Microbial transport is enhanced by mass movement of soil, produce, and ballast water, and by human tourism. The extent of this movement can be assessed by examining the spread of antibiotic resistance determinants through the Critical Zone. Key hotspots antibiotic resistance genes are: Hospitals, wastewater treatment plants (WWTPs), intensive animal farms, antibiotic manufacturers.

Figure 2: Movement of DNA through cells and ecosystems. DNA cargo can move within the cells that originally contained it, or can take advantage of the frequent lateral gene transfer that occurs between bacteria. In the case of transfer by conjugation or nanotube (LHS), DNA is passed directly from one cell to another, often on plasmids (P). When cells lyse through death or bacteriophage attack, they release their DNA content (RHS). This DNA can survive in the environment as naked DNA or encapsulated inside bacteriophage. Such extracellular DNA can be transported by physical processes, and be acquired by a new cell at locations distant in both space and time.