Human impacts on soil community mineralization of chloroaromatics

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ABSTRACT

Many organic pollutants such as pesticides are chlorinated organics, and until recently these were thought to be purely the product of anthropogenic activities. However, some chlorinated phenolics are now known to be part of the natural chemical make up of "pristine" soils, defined as those that have not previously been directly exposed to anthropogenic inputs, except through atmospheric contamination. The ability of a wide variety of these soils to mineralize a range of chlorinated aromatic chemicals has been investigated, and contrasted to the mineralization potential of agricultural soils. Our work has shown that in some instances pristine forest soils have a greater ability to degrade some chlorophenolics than agricultural soils, and that the latter harbour more specialized degradative communities. In addition to what appears to be a more generalist degradative community, pristine soils harbour high diversities of the genes encoding isofunctional enzymes important in the degradation of chloro-aromatics. In addition, these genes are often found in hosts that are quite different from those that carry them in more contaminated sites, suggesting that adaptation of soils to higher concentrations and more constant levels of chloro-organic inputs involves the loss of detectable genetic diversity.

Introduction

The ability of microorganisms to break down xenobiotics such as chlorinated aromatic compounds has long been assumed to be a recently evolved trait, selected for by the presence of pesticides and their residues in the environment. However, it is now clear that the ability to mineralize chlorinated aromatics such as 3-chlorobenzoate (3CBA) and 2,4-dichlorophenoxyacetate (2,4-D) is present in pristine soils that have not been directly exposed to xenobiotics (15,16). These findings are not surprising in the light of the fact that naturally occurring halogenated aromatic compounds have proven to be fairly diverse. Of particular note is the discovery of high levels of chlorinated anisaldehydes produced by a variety of wood-rot fungi (7). However, for a long time it was assumed that soils or waters had to be exposed to xenobiotic chemicals, essentially industrial contaminants, before chloroaromatic degradative abilities would evolve in the bacterial community. Accordingly, our understanding of this soil capability derives from studies on contaminated systems and bacteria isolated from contaminated systems. Within this older paradigm, the effect of human activity on soil degradation of chloroaromatics was to enhance its evolution. However, if the selective force behind the evolution of bacteria capable of degrading chlorinated compounds derives from natural sources, then anthropogenic effects on other biotic components of ecosystems must also be considered. Below I outline some ways in

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which human impacts may indirectly and directly affect the chloroaromatic degradative capability of soils.

By Impacting on Plant Communities

Microbiologists and soil scientists have long distinguished between the bulk soil and the rhizosphere -- that part of soil that is in close association with the active roots of terrestrial plants. The overall microbial activity of the rhizosphere is much higher than that of the surrounding soil because of the higher concentrations of organic substrates that are exuded from the roots. There is increasing evidence that the rhizospheres of some species can have a more specific effect than merely increasing general bacterial populations and activities. A number of studies indicate that specific plant species can stimulate the degradation of 2,4-D (5, 11, 25), 2,4,5-trichlorophenoxyacetic acid (5), atrazine (2, 11) and 2-chlorobenzoate (26) in rhizospheric soils. This is presumably due to enrichment of specific bacteria, or the induction of specific pathways by the species-specific phyto-exudates.

We have also recently demonstrated differences in chloroaromatic degradative abilities between different plant communities (14). Soils were sampled from two agricultural fields, two relatively pristine forests and one suburban forest in Ontario, Canada. The ability of these soils to mineralize 2,4-D, 3CBA, 4-chlorophenol, 2,4-dichlorophenol (DCP), pentachlorophenol (PCP) and atrazine was determined using ^{14}C-labeled substrates. Direct preexposure was necessary before atrazine mineralization could be detected, but it was not necessary for degradation of any of the other chemicals. 2,4-D and PCP mineralization rates were much higher in the agricultural soils relative to the pristine forest soils, but 3CBA and DCP mineralization rates showed the opposite trend. Mineralization of 4-chlorophenol was about equivalent in all soils. Suburban forests soils resembled pristine forests in their processing of only some of the chemicals - but they were indistinguishable from agricultural soils with respect to their degradation of 2,4-D and 3CBA. Additionally, they were better able than any of the soils to withstand the toxic effects of PCP. PCP mineralization was highly variable in the pristine forest soils, ranging from about 6 to 50% of substrate mineralized after 60 days. Abiotic factors such as pH, soil type, organic and moisture content did not account for these significant site differences.

There is also evidence that plant species or communities can have an effect on precisely which strains of bacteria degrade specific compounds, in addition to affecting the range of compounds mineralized. Fulthorpe *et al*. (16) isolated 610 3CBA degraders from widely separated, relatively pristine ecosystems from around the world. Samples were taken from two boreal forests and four Mediterranean systems, all of which were sites unexposed to direct sources of anthropogenic contamination. In one Mediterranean region, the strains that were isolated were sufficiently high in number for a statistical association between vegetation type and 3CBA degrader genotype to be revealed. In this region we had sampled three sites from renosterveld ecosystems, characterized by a dominance of *Restia* bushes and ericaceous shrubs, one site dominated by Proteaceae (fynbos vegetation) and another by *Eucalyptus* overgrowing a former renosterveld site. High numbers of 3CBA degraders were isolated from each of these sites and typed using REP fingerprinting (30). The renosterveld sites, in spite of the fact that they were the most widely separated of the five, were populated by identical or extremely closely related genotypes, given the name RENOS. In all we isolated 33 RENOS genotypes and only four other genotypes from the

renosterveld sites, a highly statistically significant association between genotype and vegetation type (15).

By Influencing Organic Residue Cover

No specific work has been done on investigating microbial communities that reside in exposed versus covered soils, but a specific result that we obtained leads to some speculation in this area. When the genetic makeup of chlorobenzoate degraders isolated from different soils collected around the world was studied, as described above, the greatest diversity of strains was isolated from the boreal forests of Saskatchewan, while Russian soils also collected from boreal systems exhibited diversities equivalent to that of South African soils (16). The most striking difference between the soils of the boreal and the South African areas is the amount of organic matter that overlies them. The soils sampled from the boreal systems were covered by 10 -- 20 cm of feather mosses, and below that a thick humic layer which was removed prior to sampling. In South Africa, the soils were all but bare, perhaps having small patches of organic litter or moss. The boreal soils might be experiencing a greater diversity of standing organic matter, and this might be of greater importance to bacterial community structure than the diversity of the live plant standing crop. However, another explanation might be that the bacteria of the mineral soils of boreal forests are much more spatially isolated, and greater diversification is possible. In the exposed soils, such as those typical of renosterveld vegetation, mixing of soil communities via wind, rain and animal movement must be a much more common occurrence. This leads to speculation about the effects of exposing soils and increasing rates of soil erosion. While one of the effects may be a decrease in the amount of spatial isolation and therefore bacterial diversification, another more beneficial effect might be increased opportunity for the mixing of communities, increased genetic exchange events and a greater opportunity for bacterial adaptation to truly xenobiotic chemicals.

By Altering Mammalian Fauna

A preliminary study carried out in this lab has raised the question of the possible influence of wild fauna on soil bacterial communities and their capabilities. We obtained soils that had been sampled from the sparsely vegetated ranges of Kluane National Park in the Yukon Territories, Canada. We tested two types of soils: the older unglaciated soils of the inland ranges from two sites, and the glaciated, younger soils found in small meadows perched within the rock formations emergent from glacial ice fields (known as Nunataks). The younger soils support the growth of most of the same plant species found in the older ranges. We examined several soils taken from eight locations for the mineralization of 3CBA, 2,4-D, PCP. Levels of 3CBA and PCP mineralization after 27 days are extremely low, from 1.0±0.6% to 3.9±0.3% for 3CBA, and 1.9±0.2% to 8.5±1.8% for PCP. However 2,4-D mineralization rates range from 6±0.7% to 51±16%. The significantly higher activities against both PCP and 2,4-D are seen in the soils that differ from all the other sites in one main way -- the presence of dense populations of herbivores, mostly pikas (19). While microbiologists have long studied the microbial community residing in the guts of ruminants, termites and other animals, studies on the effects of the animal fecal matter on soil microbial communities and their ability to process different wastes are limited to studies on the effects of deliberately applied manure treatments. Various types of livestock manure

have been shown to improve atrazine (12, 18, 19, 28), 2,4-D (12), lindane (8) and DDT (4) mineralization degradation when used to amend soils: Conversely, Sumasundaram *et al.* (24) report an increased persistence of six pesticides including chlorpyrifos after hog manure application. Three mechanisms may be influencing microbial mineralization rates - the bioavailability of the chemical in the presence of extra organic matter, the extra nutrients provided with the manure, but also the nature of the bacterial populations that might be enhanced, or in the latter case inhibited, during such treatment. Our very preliminary results from the Yukon soils at least hint at a new avenue for research.

By Directly Applying Selective Chemicals

The impact of chloroaromatic chemicals that are deliberately applied by humans is best illustrated by the bacteria that degrade 2,4-D, a commonly applied herbicide. Many 2,4-D degraders have been isolated from agricultural systems, and several have had their catabolic genes sequenced, or at least investigated by hybridization analyses. The best known of these is *Ralstonia eutropha* JMP134 (9). Originally isolated in Australia, it was found to carry a large plasmid pJP4, that harbors genes for 2,4-D degradation. Identical or highly similar plasmids have subsequently been identified in other strains isolated from other parts of the world (1, 3, 6) Most, if not all, of these known strains can also degrade 3CBA. 2,4-D resembles 3CBA in its physical and chemical properties, and is degraded via similar intermediates through the ortho cleavage pathway. However, it is not always processed the same way by soils. Although virtually 100 % of pristine soils sampled to date can degrade 3CBA, only some of them are capable of 2,4-D degradation (14, 16). The major difference in the degradative pathway of these two chemicals is the presence of a 2,4-D α-ketoglutarate dioxygenase that cleaves the ether bond present in 2,4-D to produce a phenol (13). This particular enzyme, encoded by a *tfdA* gene, is the rate limiting step in 2,4-D breakdown. It may be that its absence from the genomes of bacteria that otherwise carry genes for general chloroaromatic breakdown is responsible for the less efficient degradation breakdown of 2,4-D relative to 3CBA. As elaborated below, there are several pieces of evidence that 2,4-D mineralization is carried out by a particular, limited group of bacteria in pristine systems, but that enhanced horizontal gene exchange mechanisms in agricultural systems have led to acquisition of *tfdA* by organisms that previously did not have it, possibly 3CBA degraders, and/or the assembly of 2,4-D degradative genes in a more diverse group of bacteria.

2,4-D degraders from agricultural systems are largely members of the β-proteobacteria (often *Burkholderia or Ralstonia),* or are of the genus *Sphingomonas*. Some of the former closely resemble 3CBA degraders of pristine systems (17, 22, 23, 27). In various 2,4-D degraders, many variants of the pJP4-like catabolic genes (*tfdA*, *tfdB* and *tfdC*) exist (17,29). They appear to have been assembled in a mosaic fashion through horizontal transfer events. Top *et al.* (27) have shown that *tfdA* genes are mobile (plasmid borne) in soil systems and can be "captured" in Δ*tfdA Ralstonia eutropha* recipients mated with soil bacteria. However, the gene rearrangements that have led to a high diversity of 2,4-D degraders appear to have occurred after anthropogenic contamination. When soils from uncontaminated areas are sampled, 2,4-D degraders are not easily cultured (16). Kamagata *et al.* (21) report success in isolating 2,4-D degraders only after patient, repeated subculturing. They report oligotrophic α-proteobacteria similar to *Bradyrhizobium* that do

not carry the genes typically found in the β-proteobacteria 2,4-D degraders of agricultural soils. Genes are yet to be sequenced from the *Bradyrhizobium*-like degraders. The introduction of anthropogenic 2,4-D seems to have selected for the assembly of an efficient 2,4-D pathway in β proteobacteria and *Sphingomonas*.

Another effect of direct chemical application seems to be a loss of degrader diversity. Dunbar *et al.* (10) compared the 2,4-D degraders obtained via a direct plating method with those obtained by enriching the degrader populations with 377 μM of 2,4-D. The latter method produced 7 different strains, while direct plating led to the isolation of 25 different types exhibiting a greater genetic diversity. Using direct plating, Dunbar has also illustrated that enriched soils (amended with 10 - 100 ppm of 2,4-D) also become dominated by a limited number of strains and genotypes (Dunbar, PhD Thesis, Michigan State University 1996). It is difficult to assess whether or not 2,4-D amended soils have lost the populations that are present in unattended soils, or are simply dominated by fast growers. If the latter, then it is likely that intermittent application of 2,4-D enhances genetic diversity. As populations temporarily increase, the opportunities for horizontal gene exchange must also increase. However, it might be the case that only a limited number of the many variants of 2,4-D catabolic genes are favoured by this kind of chemical selection.

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