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Title: Microbial services and their management: recent progresses in soil bioremediation technology

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Abstract

There is an increasing interest in soils and sediments because their vital importance for the survival of the planet has become apparent. They assure a multitude of services such as removal of various gases from the atmosphere (methane, carbon monoxide, ...), filtering of water, removal of pathogens, degradation of organics, recycling of nutrients, ... All these processes represent an economic value, which is estimated to be about the double of the gross annual product. Yet, numerous sites (estimated at more than a quarter million in the EU alone) are polluted and need to be cleaned up. Soil biotechnology plays an ever-increasing role in this. The reason why engineers need to enhance or induce natural processes and the ways they can do this, are reviewed. Furthermore, those soil services which are of vital importance are evaluated. To understand the underlying ecological mechanisms during soil remediation, a pragmatic approach using molecular tools is proposed. Subsequently, a series of new biotechnologies for soils is examined. Also putative new dangers for soils are scouted for. Moreover a set of paradigms currently implemented in the field of soil governance is critically examined. Finally a short list of future market potentials in soil biotech is presented. Overall, it is concluded that soil biotech, driven by the economic value of the services rendered by high quality soils, is currently in a phase of extensive growth.

Keywords

microbial, contaminated, soil, services, management.

I. Introduction

Soil is an extremely complex, dynamic and living medium, formed by mineral particles, organic matter, water, air and living organisms. It establishes the interface between earth, air and water and performs many vital functions both from an ecological as non-ecological point of view. While the physical basis of human activities, source of raw materials and the geogenic and cultural heritage are among the non-ecological functionsthe soil ecological services include biomass production, filtering, buffering and transformation capacity between the atmosphere, the groundwater and the plant cover. Soils and sediments also serve as a gene reservoir, because the soil provides a biological habitat for a large variety of organisms (Blum, 2005).

Despite and due to the variety of services that it renders, there are numerous threats to soils: acid rain, exhaustion by farming, landfill seepage, mining, highway construction, reservoir flooding, irrigation, overgrazing,… All these processes have profound implications on the soil habitability. The soil system is normally endowed with a large resilience, therefore damage often only becomes apparent when it is far advanced (EEA, 2005).

According to the European Environmental Agency (EEA) it is estimated that in Europe, potentially polluting activities have occurred at about three million sites, from which more than 8% (or nearly 250 000 sites) are really contaminated and need to be remediated. Projections based on the analysis of the changes observed in the last five years, indicate that the total number of contaminated sites needing remediation may increase by more than 50% in 2025.
Remediation is progressing relatively slowly: in the last thirty years, only just over 80.000 sites have been cleaned-up in the countries where data on remediated sites is available (www.eea.europa.eu).

The main sources of soil contamination in Europe are spills during industrial and commercial operations, municipal and industrial waste treatment, oil extraction and production and inadequate storage of goods (www.eea.europa.eu). Heavy metals and mineral oil are regarded as the main soil and groundwater contaminants in Europe. Other contaminants include polycyclic aromatic hydrocarbons (PAH), aromatic hydrocarbons (BTEX), phenols and chlorinated hydrocarbons (CHC) (www.eea.europa.eu).

The high and increasing number of contaminated soils results in an unaffordable damage to the soil services. Because the valuing of soil ecosystem services is missing at the policymaking level, the costs of soil services losses can go unnoticed (TEEB, 2009). A major part of the 17 ecosystem services, as reviewed by Costanza et al. (1997) are directly depending on healthy soils. These authors estimate the overall ecosystem services (use and non-use or market and non-market values) to represent a total intrinsic economic value of twice the global gross national product. Based on this reference one can assume that the services related to healthy soils in our industrialized countries represent a total use and non-use value in the order of 10.000 € per inhabitant per year. Such estimates, although criticized (Wallace, 2007) and in need for thorough underpinning, at least stress the necessity and value of adequate soil service management.

This review looks into the ways available to enhance or induce natural processes. It tries to evaluate the vital importance of those soil services and examine a series of new biotechnologies that can be implemented in soils. It gives an overview of putative new dangers for soils. Moreover a set of paradigms currently implemented in the field of soil governance is critically examined. Finally a short list of future market potentials in soil biotech is presented.

II. Nine questions about contaminated soil and sediments

The interface between the scientific and the business community is quite strict. While for the scientists, soils are mainly an object of exploration and experimentation, industry tries to deal in a cost-effective way with the cleanup of contaminated sites. In this respect, a series of often heard questions are reflected upon to broaden both parties' insights into the complexity of the soil.

1. Why is "mother nature" not always doing the job of auto-epuration?

1.1. The physico-chemistry is wrong

Several aspects determine if a reaction can occur. In order for a reaction to take place the change of Gibbs free energy must be adequate. A bacterium needs a minimum of about -20 kJ per mol of substrate in order to exploit the free energy change in a reaction (Heimann et al., 2010; Schink, 1997). Estimates of the Gibbs free energy for several PAH (polycyclic aromatic hydrocarbons) indicate energetic yields between -209 and -331 kJ/mol for their methanogenic degradation under standard conditions, however this process has long been considered impossible (Dolfing et al., 2009). Not only thermodynamics but also the concentrations of the pollutant by itself represent a challenge, as too high concentrations may be toxic for the microorganisms and too low concentrations may be below the threshold limit. Moreover, the appropriate electron acceptors and donors have to be present. Conditions such as pH, redox potential and temperature have to be suitable and the presence of easier to degrade substrates can also represent an obstacle, as these compounds will be preferred by the microorganisms (Boopathy, 2000).

1.2. The biocatalyst is lacking or insufficiently empowered

A contaminant needs to be (bio)available in order to be taken up and become degraded. The bioavailability of a chemical is determined by the rate of mass transfer (transfer to the cell) relative to the intrinsic activity of the microbial cells (rate of uptake and metabolism) and is controlled by physical-chemical processes such as sorption and desorption (Burgos et al., 1996), diffusion and dissolution (Bosma et al., 1997). The growth of cells is negatively correlated with the distance from the substrate because of substrate diffusion limitations. Effective diffusivities of the substrates and the spatial distribution of substrates and bacteria are main determinants in degradation rates, as the distinct micro-scale distribution of pollutants and bacteria in contaminated soils implies that pollutants must diffuse to the bacteria before they can be taken up and degraded (Harms, 1996).

It is quite evident that the biocatalyst needs to be present in order for the pollutant to be degraded. However this is not always occurring, as the bacteria capable to degrade a specific chemical might not have reached the contaminated site. This has been demonstrated by introducing bacteria capable of 1,2-dichloroethane (1,2-DCA) degradation on a contaminated site, resulting in an increase of the degradation of the contaminant (Maes et al., 2006).

Microorganisms need more than just the pollutant as carbon source and/or energy source. Nutrients (macro nutrients – N, P and micro nutrients – e.g. Mo for N fixation) also need to be present at the site and in adequate amounts (Howard and Rees, 1996; Leys et al., 2005).

Another very important parameter is the water potential. The continuous water films in soils do not only allow nutrient and metabolite transfer between particles and micro-organisms, but also provide means by which organisms can move to more favourable locations, overcoming the constrains of bacterial spatial distribution (Grundmann, 2004). The lowered water activity of dry soils can inhibit microbial activity (Csonka, 1989), restrict microbial movement and decrease the diffusion of substrates (Treves et al., 2003). However it has also been observed that plenty of species of microorganisms are active in very dry soils, e. g. nitrifiers are active down to 10 molecules of water film thickness (Papendick and Campbell, 1981).

A challenge for the degradation of pollutants is the fact that it can occur only when there is a pathway to degrade it. For quaternary carbon compounds, there is no degradation pathway known. This is also the case for several other compounds such as diclofenac, diatrizoate, carbamazepine, phenazone and more, as listed in a review regarding pharmaceuticals and personal care products (Onesios et al., 2009). The existence of stereoisomers can also represent a problem as different isomers can exert a different effect on the microbial community. Mertens *et al.* (2005) demonstrated with short-term experiments that only gamma- and delta- isomers of hexachlorocyclohexane inhibited methane oxidation, evidencing the stereospecific effect of hexachlorocyclohexane on both the activity and structure of soil methanotrophic communities. Moreover one of the enantiomers can be biodegradable while the other is not. This has been observed for instance for the strong metal chelator EDDS versus its enantiomer EDTA that is poorly biodegradable (Van Devivere et al., 2001). However a missing pathway might eventually evolve. For the widely used s-triazine herbicides such as atrazine, as referred in the review by Shapir et al. (2007), the environmental half-lives have decreased dramatically (from typically 60 to 400 days in the decades following its introduction to a range from 1 to 50 days at present). Moreover, metabolite profiles have changed: while in the period between 1960 and 1990 the bacterial metabolism of atrazine was reported to occur via dealkylation of the *N*-alkyl substituents on the *s*-triazine ring, since 1995 most reports describe the atrazine degradation metabolism via hydroxyatrazine which does not involve dealkylation. Finally, the earlier reported difficulties in growing bacteria on atrazine as sole nitrogen and carbon source were overcome, as recently pure cultures of these bacteria were obtained from most soils (Shapir et al., 2007).

2. How to maximize natural attenuation?

Soil systems are not close to equilibrium and subject to significant perturbation (Crawford et al., 2005), which can be favourably used to activate natural attenuation. One can first look at the optimization of chemical and physical conditions by improving parameters such as temperature (Friis et al., 2007), moisture content, nutrients and humus. Another parameter that can be considered is the priming of the soil. This can stimulate the microbial community as extra decomposition of native soil organic matter occurs in a soil receiving an organic amendment (Bingeman et al., 1953).

Promoting gradients in soils is another aspect that can be achieved by imposing treatments such as freezing and thawing, drying and rewetting, ... (Sharma et al., 2006). Changing conditions between extremes can promote the selection of microorganisms with interesting abilities. For instance, dryer conditions prevalent at the soil surface will restrict the mobility of the individual cells leading to the outgrowth of specific microbial populations. Once isolated, more competitive species cannot compete with less fit community members at other places and as such species diversity will tend to remain high (Treves et al., 2003). During flooding and subsequent draining, cycles of aerobic/anaerobic alternation can occur which intensify microbial activity.

Bioaugmentation is a way to enhance the removal of contaminants by inoculating the contaminated site with microorganisms, either being pure cultures or mixed cultures, able to degrade the chemical of interest. This ability of the microorganisms may be natural or induced in the laboratory by introduction of the necessary degradative pathways in the carrying microorganism (Goldstein et al., 1985). Several studies have successfully applied this approach. Dejonghe et al. (2000) observed an increased 2,4 – dichlorophenoxy acetic acid (2,4-D) degradation upon inoculation of contaminated soil with a donor strain carrying plasmids encoding for the degradation pathway. The introduction of the plasmid was accompanied by a shift in the microbial community structure due to the proliferation of transconjugants. At an industrial site where groundwater contamination with 1,2-DCA was observed, injection of *Desulfitobacterium dichloroeliminans* strain DCA1 decreased the groundwater concentrations of 1,2-DCA from 93 to 0.089 mg/L in a 35 day time interval in the presence of a sodium lactate solution (Maes et al., 2006). The commercially available KB-1 mixed culture has been successfully used for bioaugmentation in order to stimulate degradation of chlorinated organic compounds (perchloroethene, trichloroethene, dichloroethene and vinyl chloride) into the end-product ethene (Hood et al., 2008; Scheutz et al., 2008). A butane enrichment culture containing predominantly two *Rhodococcus* sp. strains was used for promoting degradation of 1,1,1-trichloroethane (1,1,1-TCA) and 1,1-dichloroethene (1,1-DCE) by cometabolism (Semprini et al., 2009).

Co-metabolism is another process that can be used for removal of contaminants in soils. In this process, contaminants are fortuitously degraded by non-specific enzymes without the respective microorganisms gaining energy for microbial growth or metabolism with this process (Bradley, 2003). Several studies have reported this kind of degradation. The methane mono-oxygenase enzyme (MMO) responsible for oxidation of methane to methanol in methanotrophs has shown to be capable of cometabolic degradation of many compounds, for example trichloroethylene (TCE) (Bowman et al., 1993; Yoon and Semrau, 2008). A nitrifier enrichment culture has shown its ability to remove 17α-ethinylestradiol (EE2) at low levels by the enzyme ammonia mono-oxygenase (AMO) (De Gusseme et al., 2009). Besides co-metabolism, there is also cross-metabolism in which several microorganisms cross feed on the original molecule, or its metabolites. An example of this has been described for 4-chlorosalicilate in which the mixed culture was shown to be much more powerful in degradation than any of the single strains (Pelz et al., 1999).

3. Which microbial services in the soil are of vital importance?

Soil as a beholder of a diversity of microbial life provides a large diversity of vital microbial services. Defining which processes are related to soil quality is a difficult task, as this might depend on the use of the soil and the complexity of the soil system. Dynamics and mineralization of organic matter, soil structure formation and/or maintenance, support and control of plant production and species diversity are processes that can be distinguished (Verhoef et al., 2004).

3.1. Degradation of organic matter

The ability of the soil to degrade organic matter allows the soil to act as a "graveyard". Indeed, this process is responsible for the removal of "dead bodies", not only of higher organisms (such as ourselves) but also microorganisms themselves, for instance in the form of sewage sludges that are mineralised in the soil (Boyle and Paul, 1989; Schowanek et al., 2004). Indeed, soil microorganisms play a key role in the nutrient cycling of the soil ecosystem. The organic residues are decomposed resulting in part in microbial biomass and in part in mineral forms such as CO_2, H_2O, mineral nitrogen, phosphorus and other nutrients. Those components immobilized in biomass, will eventually be released when this microbial biomass dies off and becomes organic residue (Verstraete et al., 2004). The degradation of organic matter results in the recycling of nutrients for plants in forests, wetlands, mangroves… and supplies plants with high levels of CO_2, thus facilitating their growth (Schlesinger and Andrews, 2000). This ability also contributes to the removal of bacteria, viruses and pathogens shed by plants and animals. In this way the soil is responsible for biohygienization and helps to avoid the outbreak of pandemics (Larkin et al., 1996).

The soil capacity for organic matter degradation can be used not only for the normal cleaning of groundwater but also in a technically designed and controlled way (Fig. 1) (Haeffner and Grandguillaume, 2009).

Another interesting feature of soil microbial processes is the removal of atmospheric pollutants, which are important in the context of global warming such as CO_2, CH_4 (Seghers et al., 2005), CO, N_2O. Only soils with a very high water table are sources of CH_4, all others normally act as CH_4 sinks (Smith et al., 2000). However, conversion of natural soils to agricultural use decreases oxidation rates by two-thirds (Smith et al., 2000). Application of 2,4-D in soils has shown to inhibit the methane oxidation capacity (Seghers et al., 2003). Soils farmed with organic fertilizers have three times higher methane oxidation rates when compared with soils receiving mineral fertilizers (Seghers et al., 2005).

3.2. Nitrogen fixation

Parent rock materials and their weathering products do not contain nitrogen. Consequently most of the nitrogen demanded for biological processes has to be transferred from the atmosphere where it is present as inert N_2 into a biologically active form (NO_3^-, NH_4^+, R-NH_2). The degree of soil fertility is therefore determined by the balance between N-input and N-losses. While precipitation (atmospheric nitrogen oxygenation by thunderstorms) and biological nitrogen fixation contribute to the N-input, leaching and denitrification contribute to the N-losses (Ernst et al., 2004). It should be kept in mind that for the production of 1 kg N-fertilizer, 2 L of fossil fuel are required (Rashid et al., 2010). Moreover, the production of 1 kg of food protein requires about 40 L of fossil fuel (Pimentel and Pimentel, 2003). Hence, as the conventional fossil fuel reserves are to the best of current estimates half exploited and at the current rate of use will only last for another 20-30 years, the efficient usage of biologically fixed N will become of increasing importance.

3.3. Biomineralization

Microorganisms play a determinative role in the mineralogical characteristics of most soils and sediment environments. The geochemical role of microorganisms includes their weathering ability which liberates many essential elements (C, S, N, P) from the lithospheric reservoir within which they are largely unavailable to many living organisms (Douglas and Beveridge, 1998). Another role is the microbe's ability of producing minerals (biomineralization) by which the soil structural support is improved. The latter as well as biosorption are mechanisms that can represent an alternative for removal of inorganic contaminants. For example Hammes *et al.* (2003) demonstrated ureolytic microbial carbonate precipitation as a process for removing excess calcium from various effluents.

3.4. Reservoir/refuge of biodiversity

Bacterial diversity in pristine soils and sediments and in moderately disturbed agricultural soils is very high as numbers may reach more than 10 000 bacterial species (Torsvik et al., 1998). Microbial fungal richness increases from 5 to 120 operational taxonomic units for sampling areas varying from 10^{-4} to 10^{14} m^2 (Green et al., 2004). Protozoa can reach numbers as high as 70 000 per gram dry weight (Fierer et al., 2009). This large diversity of microbial life is observed not only in pristine soils; polluted soils have shown to be habitats of microorganisms with special abilities, as do most environments under extreme conditions. As such it stems to reason that some contaminated sites should not be cleaned but rather conserved in time for their unique biopotentials.

4. Which microbial processes in soil are ambiguous?

Apart from the essential role of microorganisms in soils, not all effects of the microbial processes naturally occurring in soils are advantageous, some are even undesired.

4.1) Nitrification The fixation of nitrogen into a biologically active form increases soil fertility. Yet, bringing it into a mobile form allows it to leach into groundwater and surface waters, thereby posing health and environmental problems. Hence, a renewed search of ways for controlling nitrification in soils is warranted.

4.2) Denitrification This process is responsible for N_2 return to the atmosphere. However it causes a release of the green house gas N_2O, with estimates indicating that about 1-10 kg N_2O-N ha^{-1} y^{-1} is produced in soils (Boeckx and Van Cleemput, 2001; Dalal and Allen, 2008). There is an absolute need to learn to manage this process in the field in an appropriate way.

4.3) Chlorination of soil organics In soils, the presence of catalytic peroxidases facilitates the chlorination of hydrocarbons and aromatic products in decomposed (humified) material using the inorganic chloride ion that dominates in fresh leaves. The knowledge about halogenation of organic matter in soil and the fate and toxicity of the resulting compounds is still very scarce (Casey, 2002).

4.4) Sulfide/S_0 oxidation The release of sulfide or the addition of sulfur can give rise to an impulse of activity of sulfur oxidising bacteria which can generate a set of secondary negative effects such as pH drop, release of metals and also of anions such as arsenate (Bayard et al., 2006; Nareshkumar et al., 2008) Yet, the inverse process, i.e. the reduction of sulfate is generally also quite undesirable since it generates toxic sulfides. Overall, the transformation of sulfur in the soil has to be monitored with care in terms of shifts in overall soil quality and ecosystem stability.

4.5) CO_2 – emission from sequestered carbon Figure 2 schematizes the overall CO_2 fluxes as calculated by Denman et al. (2007). The key message is that soil microbiota releases about 10 times more CO_2-C per year from organics present in the soil and the sediments than the amount currently emitted by cars. Clearly, any change in the former rate of microbial CO_2 production is of concern in relation to the climate change. Any method one could design to diminish that rate, e.g. in agricultural soils, would constitute a major breakthrough in agricultural engineering.

5. How can we monitor microbial processes in soils and sediments?

A microbial soil ecosystem typically contains a collection of microbial populations, i.e. a microbial community. This microbial community is normally interacting with its environment. These interactions can be of abiotic origin (temperature, nutrients, pH, substratum surface properties, …) or biotic origin (invading microbial species, grazing and predation, …). These interactions will positively or negatively influence specific microbial populations and as a result, the ecosystem functionality and stability can be affected. Indeed, it was recently shown that a high richness and a high evenness of a microbial community have positive effects on the ecosystem functionality and stress resistance (Bell et al., 2005; Wittebolle et al., 2009). To unravel the structure of the microbial community, molecular techniques are nowadays frequently used (Hanson et al., 2008). Fingerprinting techniques, such as Denaturing Gradient Gel Electrophoresis, have been used for more than 10 years and have the advantage to be rapid (both analysis as data processing) (Boon et al., 2002). However the fingerprinting techniques have a low taxonomic resolution and therefore, techniques such as microarray or 454-sequencing (Buee et al., 2009) will allow in the near future an in-depth analysis of the microbial community structure. Recently Marzoratti et al. (2008) proposed a setting-independent theoretical interpretation, based on a straightforward processing of molecular data. Using the results from these molecular methods, the different aspects such as the richness (Rr, reflecting the influx of species and the carrying capacity of the system), the dynamics of change (Dy, reflecting the specific rate of species coming into significance) and the community organization (Co, reflecting the level of population dominance within a community) can be studied (Marzorati et al., 2008). The outcome of such an analysis can allow to handle questions of who is there, who is doing what with whom and how can one adjust, control and/or steer these mixed cultures and communities leading to the implementation of a Microbial Resource Management (MRM) approach, as depicted in Figure 3 (Verstraete et al., 2007).

6. What is new in the microbial biotech of soils?

A variety of breakthroughs have recently been reported. They are subsequently organized in two groups, the first one relating to reductive processes and the second one to oxidative conversions (see Table 1).

6.1. Biologically induced chemical reductive technologies

6.1.1. Halorespiration

Several microorganisms such as *Sulfurospirillum*, *Dehalobacter*, *Desulfitibacterium,* Dehalococcoides spp. (Luijten et al., 2004; Maes et al., 2006) are able to respire halogenated compounds, i.e. use them as electron acceptors and thus dehalogenating them. However, these dehalogenating species compete for the electron donor with other soil microorganisms. The high affinity of dehalogenating bacteria for H_2 allows them to outcompete methanogenic archaea, acetogenic bacteria and sulphate-reducing bacteria at low H_2 concentrations. However, they still have to compete with nitrate- and iron reducing microorganisms, which are also able to use H_2 at low concentrations (Luijten et al., 2004).

6.1.2. The “addition of handles to unhandy substrates”

Due to the chemical inertness of specific hydrocarbons, it has been thought for decades that the biological degradation of these compounds required the presence of oxygen. Yet, about two decades ago, the first cultures of bacteria that degraded hydrocarbons under strictly anoxic conditions, were reported. Similar to the eukaryotic biotransformation pathway, these bacteria initially place a functional group on the parent molecule (hydroxylation, fumarate addition, carboxylation, methylation or reverse methanogenesis), which then serves as a “handle” to subsequently break down these compounds (Heider, 2007).

6.1.3. The Zero-Valent Iron (ZVI) donor

Zerovalent Iron (Fe(0)) is an excellent source of reducing equivalents (electrons) from a thermodynamic point of view. As such, it has been successfully employed for the transformation and subsequent detoxification of a wide range of environmental contaminants, including chlorinated organics, heavy metals, nitro-aromatics and, to some degree, perchlorate. In microbial reductive dechlorination, the electrons can be transferred both directly or indirectly via e.g. hydrogen. Dechlorinating microorganisms in the surroundings of the Fe(0) particles then have the option to directly tap the electrons from the ZVI or use the hydrogen that is expected to be present at high concentrations in the proximity of the iron surface due to the Fe(0) oxidation (De Windt et al., 2003; Dolfing et al., 2008).

6.1.4. The biocathode

In a bio-electrochemical system, bacteria interact with the electrodes, facilitating electron transport between the electron donor and the electrode (Rabaey et al., 2007). Plenty of bacteria can apparently use electrons available from a cathode and for instance generate hydrogen, methane, dinitrogen gas, … (Clauwaert et al., 2008; Rabaey et al., 2007). Thus by supplying a small current to the soil, one can actively provide energy for the desired bacteria in the soil or sediment.

6.1.5. The Anammox process

The conversion of ammonium nitrite to nitrogen gas and traces of nitrate (Kuypers et al., 2003) has also been reported to potentially occur in soils and sediments. Yet, its significance for polluted soils and its potential impact on pollutants by e.g. co-metabolism of the latter is still unexplored.

6.1.6. BioPAD

The fact that *Shewanella oneidensis* can reduce Pd(II) to Pd(0) and deposit the latter on its cell wall (De Windt et al., 2005) is quite remarkable. This process is accomplished with addition of pyruvate, formate or hydrogen gas as electron donor. Yet, even more remarkable is the fact that the latter so-called BioPAD rapidly and efficiently can reduce a variety of chemicals, such as TCE (Hennebel et al., 2009), PCB (De Windt et al., 2005), lindane and dioxins. This offers perspectives for decontamination of sites, which thus far could not be envisioned.

6.2. Biologically induced chemical oxidative technologies

6.2.1. The real humus degraders

The so-called 'old' humus is an assemblage of very heterogeneous unreactive organic matter, which is hardly biodegradable. Yet, certain termites feeding solely on this type of soil organic matter create a pH of 11 to 12 in their gut environment, resulting in the proliferation of alkaliphilic bacteria, which achieve the conversion of these otherwise quasi-inert soil organics (Ji and Brune, 2005). It remains to be examined to what extend such environments and bacteria can be used to develop powerful soil treatment technologies.

6.2.2. The radical generators

It still is a matter of discussion to what extend soil organisms are actively engaged in the production of oxidative radicals. The white rot fungi produce ligninases, which are known to produce oxidative side effects (Dzul-Puc et al., 2005). Similar effects have been suggested for the methane mono-oxygenase operated by the methanotrophs (Choi et al., 2008) and for the ammonia mono-oxygenase operated by the ammonium oxidising bacteria (Wood and Sorensen, 2001). Yet, the actual concrete engineering approaches in which such phenomena are implemented still need to be worked out. It also remains an open question to what extent archaeal nitrifiers, the AOA (Erguder et al., 2009), which are assumed to be active in soils, also can be part of such indirect oxidation of pollutants.

6.2.3. The manganese oxidizers

The manganese oxidising bacteria (MOB) produce MnO_2. Various species are known to bring about such processes e.g. *Bacillus* (Devrind et al., 1986)*, Leptothrix* (Adams and Ghiorse, 1985)*, Pseudomonas* (Caspi et al., 1998), … Figure 4 illustrates that the fine dispersion of MnO_2 under mildly acidic pH conditions results in a reactivity towards pollutants and generates breakdown products. These can subsequently be further metabolised by associated micro-organisms. This has been shown in aqueous systems for arsenate (III) (Oscarson et al., 1981), EE2 (de Rudder et al., 2004; Forrez et al., 2009), diclofenac (Forrez et al., 2010), …

6.2.4. The oxidative anode

The fact that plenty of bacteria can use a solid state electron acceptor has recently been widely documented in the domain of bio-electrochemistry (Pham et al., 2009b). In this respect, quite some opportunities are available to oxidise undesirable reduced compounds at an anodic surface inserted in the soil or sediment. The anodic oxidation of 1,2-DCA is in this respect quite inspiring (Pham et al., 2009a) Similarly, the oxidation of rhizodeposits at an anode with the concomitant production of electricity (Figure 5) is also of interest. By combining the oxidation of rhizodeposits by the active deposition of electron donors through a plant, the soil can be at the same time an energy and food production site (de Schamphelaire et al., 2008). Also, inserting an anode into the soil allows for poising of the redox potential in these environments, which can be of importance in sites where e.g. production of methane, sulfide of methylated metals should be avoided.

7. What are putative new dangers for the soil?

Soil usage has always been changing, so what new and upcoming threats can be identified when looking at the soil of the future? Most of these dangers are manmade and can be managed. The first danger is the introduction of micropollutants to the soil. Pharmaceuticals and personal care products (PPCPs) are micropollutants that have been found in waterbodies worldwide. They prevail in lakes and rivers as they are insufficiently removed in wastewater treatment plants (Onesios et al., 2009). In addition, the construction of decentralized sewage treatment systems/wetlands may contribute to a further distribution of these compounds throughout the soil.

Another type of micropollutant are the newly developed nanomaterials. Nanocarbon, nanosilver, SiO_2 and TiO_2 are widely used in varying industries and places, ranging from health products, personal care products, clothing, food, disinfectants, catalysts…etc. So far, there is little knowledge regarding the effects of these compounds once released into the environment. For instance, fullerene (C-60) seems to exert toxic effects in bacteria by inducing oxidative stress, with evidence of protein oxidation, changes in cell membrane potential and interruption of cellular respiration (Lyon and Alvarez, 2008).

Intensification of the aquaculture industry presents another potential danger for soil. More than 20 tonnes of fish per ha of aquaculture system are produced per year, thereby generating substantial amounts of polluted effluent, containing feed residues, faeces and antibiotics. The discharge of these effluents in the environment results in a high influx of nutrients, various organic and inorganic compounds, which may increase the occurrence of pathogenic microorganisms and introduce invading pathogenic species (Crab et al., 2007).

The sealing of the soil surface results in fragmentation of microbial habitats and a decrease of their soil services. Sealing of the surface can also lead to a loss in groundwater levels i.e. rain is removed either through the sewer system or over hard pavement instead of infiltrating into the soil. In order to recharge groundwater bodies, deep infiltration of rainwater has been applied. However by doing so, surface contaminants such as hydrocarbons, salts, etc. can be directly injected in the subsurface as the soil filtration capacity is not being used. They can thus create a new putative danger for the soil and the natural microbial community.

Another putative danger is the increasing use of soil as a storage medium. One can think of the heat and cold storage, but also the use of empty natural gas deposits or aquifers for the storage of excess greenhouse gas. It is not known yet how the soil can be affected both structurally and biologically by the regular changes in its temperature profile or the introduction of gasses in the deep soil.

Depletion of nutrients from the soil by intensive cropping and harvesting presents another putative danger. The bio-energy production systems (Figure 6), by which land is intensively used for biomass production might result in an exhaustion of the soil when operated in a one way route (Hanegraaf et al., 2007). It is therefore necessary to ensure soil fertilization by returning part of the nutrients present in the crop to the soil. It is necessary to take into account that this return to the soil is essential. If all crop residues and manure are used for energy production and not taken to soil fertilization, there might be a risk of reducing the soil organic matter (Lemke et al., 2010).

The putative dangers to soil quality can all be (relatively easily) managed by introducing best practices for the use and production of certain compounds or products. Another putative danger to the soil which is more difficult to manage and understand, is climate change. Climate change presents a general risk to soils. Unexpected droughts and floods may cause sudden changes in the groundwater level, which may completely alter the soil characteristics. With the changing climate, it is expected that evapotranspiration will increase and therefore soil will dry out more rapidly. Thus the frequency and intensity of soil drying/rewetting events will increase. One single dry/wetting event can kill up to 50% of the microbial community and very little is known about which organisms will survive and how fast they will recover. These alterations in the soil community will therefore have an impact on organic matter breakdown. For litter, as an example, it is hypothesised that in areas where episodic drying and rewetting become more severe, populations of cellullolytic and ligninolytic fungi could be decreased to the point where litter decomposition would decrease more than would be predicted by simply the changes in moisture (Schimel and Gulledge, 1998). Also the thawing of the tundra's poses a danger because this might release great amounts of the greenhouse gas CH_4 and possibly activate numerous of dormant microorganisms (Corradi et al., 2005).

8. What should we take into re-consideration?

It has taken a great deal of effort to establish, in the context of the Water Framework Directive (European Parliament, 2000) that the norm for nitrate in the groundwater should be 50 mg/L. Yet, it appears of interest to critically reconsider this value. Indeed the 50 mg nitrate/L is not necessarily a key consideration in terms of human (infant) toxicology since the real culprit is not nitrate but nitrite. Moreover, very appropriate technologies to remove nitrate from groundwater are existing (Liessens et al., 1993). Actually, the main reason for not tolerating higher levels of nitrate in the groundwater relate to putative disturbances of the soil microbial ecology. Underpinning of such negative impacts on the soil and sediment microbiota at high levels of nitrate is lacking however. In the near future the 'paradigm' of 50 mg nitrate per L of groundwater has to be re-discussed in relation to other effects such as mobilisation of minerals as e.g. iron, phosphate and sulfate (Lucassen et al., 2004).

Similarly, although there is a need for a thorough legislation in relation to the introduction of xenobiotics in the soil ecosystem, the current approaches as e.g. in the EU Directive rev. 91/414 will decimate the number of chemicals which will be available in terms of crop protection. This might have certain side effects such as a tendency to overuse the few that are allowed. A potential remedy could be that the latter chemicals are provided not as such, but accompanied with a proper microbiota to be seeded if necessary, whenever the remainder of the product has to be removed (Onneby et al., 2010).

In the current line of sewage treatment, the harvesting/recovery of nutrients from the domestic water line is normally vigorously rejected. Arguments such as the presence of unwanted heavy metals, personal care products, pharma residues hereby prevail. Yet, it stems to reason to completely rethink the conventional sewage treatment line e.g. in a way that products such as magnesium ammonium phosphate, nitrate and biochar can be recovered and re-used for soil fertilization (Verstraete et al., 2009). The implementation of a new class of 'Natural Standardized Fertilizers', which contain recovery products from sewage, but are standardized in terms of their composition and soil fertilizing value, needs to be given full priority in the years to come.

9. What are the future market and opportunities

The soil will become more and more valuable as a commodity. The preservation of soil quality, including its restoration in case the latter has been lost, certainly is a business opportunity, which will keep growing in all developed and industrialized countries. Actually the price per ha of agricultural land is already of the order of 25 000 € and increasing steadily (Figure 7).

In terms of specific lines of action, the soil as a system for storage of water, heat/cold, carbon, etc. will undoubtedly continue to gain importance. Society will increasingly make use of the passive storage capacity of the soil. Moreover, the active microbial capacity of the soil in combination with the specific physico-chemistry can also be thought of as a reactor.

The soil as a reactor can be seen in the context of filtration and cleaning of drinking water. Also the conversion of organic material to biogas can be a possible application of a soil reactor. Another type of soil reactor that has received quite some attention is the application of a Bio Electrochemical System (BES) in the soil (de Schamphelaire et al., 2008). This type of reactor can be used for production of electrical energy or for the control of the redox potential in the soil to stimulate a desired process (Aelterman et al., 2008).

Phytoremediation is the process of cleaning contaminated soils by making use of the metabolic properties of plants. During the last two decades this field of soil remediation has been extensively researched and developed. Thorough and in-depth reviews on this particular topic can be found elsewhere (Dzantor, 2007; Ryan et al., 2009; Wenzel, 2009)**.** Here, a brief overview will be given of the various properties of phytoremediation. Phytoremediation can be subdivided in several different processes (adapted from Vamerali et al. (2010) and Van Aken et al. (2010)). These are phyto- /rhizodegradation and phyto- /rhizovolatillisation for organic pollutants. Degradation entails de metabolisation of the product into smaller less harmful components or into biomass. This can either be done by the plant itself or by the associated rhizospheric community. Volatilisation entails the breakdown of the polluting compound into volatile compounds which are excreted through the plant or from the rhizosphere. Heavy metals are usually removed by means of 1) phytoextraction i.e. uptake in the plant, 2) rhizofiltration, the sequestering of the metals on the outside of the roots after which they are removed by harvesting the plant with the roots and 3) they can also be made less bioavailable by excretion of chelating compounds or alterations of the soil (bio)chemistry.

Phytoremediaton cannot be done alone by the plant. Just as in pristine sites, there is always a close interaction between the microorganisms in the rhizosphere and the plant (Compant et al., 2010). By studying these relationships, ‘teams’ can be created where the interplay between the plant and microbial members leads to an increased activity related to soil remediation. Examples of such interplay are 1) the plant can provide essential nutrients to beneficial bacteria and vice versa (a good example are nitrogen-fixing bacteria, either free living or in root nodules) 2) plants can provide a niche to beneficial bacteria by secreting certain anti-bacterials which repel other or hamfull bacteria. This can also go in a reverse direction.

A special relationship between bacteria and plants is in the form of an endophytic lifestyle, where the bacteria life inside the plant and provide their beneficial effects regarding phytoremediation *in Planta* (Weyens et al., 2009a; Weyens et al., 2009b) The engineering of endophytic microbial communities also lies within reach as horizontal gene transfer within these communities was detected by Thaghavi et al. (2005).

Overall a searching for and application of hyperacumulating plants in combination with a beneficial rhizo- and/or endospheric microbial community holds great promise for low cost cleaning of contaminated sites.

One certainly will continue to develop various inocula in order to speed up the biodegradation in soils. Good examples to illustrate the potential are the seeding of deep soil with vinyl chloride degraders (Scheutz et al., 2008) or with 1,2-DCA degraders (Maes et al., 2006). Yet, one could also consider the re-establishment of e.g. soil services such as the degradation of methane. Indeed, when destroyed, it may take more than 100 years to come back (Smith et al., 2000) while effective cases of bioaugmentation have been reported for certain landfill sites (Boeckx et al., 1997).

A major market potential is the concept of biochar addition to the soil (Lehmann, 2007). Biochar is the product of controlled pyrolysis of biomass. This material needs to be explored and confirmed in the years to come as it not only stores carbon in the soil, but also improves the quality of the soil as a beholder of nutrients and biodiversity. However biochar is a product of various types of biomass. It therefore needs considerable attention to determine its exact properties with respect to nutrient release, sorption of pollutants and the beneficial effect on the soil microbiology. Regarding the relation between biochar and microbiology, the biochar can also be considered as a sheltered niche or as a carrier for introduction of specific microorganisms. The fact that 1 ton of biochar carbon represents a storage equivalent to 3 ton of CO_2-carbon is of considerable importance in the light of the global carbon economy.

Thus understanding and controlling the combination of soil, microbiological and plants systems and, even more important, their interactions provides a great opportunity for various innovative approaches to improve soil cleaning and production processes.

III. Contaminated site management: “quo vadis”?

In the coming years, the management of sites will rapidly evolve from ecological risk assessment toward restoration of ecosystem services. A main driver behind ecosystem restoration is the economically quantifiable importance of the microbial soil services. It can be expected that the economic value of these soil services will become better quantified, which would already justify on shear economical facts the technological inputs.

It appears that the deep soil, simply because of its presence and putative capacity to store and act, will be more and more explored and exploited in the near future. Clearly, this exploitation of the deep soil as a reactor needs to be urgently addressed in order to come to proper governance.

Overall, as society evolves towards a bio-economy, new dangers such as the exhaustion of the essential soil services, but also new potentials as the intensive storage of carbon in soil to remediate climate change warranted close monitoring in terms of their long term socio-economic impacts.

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Figure captions:

Figure 1. Process for drinking water production. The groundwater collected in the primary production wells is injected into the soil. The soil will therefore act as a reactor, and clean groundwater is extracted in the secondary production wells (adapted from Haeffner and Grandguillaume (2009)).

Figure 2. Simplified global carbon cycle. Represented are the emissions of carbon dioxide from fossil fuel, soil respiration and vegetation as well as capture of carbon dioxide by the vegetation (adapted from Denman et al. (2007)).

Figure 3. Representation of the Microbial Resource Management principle. The molecular analyses allow determining various aspects of the microbial configuration and one can determine if the communities are endangered by abiotic or biotic stress. A direct result of such an instable community might be the loss of the ecosystem functioning and a loss of the services rendered by the ecosystem to the society. To overcome a failure of the functionality, inputs can be given to the community by for example introducing new strains or nutrients or altering the conditions in order to improve the microbial community structure.

Figure 4. The implementation of manganese oxidizing bacteria in the oxidation of pollutants by removing Mn^{2+} and providing reactive MnO_2 (adapted from de Rudder et al. (2004) and Forrez et al. (submitted)).

Figure 5. Potential implementation of oxidative anodes to capture reducing equivalents in reduced soil environments and thus steer microbial processes with controlling the redox potential.

Figure 6. Bio-energy production by means of land crop systems necessitates the return of minerals and carbon to the soil.

Figure 7. Increase in price per ha for agricultural land in a number of European countries over the last 10 years (data from epp.eurostat.ec.europa.eu/). The agricultural land price of each country in 1995 was considered as factor 1 to calculate the price increase over the analyzed years.

Figure 1:

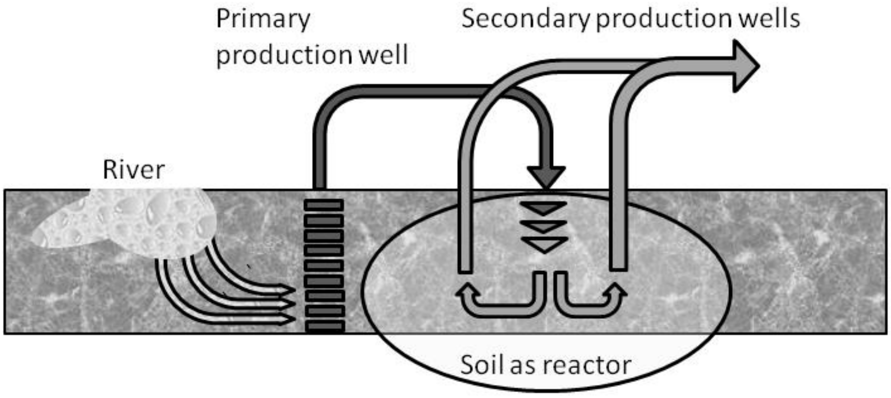
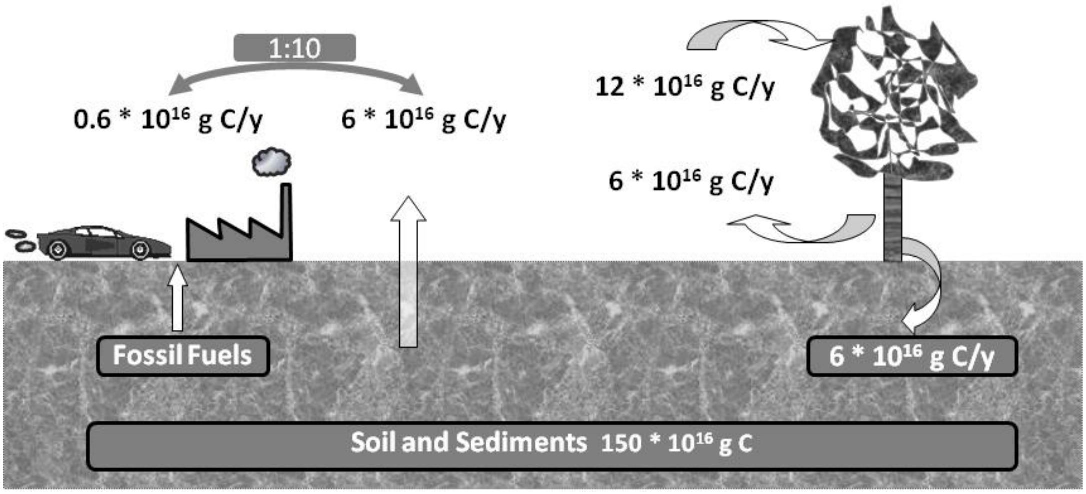


Figure 2:



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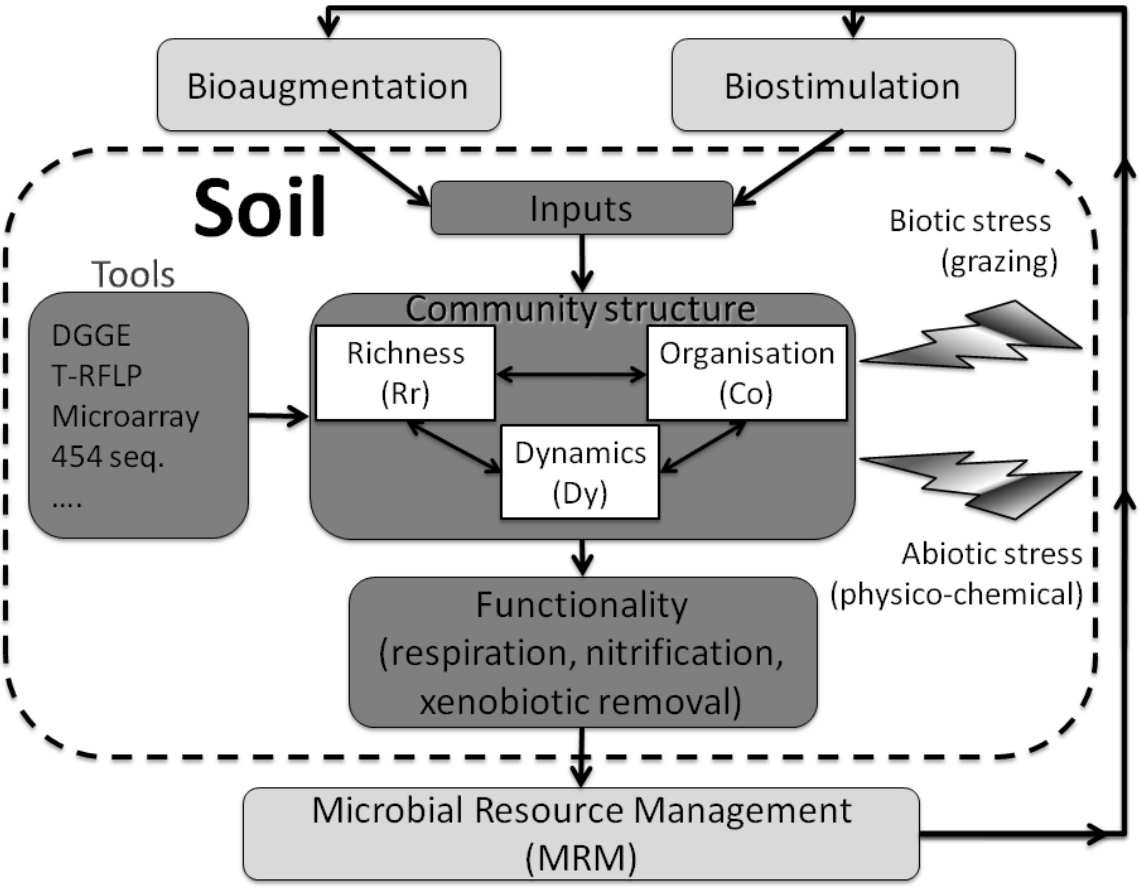

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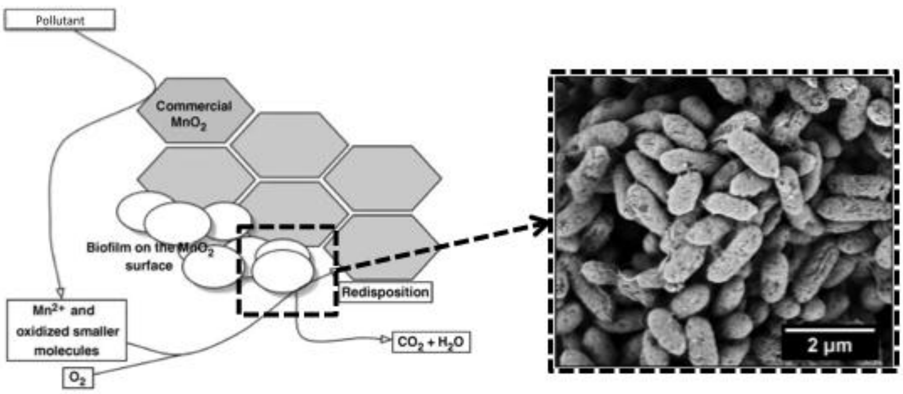

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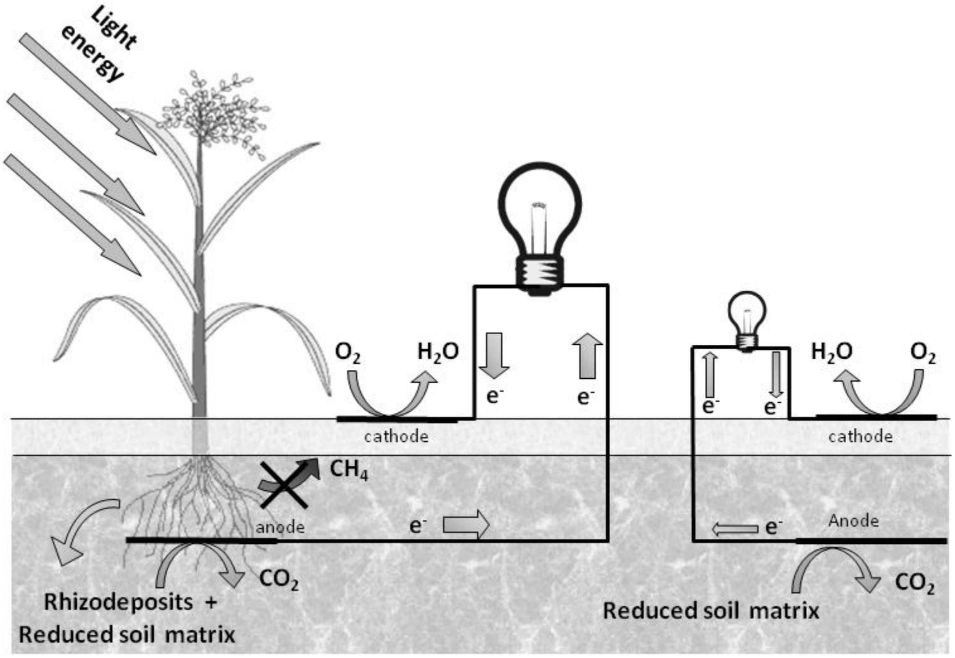

Figure 6:

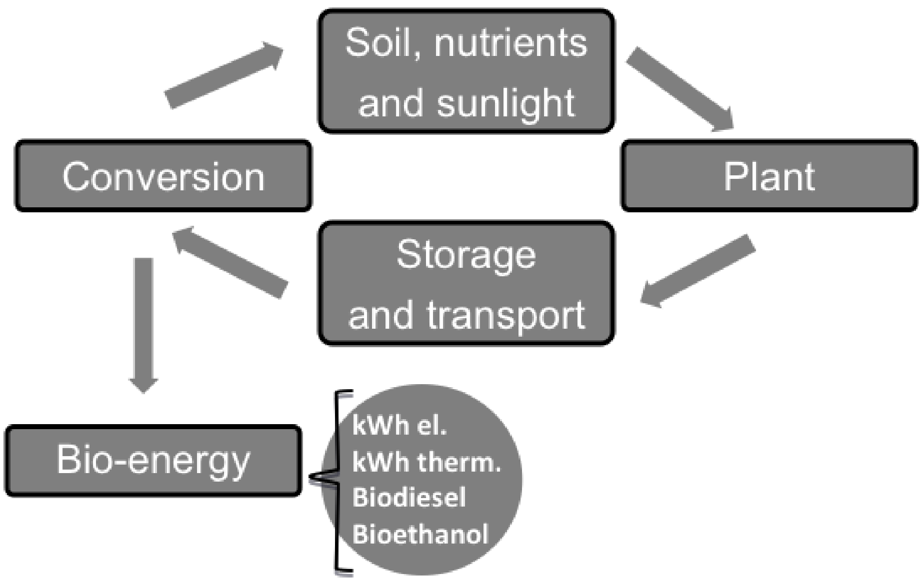

Figure 7:

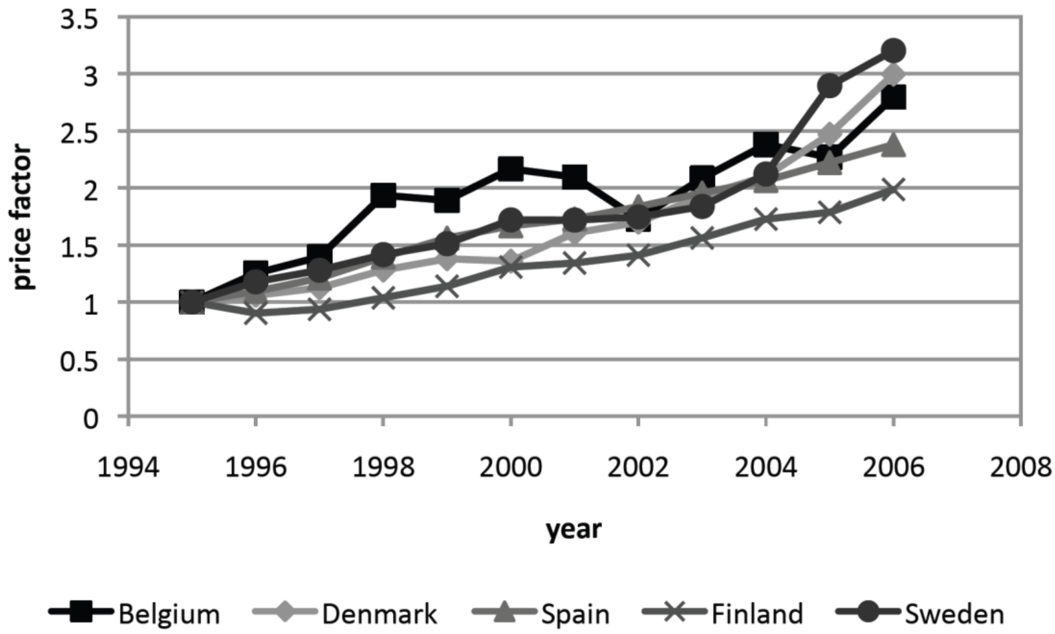

Table 1 – Overview of new biologically induced oxidative and reductive technologies in soil treatment.

Biologically induced chemical reductive technologies	**Biologically induced chemical oxidative technologies**
Halorespiration	Real humus degraders
"Addition of handles to unhandy substrates"	Radical generators
Zero-Valent Iron (ZVI) donors	Manganese oxidizers
Biocathodes	Oxidative anodes
The Anammox process	
BioPAD	