# Ecological Indicators of Ecosystem Recovery: Microbial Communities as Ecological Indicators of Ecosystem Recovery Following Chemical Pollution

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Abstract

'Ecosystem recovery' is a concept that emerged from the need to preserve our environment against increasing contamination from human activity. However, ecological indicators of ecosystem recovery remain scarce, and it is still difficult to assess recovery of ecological processes at relevant spatial and temporal scales. Microbial communities hold key relevance as indicators of ecosystem recovery as they are ubiquitous among diverse ecosystems, respond rapidly to environmental changes, and support many ecosystem functions and services through taxonomic and functional biodiversity. This chapter summarizes the state-of-the-art in knowledge on the processes driving the structural and functional recovery of phototroph and heterotroph microorganisms following chemical pollution. It covers several successful case studies providing proof of principle for the relevance of using microorganisms in recovery studies in

various ecosystems such as soil, freshwater and seawater. Questions remain for microbial ecotoxicologists to fully understand and predict how structural and functional recovery observed at microbial scale can reflect the recovery of an ecosystem. Moreover, new standards and norms taking into account recent advances in microbial ecotoxicology are now necessary in order to inform legislators and policymakers on the importance of considering microorganisms in environmental risk assessment, including ecological recovery monitoring.

Keywords
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# Chapter 10
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**Stéphane Pesce, Jean-François Ghiglione and Fabrice Martin-Laurent**

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AQ1

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Keywords ■■■

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## 10.1 Relevance of Using Microbial Communities to Assess Ecosystem Recovery

The last two decades have seen a worldwide surge in environmental regulations designed to promote effective environmental management practices to reduce anthropogenic chemical impacts in ecosystems (Depledge 1998; Hering et al. 2010). Ecological restoration has thus emerged as one of the most important issues in environmental science (Montoya et al. 2012), spurring the emergence of the concept of ecosystem recovery (Fig. 10.1), which implies that a restored ecosystem evolves towards the direction of the pre-disturbance conditions to recover healthy conditions. Ecosystem recovery is built around several paradigms (Duarte et al. 2015) and driven by complex processes involving multiple biological levels over different timescales (Adams et al. 2002). It is already a challenge to define ecosystem-healthy conditions, which revolves around the concept of normal operating range (NOR) as the range in ecological metrics observed in the ecosystem's undisturbed state under natural fluctuations in environmental conditions (EFSA Scientific Committee 2016a). It is also crucial to choose the appropriate ecological metrics for assessing ecosystem recovery, as they should not only inform on the structural recovery of ecosystems but also allow us to assess the recovery of ecosystem functions, including ecosystem services (Bullock et al. 2011; Montoya et al. 2012).

A few decades ago, no-one would have expected to see microbiologists play a role in the evaluation of ecosystem recovery. Today, though, the situation has reversed, as it is difficult to find a single ecosystem on earth where microorganisms have not been identified as key players in its functioning. Despite their small size, microorganisms are not only the most abundant organisms but are also recognized as major components of all biogeochemical element cycles (C, N, P, S, metals).

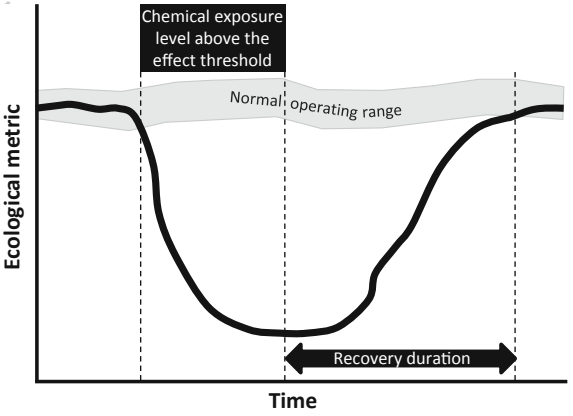


**Fig. 10.1** Schematic illustration of ecosystem recovery following chemical pollution. Adapted from EFSA Scientific Committee (2016b)

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Important recent discoveries have advanced the genomic, biochemical, physiological and ecological bases of a variety of microbiological processes, like anaerobic methane oxidation, photosynthesis, phosphorous uptake, and many aspects of the sulfur and nitrogen cycles, from anammox reaction and dissimilatory nitrate reduction to ammonia to archaeal nitrification (Madsen 2011). Indeed, it is well acknowledged that microbial communities maintain the biosphere via the biogeochemical reactions they catalyze. Moreover, recent moves to consider microorganisms along with living animals and plants—no longer viewed as autonomous entities but rather as assemblages of different species forming ecological units called holobionts—has shaken up the life sciences (Bordenstein and Theis 2015).

Advances in microbial ecology allow us to extend the mechanistic understanding of relatively simple biological systems to complex naturally-occurring microbial communities that dwell in soils, air, sediments and waters. The emerging discipline of microbial ecotoxicology is now facing the challenge of evaluating the relevance of microbial communities for assessing ecosystem recovery after pollution (Ghiglione et al. 2016).

## 10.2 Structural and Functional Recovery Potential of Microbial Communities Following a Decrease in Chemical Exposure

The potential of microbial communities to recover from disturbances depends on both the internal and external recovery capacities of their constitutive populations through population growth of surviving organisms or propagules and recolonization following passive or active dispersal, respectively (EFSA Scientific Committee 2016b; Gergs et al. 2016). To gain an overview of how microbial communities can recover from chemical exposure and be able to predict recovery trajectories, it is first necessary to better understand the mechanisms underpinning internal and external recovery. Such investigations can be conducted at population and community levels using laboratory or in situ experimental studies.

AQ3

### *10.2.1 Internal Recovery Potential of Microbial Populations: The Case of Photosynthetic Microorganisms*

Among microorganisms, algae and cyanobacteria are the most intensively studied model organisms in aquatic ecotoxicology. Several studies assessing microbial recovery potential at population level have been performed using freshwater photosynthetic microbial species (Table 10.1). Vallotton et al. (2008a, b) evaluated the capacity of the Chloropyceae *Scenedesmus vacuolatus* to recover following acute

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**Table 10.1** Laboratory studies of the recovery potential of algal and cyanobacterial populations after exposure to various pesticides and heavy metals

| Algal species | Structural metrics | Functional metrics | Contaminant(s) | Nominal concentrations | Maximal exposure/recovery duration | References |
|---|---|---|---|---|---|---|
| *Planothidium frequentissimum Pseudokirchneriella subcapitaa* | Teratologica l forms | Growth, viability | Metal (Cd) | 20–100 µg/L | 21 day/28 day | Arini et al. (2013) |
| *Anabaena flos-aquae Navicula pelliculosa* | Cell densities | Growth, photosynthesis | Herbicide (atrazine) | 5–1000 µg/L | 48 h/48 h | Brain et al. (2012) |
| *Phaeodactylum tricornutum* | | Growth, phytochelatin synthesis | Metals (Cd, Pb, Zn) | 112 µg/L (Cd) 207 µg/L (Pb) 65 µg/L (Zn) | 8 h/24 h | Morelli and Scarano (2001) |
| *Selenastrum capricornutum Chlorella vulgaris* | | Growth | Metal (Zn) | 65 µg/L | 100 day/10 day | Muyssen and Janssen (2001) |
| *Selenastrum capricornutum* | Chlorophyll a content | Growth, carbon assimilation | Metal (Cd) | 30–100 µg/L | 48 h/96 h | Thompson and Couture (1993) |
| *Scenedesmus sp.* | | Growth, photosynthesis, respiration, uptake and assimilation of nitrate | Metals (Cu and Zn) | 159–635 µg/L (Cu) 327–1635 µg/L (Zn) | 48 h/96 h | Tripathi et al. (2004) |
| *Scenedesmus sp.* | Photosynthetic pigments, protein, carbohydrate and lipid content | Growth, photosynthesis, respiration, uptake and assimilation of nitrate | Metals (Cu and Zn) | 158–635 µg/L (Cu) 327–1635 µg/L (Zn) | 48 h/96 h | Tripathi and Gaur (2006) |

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**Table 10.1** (continued)

| Algal species | Structural metrics | Functional metrics | Contaminant(s) | Nominal concentrations | Maximal exposure/recovery duration | References |
|---|---|---|---|---|---|---|
| *Scenedesmus vacuolatus* | | Photosynthesis, growth | Herbicides (isoproturon and atrazine) | 60–320 µg/L (isoproturon) 80–510 µg/L (atrazine) | 25 h/48 h | Vallotton et al. (2008a) |
| *Scenedesmus vacuolatus* | | Growth | Herbicide (S-metolachlor) | 750 µg/L | 24 h/48 h | Vallotton et al. (2008b) |
| *Thalassiosira nordenskioeldii* | | Growth, phytochelatin synthesis | Metal (Cd) | 0.001–10 µg/L | 7 day/15 day | Wang and Wang (2011) |
| *Microcystis aeruginosa* | | Growth (sensitivity tests) | Metals (Cd and Zn) | 3.37 µg/L (Cd) 0.65 µg/L (Zn) | 5 day/5 day | Zeng et al. (2009) |

pulse exposure to various herbicides. The effective quantum yield recovered completely within 4 h after removal of atrazine and isoproturon, leading to rapid recovery of photosynthetic microorganism growth independently of the magnitude of the effects induced by these two photosystem-II inhibitors (Vallotton et al. 2008a). By testing different exposure levels to atrazine (5–1000 µg/L for 48 h), Brain et al. (2012) observed that the resulting effects on photosynthesis and growth were transient and fully reversible within 48 h in three tested photosynthetic microorganism species of chlorophyceae, cyanobacteria and diatoms, respectively. However, the recovery of *S. vacuolatus* following an acute exposure to the chloroacetanilide S-metolachlor was delayed, occurring only after 29 h, revealing that the extent and time-to-reversibility of the toxic effects may be dependent on the nature of the toxicant (Vallotton et al. 2008b).

An important parameter to consider here is the kinetics of toxicant elimination from the cells. Metals are well known to bioaccumulate in photosynthetic microorganisms. The potential of photosynthetic microbial populations to recover following metal exposure was investigated using various species belonging to the chlorophyceae (Morelli and Scarano 2001; Muyssen and Janssen 2001; Thompson and Couture 1993; Tripathi and Gaur 2006; Tripathi et al. 2004), diatoms (Arini et al. 2013; Morelli and Scarano 2001; Wang and Wang 2011) and cyanobacteria (Zeng et al. 2009). Most of these studies reported a significant decrease in intracellular concentrations of cadmium (Cd) (Arini et al. 2013; Thompson and Couture 1993; Wang and Wang 2011), copper (Cu); (Tripathi and Gaur 2006) and zinc (Zn) (Tripathi and Gaur 2006), whatever the model species. However, the extent of recovery proved variable according to the exposure conditions (duration and concentrations), parameters measured, and duration of the recovery period. This is clearly illustrated by Tripathi et al. (2004, 2006) who assessed the recovery of *Scenedesmus sp.* using a set of structural (i.e. photosynthetic pigments, protein, carbohydrate and lipid contents) and functional parameters (i.e. growth, cell viability, photosynthesis, respiration, uptake and assimilation of nitrate) following a 48 h exposure to Cu and Zn tested at two nominal concentration levels each (2.5–10 and 5–25 µM, respectively). Photosynthesis and respiration recovered quickly without any immediate change in cell density, suggesting an adaptive response for producing energy and returning to normal catabolism conditions. This functional recovery was accompanied by a slight decline in lipid contents as well as an increase in carbohydrates, which are a preferred source of energy. Nitrate reductase activity recovered much earlier than nitrate uptake, but both these processes were dependent on the recovery of photosynthesis and respiration which provide the energy required to recover microbial activities. This is consistent with the results of Tripathi et al. (2004) who observed that recovery from metal stress was slower when algae were previously exposed for 72 h to dark conditions, whereas no recovery was found in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), a transformation product of the herbicide diuron, which acts as a photosynthetic inhibitor. When photosynthesis was possible, the resulting chain of metabolic events stimulated algal growth, allowing enhanced dilution of intracellular level of metals. However, the relatively high intracellular levels of Cu or Zn maintained in

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algal populations exposed to the highest metal concentrations precluded complete recovery of some processes during the 96 h recovery period, which was probably too short given the concentrations tested (i.e. 10 and 25 µM, respectively). Based on a study of teratological forms, and despite complete depuration of intracellular Cd, Arini et al. (2013) also observed incomplete recovery of *Planothidium frequentissimum* diatoms, even at 23 days after removal of Cd contamination (at 20 and 100 µg/L).

Recovery assessment at population level can also be conducted by studying the adaptive processes of photosynthetic microorganisms in response to toxicant exposure. Indeed, microbial adaptation leading to the ability to tolerate toxicants is a defense strategy that generally generates an energetic cost that weakens the microorganisms' ability to cope with supplementary disturbances (Congdon et al. 2001). This means that from an ecological point of view, loss of adaptation to toxicants, at population or community level, can be perceived as an indication of microbial recovery (Pesce et al. 2013, 2016). To that effect, Morelli and Scarano (2001) and Wang and Wang (2011) studied phytochelatins, which are metal-binding thiol-containing peptides, in response to heavy metals exposure and observed a rapid decrease in the phytochelatin pool in diatoms exposed to various metals, confirming a recovery process within the populations. Another approach consists in studying the evolution of tolerance capacities of photosynthetic microorganisms towards toxicants by performing short-term toxicity tests based on functional parameters. Using this approach, and by measuring growth rates, Zeng et al. (2009) evidenced an increase in the tolerance of the cyanobacteria *Microcystis aeruginosa* towards Cd or Zn according to the pre-exposure conditions (concentration and duration) used. In the metal-free medium, an increase in sensitivity to metals was observed following 1-day recovery while a 5-day recovery led to complete loss of tolerance capacities. The same trend was observed by Muyssen and Janssen (2001) in the two chlorophyceae species *Selenastrum capricornutum* and *Chlorella vulgaris* which showed a maximal 3-fold increase in zinc tolerance (based on growth inhibition tests) after 100 days of exposure to 65 µg Zn/L followed by a significant decrease in tolerance after a 10-day recovery period in a metal-free medium. Note that the rapid decrease in the tolerance following recovery in these two studies may indicate that the tolerance involves physiological acclimatization rather than genetic adaptation, such as the production of intracellular ligands (e.g. phytochelatins or metallothioneins) which can complex or detoxify intracellular metals (Zeng et al. 2009).

### *10.2.2 Internal and External Recovery Potential of Microbial Communities*

Even if the study of microbial recovery potential at population level may be relevant to evaluate the internal capacities of microorganisms to recover and to characterize the mechanisms involved, it is now well acknowledged that

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ecotoxicological studies hold stronger ecological relevance when they consider biological responses at community level, applying community ecology concepts (Clements and Rohr 2009; Geiszinger et al. 2009; Schmitt-Jansen et al. 2008). This statement also holds for recovery studies especially when the aim is to study an ecosystem's capacity to recover from disturbances (Admiraal et al. 2000; EFSA Scientific Committee 2016b).

#### 10.2.2.1 Microcosm and Mesocosm Experiments

Using microcosm or mesocosm approaches to address ecological recovery offers several advantages, including the possibilities for controlling and standardizing exposure and habitat conditions, allowing replication and statistical evaluation, and taking into consideration certain ecological interactions.

Several studies have been performed to study the potential of freshwater phototrophic microbial communities to recover following herbicide exposure (Pesce et al. 2011). Some of these works aimed specifically at evaluating short-term recovery of periphyton in herbicide-free water after an acute pulse exposure to photosystem inhibitors (i.e. *s*-triazine and substituted phenylurea herbicides, varying between 1 and 48 h (Gustavson et al. 2003; Laviale et al. 2011; Prosser et al. 2013, 2015). All of these studies showed high short-term potential recovery of photosynthesis, even after exposure to toxic concentrations significantly inhibiting this function. However, functional recovery trajectories varied according to exposure duration (Gustavson et al. 2003; Laviale et al. 2011), tested concentrations (Gustavson et al. 2003; Laviale et al. 2011; Prosser et al. 2013, 2015) and kind of toxicants, even for those having the same mode of action (Gustavson et al. 2003; Laviale et al. 2011). Gustavsson et al. (2003) also pointed out that functional recovery is sometimes not associated with structural recovery. Indeed, while the recovery of photosynthetic activity in periphyton after an acute exposure to metribuzin was almost complete after 48 h in herbicide-free water, even after exposure at the concentration of 50 µg/L where photosynthesis was inhibited by 80%, the composition of the periphyton remained impacted, even at the lowest concentration of 0.4 µg/L. This was due to the fact that chlorophytes were severely affected by exposure and failed to recover whereas diatoms and especially cyanobacteria recovered well. This report clearly illustrates that functional redundancy can contribute to the rapid recovery of some ecological functions. A delay in chlorophytes recovery following a chronic exposure to the herbicide metazachlor was also observed by Mohr et al. (2008a), confirming that different microbial populations within a complex community can exhibit different recovery trajectories following chemical exposure, due to their intrinsic properties.

However, these trajectories can also be highly influenced by the existence or not of microbial immigration processes. This was clearly demonstrated in studies by Lambert et al. (2012) and Morin et al. (2012) who observed no structural recovery of periphytic diatom communities within 6 weeks following a chronic exposure to copper when immigration process from non-exposed communities were impossible,

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whereas recovery was complete when these same processes were enhanced. This report was confirmed by a pollution-induced community tolerance (PICT) approach showing that the Cu phototrophic tolerance that had been induced during the exposure period was only lost when immigration was possible (Lambert et al. 2012). Moreover, photosynthesis measurements revealed that the lack of immigration precluded functional recovery of phototrophic communities (Lambert et al. 2012). Arini et al. (2012b) also suggested that the limited recovery they observed in the structure of periphytic diatom communities 8 weeks after a chronic exposure to metals may have been due, at least partially, to the difficulty of non-impacted species to invade the pre-exposed biofilms.

Nevertheless, immigration processes seem to be less important to the structural recovery dynamics of periphytic bacterial communities following a metal stress. Lambert et al. (2012) observed that, in contrast to diatoms, the structure of bacterial communities in metal-exposed samples remained quite different from controls throughout the 6-week recovery period, even when species immigration was possible. This is consistent with other reports of weak structural recovery of periphytic bacterial communities within several weeks after a decrease in metal and pesticide exposure despite the possibility of immigration of non-exposed species (Boivin et al. 2006; Dorigo et al. 2010b). Despite the lack of structural recovery, the functional recovery of heterotrophic communities (estimated from β-Glucosidase activity) was accelerated when immigration processes were possible (Lambert et al. 2012). Boivin et al. (2006) also showed that functional changes in bacterial communities (estimated from community-level physiological profiles) following Cu exposure were reversible within 28 days. All these results illustrate the crucial importance of functional redundancy acting as an ecological insurance allowing the functional recovery of microbial communities following exposure to chemicals.

Recovery in aquatic microbial communities depends not just on type of microorganisms (e.g. diatoms *vs* bacteria) and feasibility of immigration processes but also mode of life (i.e. benthic or planktonic). Mohr et al. (2008b) observed no structural recovery in periphytic phototrophic communities within 150 days following single applications of 1 and 5 µg/L of the herbicide Irgarol whereas phytoplankton recovered after just a few weeks. This suggests that Irgarol bioaccumulation in periphyton may have prolonged the exposure duration. In contrast, the recovery dynamics of phytoplankton communities generally co-occurs with toxicant dissipation in water (Brock et al. 2004; Knauert et al. 2009).

Compared to the numerous aquatic microcosm studies, soil microcosm studies assessing microbial recovery following chemical pollution are scarce. To the best of our knowledge, only a few studies have attempted to evaluate the effects of various fungicides on soil microbial communities and soil ecological processes (Bending et al. 2007; Chen and Edwards 2001; Chen et al. 2001). These studies suggest that both the magnitude of the effects and the dynamics of recovery are dependent on several factors, including kind of fungicide and soil physicochemical properties, which can be affected by management practices such as organic amendment driving soil organic matter content. For example, a significant negative effect of fungicides on dehydrogenase activity was observed only in soils exhibiting the lowest levels of

organic matter and microbial biomass (Bending et al. 2007). Moreover, in these soils, chlorothalonil had a greater and more prolonged impact on the microbial community than azoxystrobin and tebuconazole. Similarly, Chen and Edwards (2001) observed only transient effects of benomyl and chlorothalonil on soil microbial activity and nitrogen dynamics while these effects were more pronounced and prolonged following captan treatment, with a significant influence of type of soil. Kostov and Van Cleemput (2001a, b) also observed that the magnitude of the inhibition of basal nitrification and N mineralization by Cu and the subsequent recovery was strongly influenced by type of soil (i.e. sandy soil *vs* sandy loam soil). Moreover, they showed that recovery of microbial activity and fertility in Cu-contaminated soils was enhanced following lime and compost amendments (Kostov and Van Cleemput 2001a, b). This may be due to the fact that compost amendment increases soil organic matter content, which improves the heavy metal binding capacity of the soil (Martinho et al. 2015). Functional recovery potential depends not just on soil physicochemical properties but also soil microbial community characteristics. For example, Griffiths et al. (2000) demonstrated that soil functional recovery can be significantly impaired by a loss of microbial diversity (estimated with a diversity index including various kinds of microorganisms, i.e. bacteria, flagellate protozoa and nematodes). This result underlines the importance of microbial diversity, which is one of the keystones of ecological insurance allowing the recovery of microbial functions following a stress.

#### 10.2.2.2 In Situ Experiments: Translocation Studies in Lotic Ecosystems

Over the past decade, several in situ studies have set out to evaluate the potential of river periphytic communities to recover from chemical pollution using translocation approaches (Table 10.2). Translocation approaches use experimental transfers of microbial communities from a contaminated station to a reference station (i.e. pristine or less-contaminated station) to assess their trajectories of recovery. Most of these studies have focused on the capacity of phototrophic communities to recover from exposure to metals or pesticides, using structural metrics such as microbial biomass, distribution of photosynthetic microbial classes and diatom community composition (Arini et al. 2012a; Dorigo et al. 2010a, b; Fechner et al. 2012; Ivorra et al. 1999; Morin et al. 2010; Rimet et al. 2005; Rotter et al. 2011). These studies generally evidenced shifts in community structure towards the reference community following transfer from contaminated-station to reference-station, but community structure recovery times differed between studies, from a few days (Rotter et al. 2011) to a few weeks (Arini et al. 2012a; Morin et al. 2010), and also varied with type of structural metrics or indices used (Rimet et al. 2005). For example, quantitative parameters (total and photosynthetic biomasses) recovered rapidly within 4 weeks whereas biological diatom index (BDI) did not recover at all (Morin et al. 2010). Likewise, Ivorra et al. (1999) showed that diatom community compositions of biofilms transferred from metal-polluted to reference sites were still different

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**Table 10.2** In situ translocation studies of the recovery potential of microbial periphytic communities following a decrease in chemical exposure

| Structural metrics | Functional metrics | Contaminant (s) | Exposure/recovery duration | References |
|---|---|---|---|---|
| Diatom community structure, teratological forms | | Metals (Zn and Cd) | 24 day/63 day | Arini et al. (2012b) |
| Microbial biomass, eukaryotic community structure | Photosynthesis (PICT approach) | Pesticides (diuron) | 5 week/5 week | Dorigo et al. (2010a) |
| Diatom community structure, algal biomass, eukaryotic and bacterial community structure | Photosynthesis (PICT approach), respiration (PICT approach) | Pesticides (diuron), metals (Cu) | ND/9 week | Dorigo et al. (2010b) |
| Eukaryotic and bacterial community structure | Beta-glucosidase (PICT approach) | Metals (Cu) | 23–34 day/30 day | Fechner et al. (2012) |
| Diatom community structure, algal biomass, microbial biomass | | Metals (Zn and Cd) | 7–16 day/14–18 day | Ivorra et al. (1999) |
| Diatom community structure, algal class composition, microbial biomass | | Pesticides | 4 week/8 week | Morin et al. (2010) |
| Diatom community structure | | High organic laod | 20 day/60 day | Rimet et al. (2005) |
| Diatom community structure, algal class composition | Photosynthesis (PICT approach) | Pesticides (prometryn) | 26 day/44 day | Rotter et al. (2011) |

after two weeks. Using molecular fingerprinting approaches, Dorigo et al. (2010a, b) and Fechner et al. (2012) also reported divergent results on the capacity of eukaryotic and bacterial biofilm communities to recover their reference structure within a few weeks. Indeed, while Fechner et al. (2012) observed good recovery of the genetic structure in microbial communities only 15 days after translocation, Dorigo et al. (2010a, b) observed only delayed and partial structural recovery, which was still incomplete after 9 weeks after their translocation.

These differences in time response between studies are strong evidence that in-field structural recovery trajectories of periphytic communities are influenced by a number of environmental parameters, some of which being directly related to the exposure conditions in the contaminated site, especially in terms of types of toxicants, which are more or less likely to bioaccumulate in the periphyton matrix and

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cells. Bioaccumulation can indeed prolong the toxicant pressure in the uncontaminated reference sites, thus delaying post-translocation microbial recovery (Dorigo et al. 2010b; Morin et al. 2010). Among toxicants, metals are well known to bioaccumulate within periphytic biofilms and several translocation studies have confirmed that depuration of metals from biofilms in reference sites can sometimes take several weeks before significant recovery becomes possible (Admiraal et al. 2000; Arini et al. 2012a; Dorigo et al. 2010b; Ivorra et al. 1999). Depuration time is influenced by several parameters, such as type of metals, microbial growth in biofilms (dilution process) and/or biofilm detachment and grazing (Arini et al. 2012a). It is also well known that following chemical exposure, the recovery of populations and communities depends on their connection to undisturbed environments conditioning migration processes (Gergs et al. 2016; Lambert et al. 2012; Morin et al. 2012). Even if lotic systems are usually well connected to undisturbed sections, allowing faster recovery than in lentic systems (Gergs et al. 2016), several authors have pointed out that recovery processes are probably facilitated in translocation studies, where exposed biofilms are directly transplanted into river sections inhabited by unexposed communities, thus facilitating migration (Arini et al. 2012a; Ivorra et al. 1999; Lambert et al. 2012). Toxicant releases and migration processes are key drivers of periphytic recovery and both are highly dependent on maturity stage of the translocated biofilms, as thicker biofilms may accumulate higher amounts of toxicants than thinner ones (Lawrence et al. 2001) while microbial immigration processes may be facilitated in early biofilm development stages (Dorigo et al. 2010b). AQ4

Some translocation studies also set out to investigate the link between structural recovery and possible changes in sensitivity towards the main pollutants identified in the contaminated sites using PICT approaches. Short-term photosynthetic bioassays applied to investigate phototrophic community recoveries after a decrease in exposure to herbicide (Dorigo et al. 2010a, b; Rotter et al. 2011) or copper (Dorigo et al. 2010b) following translocation showed a significant decrease in herbicide and copper tolerance with changes in phototrophic community composition. Likewise, PICT measurement with heterotrophic functions such as substrate-induced respiration (Dorigo et al. 2010b) and β-glucosidase activity (Fechner et al. 2012) combined with monitoring of bacterial community structure revealed that changes in community tolerance occurred concomitantly with changes in community structure. Indeed, Fechner et al. (2012) observed 15 days after translocation that the fast recovery of low tolerance levels of hetrotrophic communities towards copper was accompanied by significant modifications in bacterial community structure. Conversely, Dorigo et al. (2010b) reported limited recovery of tolerance to copper and structure in the bacterial community 9 weeks after translocation.

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## 10.3 Case-Studies of the Use of Microbial Communities to Evaluate Ecosystem Recovery Following a Decrease in Chemical Exposure

As recently underlined by the EFSA Scientific Committee (2016b), assessing recovery in natural complex ecosystems exposed to multiple stressors and where the connection to undisturbed areas may influence recovery trajectories is far from trivial. Moreover, and in contrast to experimental studies, the lack of system replication in such approaches makes it necessary to define reference conditions for each of ecological metric measured, based on the state of the disturbed system prior to disturbance, or the state of similar but undisturbed systems, or theoretically-derived system states (Gergs et al. 2016). Nevertheless, despite these recognized weaknesses, field studies provide the most realistic assessment of 'real-life' environmental risks of chemicals. Furthermore, when conducted over a long period of time, field studies provide relevant information depicting effective ecological recovery trajectories. This section provides illustrative examples of in-field case studies designed to assess autochthonous microbial community recovery in different kinds of ecosystems.

### *10.3.1 Structural and Functional Recovery of Microbial Communities*

Soil remediation and rehabilitation processes offer practical case-studies to assess ecosystem recovery following an improvement in chemical quality. Worldwide pollution of soils by heavy metals has prompted the development of various biotechnological strategies for remediating metal-contaminated soils, such as chemical- and bio-remediation, including phytoremediation and bioaugmentation (dos Santos et al. 2016; Kavamura and Esposito 2010). However, ultimately, the goal of soil remediation and rehabilitation is not only to eliminate the contamination but also to allow restoration of soil quality and functioning. Within this context, microbial community monitoring (e.g. Ritz et al. 2009; Schloter et al. 2003) is viewed as a way to assess the recovery of soil quality during the remediation process (Gomez-Sagasti et al. 2012). Various methods have been applied to achieve this objective, chiefly analyses of microbial biomass, basal and substrate-induced respiration, and enzymatic activities (such as urease, β-glucosidase, phosphatase, dehydrogenase, protease, invertase, etc.; Alvarenga et al. 2009; Ciarkowska et al. 2014; Epelde et al. 2008, 2009; Goupil and Nkongolo 2014; Jiang et al. 2010). These measurements of microbial abundance and activity are sometimes supplemented by the assessment of functional diversity using community-level physiological profiles (Castaldi et al. 2009; Epelde et al. 2009; Kelly and Tate 1998) and microbial community structure using phospholipid fatty acid analysis (Kelly et al. 2003) or 16S rRNA-based analyses (dos Santos et al. 2016). Taken together, these

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different methodologies serve to assess the recovery of soil quality supported by soil microorganisms all along the remediation and rehabilitation processes. Gomez-Sagasti et al. (2012) proposed that a better interpretation of microbial properties as indicators of soil quality could be gained by grouping microbial indicators into categories of high ecological relevance, such as soil ecosystem functions and services.

Although there is a long history of using biological indicators of anthropogenic disturbance in surface freshwater ecosystems (Kelly and Harwell 1990), this trend has really taken off over the last decade due to strong regulatory pressure exerted by the European Water Framework Directive (WFD, Directive 2000/60/EC of the European Parliament), which aims at achieving a good ecological and chemical status of surface waters. The evaluation of ecological status of water ecosystems is based on the use of several indices, including the Biological Diatom Index (Coste et al. 2009) for microbial communities. These indices, primarily based on the analysis of species characteristics such as taxonomy, abundance and identification of key species, do not reflect the ecological effects induced in response to toxicant exposure (Montuelle et al. 2010; Tlili et al. 2015). Moreover, even though the WFD was first focused on characterizing the chemical and ecological status of aquatic ecosystems, its ultimate goal is to monitor gain in ecological quality during ecological recovery following restoration measures to decrease chemical pressure (Hering et al. 2010). Surprisingly few studies have been led to assess structural and functional recovery of microbial communities in aquatic ecosystems subjected to chemical remediation (Adams et al. 2002; Arini et al. 2012c; Cherry et al. 1977). Arini et al. (2012c) assessed the ecological impact of remediation in a river subjected to an industrial contamination and did not observe significant change in periphytic diatom composition within two years due to the lack of decrease in metal accumulation (Cd and Zn) in periphyton. This study pointed out that recovery of aquatic microbial communities after industrial site remediation can sometimes be delayed. Cattaneo et al. (2004) arrived at the same conclusion after studying diatom communities along a sediment core collected in a lake with a long history of mining pollution. Indeed, by analyzing diatoms in the upper sediment layers, they detected indications of successful ecological recovery, but only 20 years after the start of remediation. However, it must be kept in mind that new diatom species can develop during the course of recovery, thus leading to the establishment of new community structures that may differ from those prevailing before disturbance (Hynynen et al. 2004). The functional consequences of these changes remain generally unknown, which highlights the limits of only assessing structural recovery of microbial communities. Adams et al. (2002) pointed out the need to combine various biological metrics to assess recovery in aquatic ecosystems. Studying recovery dynamics in a stream previously exposed to various contaminants from a nuclear weapons production facility (including heavy metals, chlorinated organics, and residual chlorine), they observed that the evolution of periphytic photosynthetic biomass (based on chlorophyll a measurement) reflected the general decrease of chlorine and mercury in the water, being more responsive than photosynthesis to recovery processes.

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In marine ecosystems, there is plenty of literature on ecosystem recovery after pollution, mainly dominated by studies after oil spill. Recovery of the bacterial communities after oil pollution is closely linked to the pollution history, being much higher in ecosystems that have previously faced accidental spill or human activities compared to pristine sites (Head et al. 2006; Sauret et al. 2012). Nutrient and surfactant amendment is a widely accepted practice in oil-spill bioremediation, where resource-ratio theory (based on carbon/nitrogen/phosphorus ratios) is an important factor to determine recovery speed of the contaminated ecosystem both in terms of diversity of organisms and ecosystem functions (Delille et al. 2009; Sauret et al. 2015). Several studies used the non-specific Microtox® test based on measuring the decrease of bioluminescence of *Vibrio fisheri* to assess the toxicity stress of oil and its residues for ecosystem recovery. For example, with this test Pelletier et al. (2004) showed that intertidal sediments were still under toxicity stress one year after oil spill, whereas chemical analysis showed over 90% degradation of n-alkanes and disappearance of most light aromatics. Spectacular evidence of bacterial community resilience after pollution in marine environments comes from bacteria associated to corals. Shifts in microbiota composition often correlate with the appearance of signs of coral disease and/or bleaching, thus suggesting a causal link between microorganisms, coral health and stability of reef ecosystems (Krediet et al. 2013). For example, Garcia-Armisen et al. (2014) evidenced resilience of bacterial communities together with coral health under the influence of a sewage-polluted river. It is thus vital to evaluate both the resistance (insensitivity to disturbance) and resilience (the rate of recovery after disturbance) of microbial communities to understand the mechanisms that dictate the outcomes of host–microbial interactions and impact resilience of the host.

### *10.3.2 The Study of Microbial Adaptation to Toxicants for in Situ Assessment of Recovery*

A major challenge in environmental risk assessment of pollutants is to establish causal relationships between chemical exposures and resulting community responses within complex ecosystems (Blanck and Dahl 1998; Tlili et al. 2015). A recent study using a large set of environmental parameters along several pollution gradients showed that this link is difficult to find, even when using multivariate statistical analysis (Sauret et al. 2016). Likewise the reliability of biological metrics for assessing recovery depends, among other things, on their causal relationships to stressors (Adams et al. 2002). Recent papers highlight the need to develop specific ecological indicators to monitor biological recovery following a decrease in toxic chemical pollution (Pesce et al. 2016; Tlili et al. 2015). As mentioned above, this need is particularly acute now that each EU member state is expected to implement the WFD, since one of the key as-yet-unresolved challenges is the evaluation of ecological recovery following water chemical quality improvement (Hering et al. 2013).

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It is now well admitted that complex microbial communities are able to cope with chronic exposure to toxicants in various ecosystems through intra- or inter-specific adaptation processes. Such adaptations can lead to an increase not only in toxicant tolerance (according to the PICT concept, e.g. Pesce et al. 2010) but also in toxicant biodegradation capacities in the exposed communities in both soil and aquatic systems (Pesce et al. 2009). Given their relative specificity to various classes of toxicants (generally according to their mode of action and/or molecular structure), adaptation processes offer new insights for developing new ecological indicators to monitor microbial recovery.

Real-world case studies investigating the relevance of such approaches to evaluate community recovery from environmental contamination (i.e. in a context of long-term and progressive change in chemical quality) remain rare (Table 8.4). Blanck and Dahl (1998) performed a 4-year PICT approach to assess the recovery of marine periphyton communities on the Swedish west coast after the 1989 ban on the use of tri-*n*-butyltin (TBT) in antifouling paint. The observed decrease in TBT tolerance of field-sampled periphyton communities in response to the decrease in TBT concentrations in the water confirmed that PICT approaches are suitable for assessing recovery in natural microbial communities. More recently, PICT approaches have successfully been used to assess the recovery of phototrophic microbial communities (phytoplankton and periphyton, respectively) in lake (Larras et al. 2016) and stream (Pesce et al. 2016) ecosystems in a context of chemical restoration from herbicide contamination. These studies offer evidence that PICT has potential as a powerful microbial metric to assess ecological recovery. However, prior its implementation in a regulatory framework, further work is required to standardize PICT measurement (Lambert et al. 2015; Tlili et al. 2015) and acquire baseline tolerance levels at large geographical scales (Pesce et al. 2016).

Besides PICT approaches, Pesce et al. (2013) also proposed the use microbial biodegradation potential of sediment to assess ecological recovery following a decrease in chronic exposure to organic pollutants. In a 4-year case study conducted in a small agricultural stream, the post-ban decrease in level of chronic diuron exposure in the river led to a strong decrease in sediment diuron-mineralizing capacities, revealing the recovery of the microbial community. This result brings further evidence that the study of microbial adaptation to toxicants can serve to demonstrate community recovery from environmental contamination, reflecting its relevance as an indicator in ecosystem restoration. Indeed, such approaches are generally specific to one substance, or one class of substances (according to their mode of action or their chemical structure), as shown by the results of Pesce et al. (2013, 2016) that reflected the resulting progressive decrease in diuron concentrations in the Morcille River despite the persistence of a multi-contamination context.

However, as previously stated with the PICT approach, further research is still required before the assessment of microbial biodegradation potential can be proposed as a routine protocol for evaluating ecological recovery in contaminated ecosystems. One major limitation is the use of radiorespirometry which requires specific authorization to manipulate radiolabeled contaminants. A promising

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alternative is the use of molecular approaches to study functional genes encoding enzymes involved in degradation pathways (Smith and Osborn 2008; Bombach et al. 2010; Monard et al. 2013), which could be potential biomarkers for the detection of organic xenobiotics (Sipilä et al. 2008). A prerequisite for applying such approaches is knowledge of the genes coding degrading enzymes, and the number of these genes known to date is still relatively limited. Rapid advances in functional genomics, such as transcriptomics and proteomics complementing traditional genetic approaches, which make it more feasible to understand gene functions, are providing methodological tools to overcome this constraint (Ortiz-Hernández et al. 2013; Karpouzas et al. 2016).

## 10.4 Challenges and Perspectives

As recently underlined by the EFSA Scientific Committee (2016a) and touched on briefly in the first section of this chapter, the assessment of ecosystem recovery is no trivial challenge. Microbial communities are identified as major ecological engineers in the recovery of degraded ecosystems (Singh 2015) and the numerous examples cited in this chapter clearly show that microbial ecologists and ecotoxicologists have a large variety of tools and methods to study the structural and functional recovery of phototroph and heterotroph microorganisms following chemical exposure, at population and community scales and in different kinds of ecosystems. The next challenge for scientists is to translate the microbial response at ecosystem scale, or in other words to understand how structural and functional recovery observed at microbial scale can reflect wider ecosystem recovery.

Pesce et al. (2013) offers an interesting case study to illustrate the magnitude of this issue. Indeed, in their survey, although the decrease in the diuron biodegradation potential of microbial communities reflected an improvement in chemical quality of the river, it also indicated a decrease in the capacity of the microbial community to help dissipate organic toxicants. Paradoxically, this can somehow be viewed as a decrease in the efficiency of the ecosystem function supported by microbial degradation in driving natural attenuation of organic pollutants in the environment. Another point, which was raised by Gomez-Sagasti et al. (2012) and is clearly highlighted here, is that microbial properties are highly context-dependent, making each study case unique. This statement outlines the need to define ecosystem recovery targets as well as the microbial metrics needed to assess the course of recovery accordingly (Duarte et al. 2015). Such a process should be facilitated by combining (i) microbial metrics of high ecological relevance (i.e. microbial functions supporting a range of ecosystem functions and services) and (ii) microbial metrics that could serve to establish a direct link between improvement of chemical quality and microbial recovery (e.g. study of structural and functional microbial adaptation to toxicants).

Several examples cited above offer successful case studies of using microbial indicators to assess recovery following improvement in chemical quality in

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ecosystems ranging from soils and freshwaters to seawaters. Such case studies are particularly important to provide proof-of-principle for the relevance of considering microbial communities in recovery studies (EFSA Scientific Committee 2016a). Based on this set of demonstrations, and to successfully implement a strategy for better assessing ecosystem recovery in various environments and at a larger geographical scale, there is a need to educate legislators and policymakers on the importance of considering microbial communities in environmental risk assessment, including ecological recovery monitoring.

Indeed, despite the recognized importance of microorganisms in supporting a range of ecosystemic services, they are barely protected by any regulations or legislations. For example, despite a proposal in 2006, the European Commission did not ratify the soil protection directive (Van Camp et al. 2004). Until now, only EU directive 91/414 for placing plant protection products (pesticides) on the market evaluates, at least in principle, the ecotoxicological impact of pesticides on soil microorganisms, but only using two global tests assessing their impact on the mineralization of carbon and nitrogen (EU-Regulation 1107/2009/EC). However, referring to recent work assessing the resistance and resilience of microbial communities and considering their functional redundancy, Martin-Laurent et al. (2013) suggested that carbon and nitrogen mineralization provide only a rough estimate of the possible impact of pesticides on soil microbiota. More recently, Karpouzas et al. (2016) further affirmed that these two out-of-date tests are not sensitive enough to reliably assess the impact of pesticides on the diversity and functioning of soil microbial communities and on supported ecosystemic functions. However, the tools required to monitor a range of ecosystemic functions relying on microbial communities, are still missing or remain unstandardized (e.g. Philippot et al. 2012). The absence of standardized methods means that there is no consistent dataset available that could be used to define normal operating ranges of microbial indicators, which is an important prerequisite for assessing microbial recovery in various ecosystems. This is probably due to the fact that although microbial ecologists have made huge steps forwards by developing an impressive toolbox for measuring the abundance, diversity and activity of microorganisms, they are less involved in the next-step technology knowledge transfer, mobilization and outreach to society. It thus precludes their implementation in regulatory frameworks which would better preserve environmental resources by taking into account the ecological role of microbial communities and their potential use as ecological indicators of ecosystem recovery following chemical pollution.

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