



**HAL**  
open science

## Resistance and resilience of removal efficiency and bacterial community structure of gas biofilters exposed to repeated shock loads

Léa Cabrol, Luc Malhautier, Franck Poly, Xavier Le Roux, Anne-Sophie Lepeuple, Jean-Louis Fanlo

### ► To cite this version:

Léa Cabrol, Luc Malhautier, Franck Poly, Xavier Le Roux, Anne-Sophie Lepeuple, et al.. Resistance and resilience of removal efficiency and bacterial community structure of gas biofilters exposed to repeated shock loads. *Bioresource Technology*, 2012, 123, pp.548 - 557. 10.1016/j.biortech.2012.07.033 . hal-01427048

**HAL Id: hal-01427048**

**<https://hal.science/hal-01427048>**

Submitted on 31 May 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Resistance and resilience of removal efficiency and bacterial community structure of gas biofilters exposed to repeated shock loads

Léa Cabrol<sup>a,b,\*</sup>, Luc Malhautier<sup>a</sup>, Franck Poly<sup>c</sup>, Xavier Le Roux<sup>c</sup>, Anne-Sophie Lepeuple<sup>b</sup>, Jean-Louis Fanlo<sup>a</sup>

<sup>a</sup>Laboratoire Génie de l'Environnement Industriel, Ecole des Mines d'Alès, Rue Jules Renard, 30100 Alès, France

<sup>b</sup>Veolia Environnement Recherche et Innovation, Chemin de la Digue, BP76, 78600 Maisons Laffitte, France

<sup>c</sup>Laboratoire d'Ecologie Microbienne, Université de Lyon, Université Lyon 1, CNRS, INRA, UMR CNRS 5557, USC INRA 1193, Bâtiment Gregor Mendel, 43, Boulevard du 11 Novembre 1918, 69622, Villeurbanne Cedex, France

## H I G H L I G H T S

- ▶ Four biofilters were exposed to repeated substrate pulses of increasing intensity.
- ▶ New quantitative indices of resistance and resilience capacity were proposed.
- ▶ We highlighted different robustness levels according to the contaminants.
- ▶ There was an impact of the perturbation regime at the community level (DGGE).
- ▶ Bacterial community structure partially coupled to removal efficiency robustness.

## A B S T R A C T

Since full-scale biofilters are often operated under fluctuating conditions, it is critical to understand their response to transient states. Four pilot-scale biofilters treating a composting gas mixture and undergoing repeated substrate pulses of increasing intensity were studied. A systematic approach was proposed to quantify the resistance and resilience capacity of their removal efficiency, which enabled to distinguish between recalcitrant (ammonia, DMS, ketones) and easily degradable (esters and aldehyde) compounds. The threshold of disturbing shock intensity and the influence of disturbance history depended on the contaminant considered. The spatial and temporal distribution of the bacterial community structure in response to the perturbation regime was analysed by Denaturing Gradient Gel Electrophoresis (DGGE). Even if the substrate-pulses acted as a driving force for some community characteristics (community stratification), the structure-function relationships were trickier to evidence: the distributions of resistance and composition were only partially coupled, with contradictory results depending on the contaminant considered.

### Keywords:

Biofiltration

Resistance

Resilience

Community structure

DGGE

## 1. Introduction

As a result of fluctuating industrial activity, full-scale biofilters are often submitted to unstable influent composition, concentrations and flow rates, which can alter the bioprocess in terms of both functional performance and community structure (Kraakman, 2003), especially when applied to the treatment of sludge composting emissions. It is thus of prime importance to identify the changing conditions that can alter the biofiltration, and to deter-

mine to what extent biofilters are able to cope with disturbance and to recover after disturbance.

Among other temporal variations, substrate pulses are ephemeral events of resource superabundance which are often observed in industrial biofilters. They induce microbial responses in terms of growth, decay or enzymatic activity and likely lead to shifts in microbial community structure (Chesson et al., 2004). However, whether the responses of the biological component to resource pulses reflect adaptive behaviours (genotype selection) and/or physiological effects is still unclear (Yang et al., 2008), which restricts the optimal management and operation of biofilters. In addition, as they can be controlled and monitored, lab-scale biofilters are model engineered ecosys-

\* Corresponding author at: Laboratoire Génie de l'Environnement Industriel, Ecole des Mines d'Alès, Rue Jules Renard, 30100 Alès, France. Tel.: +56 32 2274178.  
E-mail address: lea.cabrol@gmail.com (L. Cabrol).

tems that are relevant for assessing resistance and resilience capacities of complex microbial communities facing disturbances, and for exploring the relationships between the functional characteristics and structural and molecular features of these microbial communities (Graham and Smith, 2004). Indeed, unravelling the biodiversity-ecosystem function relationship in engineered ecosystems such as biofilters has been a challenging Microbial Ecology-based issue during the last decade (Cabrol and Malhautier, 2011).

Many definitions of resilience, resistance and stability are proposed in the ecological literature, without consensus on the way to quantify them. The stability properties differ according to the component considered (either physico-chemical and biological status of the system, or its functioning level), the variable chosen to describe it (e.g. biomass density or system performance), the nature of the disturbance and the environmental conditions prevailing before the disturbance (Carpenter et al., 2001). To avoid confusion, the conditions defining stability have to be clearly specified. Here, the stability will be defined as the ability of a disturbed system to stay in or return to an equilibrium state. In this context, the resistance represents the capacity of the system to withstand a disturbance, which is quantified by the magnitude of deviation of an ecosystem variable from its equilibrium state (Hashsham et al., 2001). The “engineering resilience” is the speed of recovery (or the time required for recovery) of a given variable after a disturbance, while the “ecological resilience” is the maximum magnitude of disturbance that can be absorbed before the system experiences a transition toward an alternative stability domain (Pimm, 1984; Hashsham et al., 2001).

The goal of the present study was to investigate the functional and microbial behaviour of gas biofilters applied to the treatment of composting emissions and experiencing repeated substrate pulses of increasing intensity. The key objectives and hypotheses of the work were:

- (i) to accurately quantify the resistance and engineering resilience of perturbed biofilters facing such substrate pulses, in terms of contaminant removal efficiency (RE). Biofilter resistance is supposed to decrease with increasing disturbance intensity, and a threshold of the disturbance amplitude should exist, above which resilience capacity would be lost,
- (ii) to compare the resistance and resilience of RE for a range of gaseous compounds present in the composting gas mixture. Competition between substrates was assumed to lead to higher RE resistance and resilience levels for preferential compounds at the expense of recalcitrant substrates, under excess conditions,
- (iii) to assess the history effect, i.e. to determine if previous application of disturbances influences the resistance and engineering resilience to the same type of disturbance. In biofilters that have previously experienced disturbances, physiological responses and selection of microbial populations were supposed to lead to higher resistance and resilience to the same kind of disturbance
- (iv) to determine the impact of disturbances on the spatial and temporal distribution of microbial community structure in order to analyse the possible links between the functional and microbial responses. For disturbances of low amplitude, the observed responses in RE could be explained mainly by transient, physiological changes of the microbial component, whereas changes in abundance and composition of the microbial component would become of major importance for disturbances of higher amplitude.

## 2. Materials and methods

### 2.1. Pilot-scale biofiltration unit

Four biofiltration columns (15 cm internal diameter, 230 cm height), packed with pine bark woodchips, were operated in downward flow mode for 290 days, under previously described conditions (Cabrol et al., 2012). The total bed height of 150 cm (packing volume 26.5 L) was divided into five compartments of 30 cm-height of material. Gas-sampling ports were located at 0, 30, 60, 90, 120 and 150 cm from the gas inlet, and biomass-sampling ports were located at the middle of each compartment (15, 45, 75, 105, 135 cm from the gas inlet).

### 2.2. Inlet stream and disturbance strategy

The biofilters were continuously fed by a synthetic gaseous mixture representative of composting emissions, containing ammonia and six Volatile Organic Compounds (VOCs): butyraldehyde, acetone, methyl ethyl ketone (MEK), methyl butanoate (MB), methyl valerate (MV) and dimethyl disulfide (DMDS). The dynamic generation system (Cabrol et al., 2012) allowed to maintain stable target concentrations (<10% standard deviation).

The gas flow rate, empty bed residence time (EBRT) and superficial gas velocity were 30 L min<sup>-1</sup>, 54 s and 100 m h<sup>-1</sup>, respectively, which was representative of full-scale biofilter operation. The four columns consisted of two control biofilters (C1 and C2) and two perturbed biofilters (P1 and P2). Since such systems are strongly money- and time-consuming, it was not possible to increase the number of biofilters. P1 and P2 were run under alternation of stable operation and transient perturbations (Table 1). During stable operation, all four biofilters were fed with standard concentrations, i.e. NH<sub>3</sub> at 21 ± 3 mg m<sup>-3</sup> and each VOC at 4.9 ± 0.6 mg m<sup>-3</sup>. The perturbations consisted in six periodic shock loads (applied at days 42, 58, 91, 121, 169 and 224), during which the inlet concentration was progressively increased from 2 to 44 times the standard concentration level, without changing the flow rate (Table 1). Each shock was only applied when all biofilters experienced steady state, and lasted for 6 days, after which the standard concentrations were restored. C1 and C2 were run under stable conditions until day 231, afterwards they were submitted to a single 6-d disturbance, similar to the last shock applied to P1 and P2 (Table 1), to assess the disturbance history effect.

### 2.3. Characterization of biofilter functional robustness

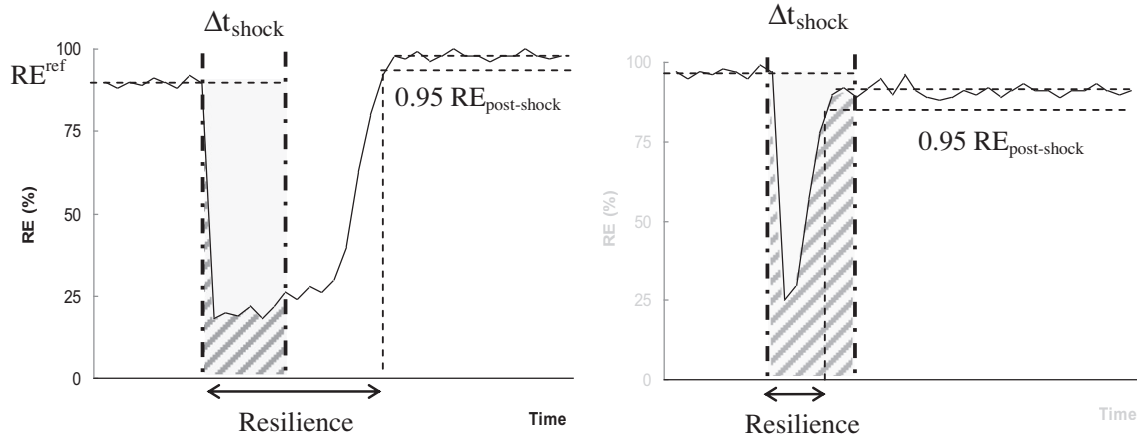
Gaseous concentrations of VOCs and ammonia at the different biofilter heights were quantified daily as previously described (Cabrol et al., 2012). For each compound *i*, the removal efficiency (RE<sub>*i*</sub>) was calculated at the biofilter height *h*:  $RE_{i,h} = \frac{C_{i,inlet} - C_{i,h}}{C_{i,inlet}}$ , where *C<sub>i,inlet</sub>* could be either the concentration at the entrance of the biofilter (global RE), or at the entrance of each 30-cm compartment (local RE).

#### 2.3.1. Resistance index

The resistance was assessed by quantifying the level of RE that was maintained during the whole perturbation period ( $\Delta t_{shock}$ ). As described in Fig. 1, the resistance index was computed as the ratio of the Effective RE (based on the actual, time-integrated RE during the shock) to the Reference RE (based on the potential, time-integrated RE that would have been obtained during the same period without any shock, i.e. if the stable RE<sup>ef</sup> level observed before the shock had been maintained). A similar envelop-approach has been proposed by Hashsham et al. (2001). The Resistance Index

**Table 1**  
Inlet concentrations of ammonia and VOCs in the four biofilters during steady states (without disturbance) and during the last periodic shock (S6), as well as in the disturbed biofilters during the first five periodic shocks (S1–S5). Each shock lasted for 6 days. The values are averaged upon the period considered, the six VOCs, and the biofilters (i.e.  $n = 4$  for steady state and S6, and  $n = 2$  for S1–S5). Standard deviations in brackets.

	Steady states	S1	S2	S3	S4	S5	S6
Each VOC ( $\text{mg m}^{-3}$ )	5 (0.6)	10 (0.7)	22 (1)	44 (2)	97 (6)	125 (9)	218 (25)
$\text{NH}_3$ ( $\text{mg.m}^{-3}$ )	21 (3)	42 (1)	77 (1)	189 (6)	390 (10)	504 (41)	876 (65)
Increasing factor	–	2	4	9	19	25	42–44



**Fig. 1.** Quantification of the resistance and resilience index of biofilter removal efficiency (RE) in response to a shock load. The bold dotted vertical lines represent the beginning and the end of the shock. The engineering resilience index is the time required, since the beginning of the shock, to reach 95% of the stable post-shock RE. It can be longer (left) or shorter (right) than the total shock period. The resistance index is the ratio of the effective RE during the shock (hatched area) to the reference RE ( $RE^{\text{ref}}$ ) during the same period assuming there were no shock (gray area). Note that the post-shock RE could be either higher (left) or lower (right) than  $RE^{\text{ref}}$  before the shock.

was computed on the basis of the local  $RE_i$ , i.e. relative to the inlet gas concentration for each layer:

$$\text{Resistance Index}_{i,h} = \frac{\text{Effective } RE_{i,h} \text{ during shock}}{\text{Reference } RE_{i,h} \text{ assuming no shock}}$$

$$= \frac{\int_{\Delta t_{\text{shock}}} RE_{i,h}(t) \cdot dt}{\Delta t_{\text{shock}} \cdot RE_{i,h}^{\text{ref}}}$$

For each compound  $i$ , the Resistance Index was calculated for the 6 shocks and the 5 biofilter heights  $h$ , thus providing 30 couples of resistance versus inlet compartment concentration, in each of the duplicated biofilters.

### 2.3.2. Resilience index

As illustrated in Fig. 1, engineering resilience was defined as the time (since the beginning of the perturbation) required to reach 95% of the asymptotic stable RE after a perturbation (which could be higher or lower than the stable RE before the shock). This index was computed on the basis of the global RE of each biofilter, i.e. relative to the inlet gas concentration. Based on the extent of functional stability observed in control biofilters (Cabrol et al., 2012), a state was considered as stable when the relative standard deviation of RE was <15% over time.

## 2.4. Microbial community investigation

### 2.4.1. Biomass sampling and DNA recovery

DNA was recovered from the woodchip biofilm according to a previously optimized protocol (Cabrol et al., 2010). In P1 and P2, biomass was sampled at five height levels (15, 45, 75, 105 and 135 cm from the gas inlet), before and after each of the 6 shocks, i.e. once at the end of the stable state just before the perturbation, and once on the last day of the perturbation (Fig. 2). In C1 and C2, biomass was sampled at days 42, 47, 58, 64, 90, 97, 121, 127, 173

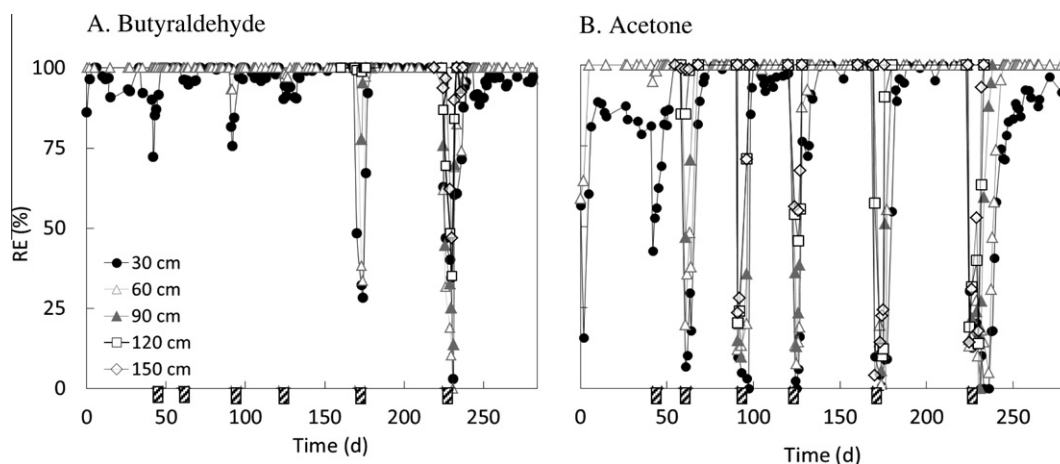
and 231, at four height levels (15, 45, 75 and 135 cm from the gas inlet).

### 2.4.2. 16S rDNA PCR-DGGE

The V3 region of the bacterial 16S rRNA gene was PCR amplified according to Cabrol et al. (2010). PCR products were quantified by fluorescence at 535 nm after PicoGreen staining (Quant-iT ds DNA HS reagent, Invitrogen, Carlsbad, CA, USA). DGGE was performed with Ingeny phor U-2 system (Goes, The Netherlands) according to the protocol of Muyzer et al. (1993), with denaturing gradient ranging from 43% to 63% as previously described (Cabrol et al., 2010). Gels images were analyzed with the Bionumerics software (Applied Maths, Gent, Belgium). The analysis of bacterial community by DGGE is limited by the quantitative bias inherent to the different steps of the methodology (non-exhaustive DNA extraction, preferential PCR amplification, DGGE detection limit and saturation impairing the detection of minority species, the multiple operation sequences resulting in multiple bands for a single species and the co-migration resulting in artefactual unique band for several different species) (Muyzer et al., 1993; Muyzer and Smalla, 1998). However, despite these limitations, DGGE is a good tool to comply with the present objective, which is to compare the community structures between a relatively large number of samples, undergoing the same methodological treatment, based on the major populations detected (Loisel et al., 2006).

### 2.4.3. Analysis of community genetic structure

After exclusion of the rarest bands (i.e. <5% intensity in all samples), the initial data matrix (relative intensities according to band position) was standardized and square-root transformed to down weight the influence of more abundant species. The pair-wise similarity index  $S_{ij}$  between community profiles  $i$  and  $j$  was calculated by the Bray-Curtis coefficient. The percentage of dissimilarity is given by 100-Similarity. Hierarchical clustering, using UPGMA



**Fig. 2.** Examples of the temporal variations in removal efficiency (RE) of the whole perturbed biofilter P1, for butyraldehyde (A) and acetone (B), measured at 5 different heights from the gas inlet. The hatched boxes on the x-axis represent the time and duration of periodic shock loads of increasing intensity.

linking, was performed with Matlab® software (The Mathworks, Natick, MA, USA).

### 3. Results and discussion

#### 3.1. Overall variations in removal efficiency

The undisturbed control biofilters C1 and C2 maintained high and stable removal efficiency (RE) during the whole experiment (not shown, but see Cabrol et al., 2012). The two perturbed biofilters P1 and P2 exhibited similar RE patterns (Figs. 3 and 4). During the stationary periods between shocks, RE was stable and high for all compounds: the contaminant took place mostly within the first 30 cm, as shown for two VOCs in Fig. 2. During the intermittent shock loads, RE dropped more or less dramatically according to the contaminant considered, height level, and shock intensity (Fig. 2). The lower compartments provided buffer capacity to absorb the shocks: the substrate pulses had been gradually dampened through the successive compartments, resulting in reduced performance loss and recovery times. The more intense the shocks were, the deeper the contaminants penetrated down the filters, until breakthrough. For all compounds, the biofilters were able to recover after all the disturbances tested in this study (Fig. 2).

#### 3.2. Compound effect on resistance and resilience capacities of biofilter removal efficiency

##### 3.2.1. Resistance capacity

The patterns of resistance capacity of RE as a function of the inlet gas concentration (Fig. 3) revealed two distinct behaviours between recalcitrant and easily degradable compounds. The biofilters had a poor resistance capacity for removal of DMDS, acetone, MEK and ammonia. For those four compounds, the resistance capacity dropped dramatically as soon as the inlet concentration increased, according to a hyperbolic trend, independently of the layer considered (Fig. 3A–D). An increase of the inlet concentration by a factor 2–3 was sufficient to induce a 50% loss of performance. For the highest inlet concentrations (VOCs > 200 mg m<sup>-3</sup> or ammonia > 800 mg m<sup>-3</sup>), the biofilter RE could only maintain a residual resistance level of 5%, 10%, 11% and 15% for DMDS, acetone, MEK and ammonia, respectively.

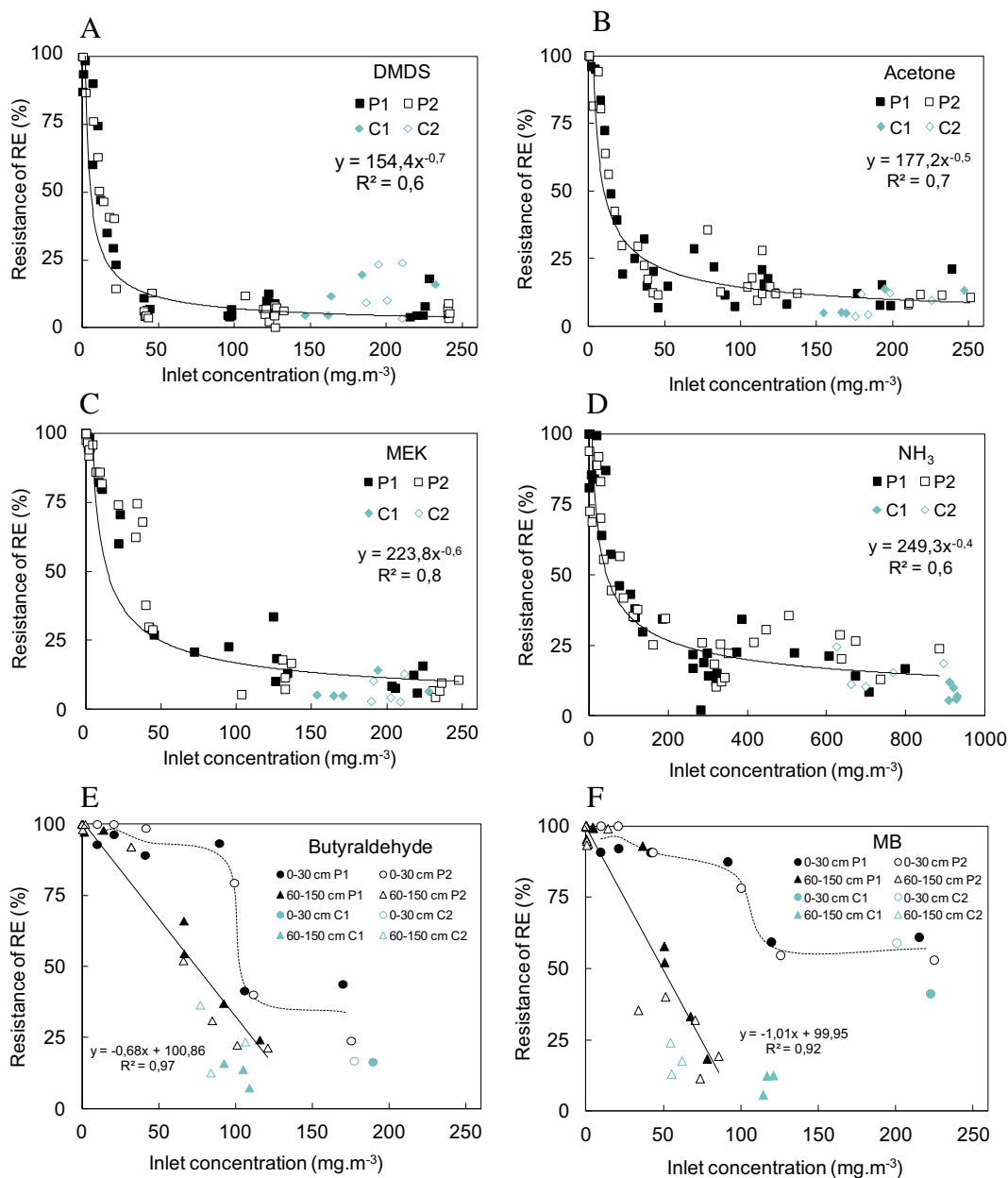
The biofilters had a higher resistance capacity for butyraldehyde, methyl butanoate (MB) and methyl valerate (MV) removal

(Fig. 3E and F; the resistance plot for MV is similar to MB, see Supplementary Information). For these three compounds, the resistance capacity differed according to the biofilter layer considered. The inlet layer (0–30 cm), which was acclimatized to a continuous contaminant exposure during the whole experiment, was the most resistant (circles in Fig. 3E and F). Its resistance index remained high (>80%) for inlet concentrations up to 100 mg m<sup>-3</sup> (i.e. 20-fold the standard concentrations) and decreased to 37 ± 9% for butyraldehyde and 57 ± 4% for MB and MV for inlet concentrations ranging from 110 to 225 mg m<sup>-3</sup> (i.e. up to 45-fold the standard concentrations). In contrast, the 3 lower layers (60–150 cm), which were only temporarily exposed to the contaminant load, were less resistant (triangles in Fig. 3E and F). Their resistance index decreased linearly with increasing inlet concentration, and a loss of 50% of RE was observed for inlet concentrations of 75 and 50 mg m<sup>-3</sup> (respectively 13 and 10-fold the standard concentrations) for butyraldehyde and for MB and MV, respectively.

Previous works already reported the lower biodegradability of ketones compared to esters, of sulphur compounds compared to ammonia and oxygenated compounds, of acetone compared to MEK (Sempere et al., 2008; Smet et al., 2000). As reported by several authors, the differences of RE between different compounds mainly lies in mass transfer characteristics, with a positive correlation between the degradation order and the substrate availability in the liquid phase (Deshusses and Johnson, 2000). In the present work, the transfer limitation could partly explain the poorest resistance for the least soluble compound (DMDS). However, a significant difference in resistance capacity of biofilter RE was observed for ketones and aldehyde, despite their rather similar Henry coefficients.

The different resistance levels of biofilter RE between compounds can also be attributed to biological factors (Girvan et al., 2005). The observed low resistance capacity of biofilter RE for recalcitrant compounds (DMDS, NH<sub>3</sub> and ketones) can thus be explained by a competition between substrates: as already reported (Santos et al., 2000), a microbial community of generalists in biofilters, potentially able to consume all the different substrates under limited-substrate conditions, may shift its metabolism towards the consumption of preferential substrates (here aldehyde and esters) under excess-substrate conditions. The observed high resistance capacity of biofilter RE for aldehyde and esters could also be due to competition between bacterial taxa, through the selection of specialised populations able to degrade those compounds and to largely out compete the other taxa for space, energy and oxygen competition (Juhler et al., 2009; Mohseni and Grant Allen, 2000).



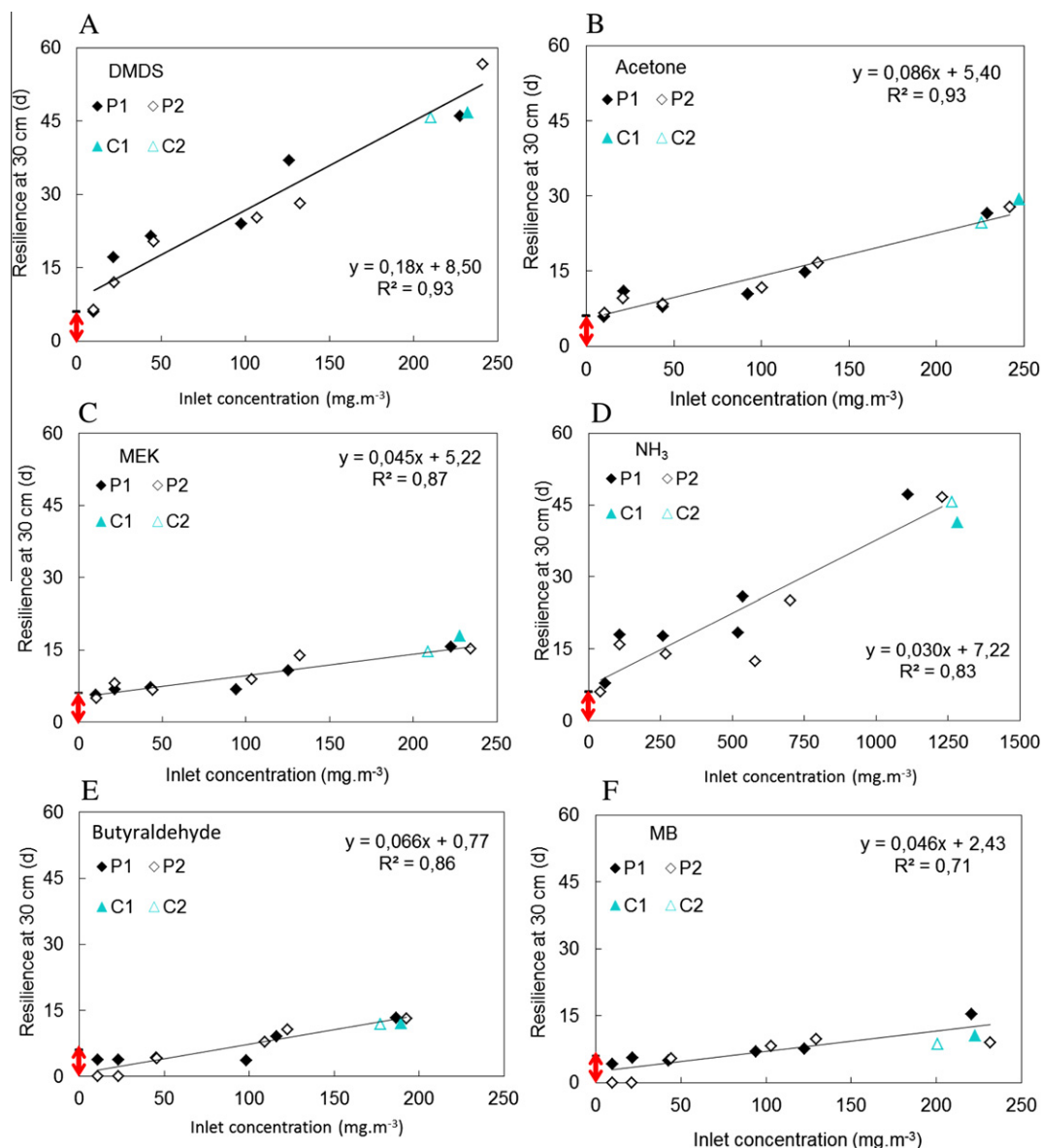


**Fig. 3.** Changes in the resistance index of local removal efficiency, RE, as a function of inlet concentration for all layers in P1 (closed symbols) and P2 (open symbols), for DMSO (A), acetone (B), MEK (C), ammonia (D), butyraldehyde (E) and methyl butanoate MB (F). Each point represents the resistance calculated for a given layer and a given shock, plotted against the inlet concentration of that layer at that time. For the first four compounds (plots A-D), the relationship presented was computed considering both columns and all layers. For butyraldehyde and MB (plots E and F), two distinct relationships were observed: one for the first layer between 0 and 30 cm (circles) and another one for the lower layers between 60 and 150 cm (triangles). The observed resistance index of the control biofilters C1 and C2 which experienced only the last perturbation is presented in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.2.2. Resilience capacity

In the first layer (0–30 cm) of perturbed biofilters, the time required to reach functional stability after a shock increased proportionally to the shock intensity and depended on the contaminant considered (Fig. 4). For DMSO, acetone, MEK and ammonia, the functional recovery only occurred after the end of the shocks, *i.e.* once the inlet concentrations had returned to the standard level. High RE recovery could require up to 45–50 days for the strongest shocks for DMSO and ammonia. For butyraldehyde and MB (Fig. 4E and F) as for MV (Supplementary information), the functional recovery occurred before the end of the shock for the first three perturbations. The recovery time increased linearly with increasing disturbance intensity, but

remained lower than values observed for the first 4 compounds at a given concentration. The resilience capacity for MV was similar to MB (Supplementary information). In response to the last shock, the control and perturbed biofilters exhibited similar levels of resilience capacity in the first layer for all compounds (blue symbols in Fig. 4). The resilience capacity values of the whole 150 cm-columns are presented in Table 2. In the perturbed biofilters, the recovery time when considering the whole biofilter height was approx. Two to three times lower than the recovery time for RE at 30 cm. There was even no shock impact at the outlet for butyraldehyde, MB and MV after the four least intense perturbations. As in the first layer, the resilience index increased linearly with inlet concentration.



**Fig. 4.** Changes in the resilience index of removal efficiency, RE, of the first 30-cm-layer of biofilters as a function of inlet concentration, in P1 (◆) and P2 (◇), for DMDS (A), acetone (B), MEK (C), ammonia (D), butyraldehyde (E) and methyl butanoate MB (F). The resilience index was calculated as the time required (in days after shock start) to reach 95% of the stable RE observed after a shock, at 30 cm from the gas inlet. The red arrow on the y axis represents the duration of the shock. The observed resilience index of the control biofilters C1 and C2 following the last perturbation is also presented, in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 2**

Resilience index of the whole biofiltration column, after each of the 6 periodic shocks (S1–S6) in the two perturbed biofilters, and after the last shock (S6<sub>control</sub>) in the two control biofilters. The resilience index was calculated as the time (in days after shock start) required to reach 95% of the stable global RE after a shock, at 150 cm from the gas inlet. Average values and standard deviations are presented for the duplicated biofilters.

	S1	S2	S3	S4	S5	S6	S6 <sub>Control</sub>
MB, MV	0	0	0	0	1.3 ± 0.5	7.6 ± 0.1	6.6 ± 0.6
But	0	0	0	0	1.4 ± 0.5	7.8 ± 0.4	8.1 ± 3.5
MEK	0	0	4.2 ± 1.2	0	6.7 ± 0.1	11.4 ± 0.3	12.6 ± 0.9
Acetone	0	3.0 ± 2.0	5.9 ± 1.5	6.7 ± 0.1	6.9 ± 0.1	7.7 ± 0.1	8.5 ± 1.1
DMDS	0	7.9 ± 2.6	7.9 ± 0.8	11.6 ± 0.3	16.6 ± 2.1	25.5 ± 0.5	27.4 ± 0.9
NH <sub>3</sub>	0	0	13.0 ± 0.1	14.2 ± 2.3	22.0 ± 2.8	23.5 ± 4.9	48.1 ± 0.6

MB, methyl butanoate; MV, methyl valerate; But, butyraldehyde; MEK, methyl ethyl ketone; DMDS, dimethyl disulphide.

The impact of load shocks on biofilter RE was not the same in terms of resistance and resilience between compounds. Particularly for ketones, biofilter RE was poorly resistant (resistance close

to that observed for DMDS), while it exhibited a much higher resilience capacity, almost similar to that observed for other oxygenated compounds. This observation is consistent with the

preferential consumption exposed before, which favoured aldehyde and esters metabolic pathways during the shocks. Indeed, after the shocks, these metabolic routes may be more easily and rapidly reversible towards ketone- than sulphur-metabolism, because of the higher similarity between oxygenated compounds metabolism than with sulphur metabolism.

### 3.2.3. Contrasted disturbance threshold values for resistance and resilience

The threshold of the disturbance amplitude above which the resistance of biofilter RE dropped varied strongly according to the compounds considered. This threshold was very low for the recalcitrant compounds in all biofilter layers (Fig. 3A–D). In contrast, the threshold was much higher for the more easily degradable compounds in the 0–30 cm layer, whereas no clear threshold was observed for the other biofilter layers (Fig. 3E and F). The existence of clear threshold for the resistance of biofilter RE has been reported to be due to catabolic repression (Santos et al., 2000), self- and/or cross-inhibition of enzymatic activities by a substrate (Lee and Cho, 2009), by a degradation intermediate (Muñoz et al., 2007), or by the pH (Smet et al., 2000). For example, the toxic effect of DMDS has been reported for concentrations above a threshold value (Ito et al., 2007). Similarly, the degradation of a sulphur compound (DMS) was completely inhibited when ammonia concentration reached  $100 \text{ mg m}^{-3}$  (Smet et al., 2000).

There was no threshold of the disturbance amplitude above which no resilience of biofilter RE would occur, for all compounds. The biofilter RE was resilient for all the perturbations tested, i.e. the loss of degradation capacity was not permanent at the microbial community level, even though it could have been permanent at a single microbial species level. This resilience ability may be linked to the high microbial diversity and associated high level of functional redundancy, which enabled the emergence of functionally equivalent species more resistant in case of disturbance, according to the insurance hypothesis (Girvan et al., 2005).

### 3.3. Influence of disturbance history on biofilter resistance and resilience capacities

In response to the last shock, the control and perturbed biofilters exhibited the same level of resistance capacity (blue symbols in Fig. 3) for all compounds and all compartments. In addition, when considering the whole biofilter height, control and perturbed biofilters exhibited a similar resilience capacity for all compounds, excepted for ammonia (Table 2): the recovery time required to reach high and stable ammonia RE at 150 cm after the most intense shock in C1 and C2 was approx. two-fold the time required in P1 and P2.

It was speculated that the disturbance history would improve the resilience capacity of biofilters, as sometimes reported (Chesson et al., 2004; Ranjard et al., 2008). In the present study, this was only verified for ammonia at the whole biofilter scale (Table 2). Indeed, when considering the whole column, the recovery time for ammonia RE in response to a new substrate pulse was lower in biofilters which had been previously submitted to periodic substrate pulses of low intensity, compared to biofilters which had been previously maintained under constant feeding. The succession of perturbations of increasing intensity may (i) have selected a more versatile bacterial community, including taxa able to cope with high ammonia concentrations, and/or (ii) have induced physiological changes improving the robustness of existing taxa in case of high ammonia concentrations.

However, there was no evidence for an effect of disturbance history on the resistance and resilience capacity of biofilter RE for VOCs (Table 2, Figs. 3 and 4). First, the turnover of the VOCs-degraders may be higher than that of the ammonia oxidizers,

thus explaining that the potential species selection and/or physiological adaptation to disturbance history did not persist until the last shock in the case of VOCs RE. In addition, the environmental conditions at a given time may be a stronger driving force than the history, and/or the repeated stresses might even have altered the biofilter response to the last stress (Langenheder et al., 2005).

Excepted for the particular community of ammonia degraders which would need further specific investigations, the assumptions on the mechanisms leading to the observed functional responses in perturbed biofilters can be partly enlightened by the investigation of bacterial community structures.

### 3.4. Spatial and temporal distribution of microbial community structures in biofilters

#### 3.4.1. Community structure stratification in perturbed biofilters

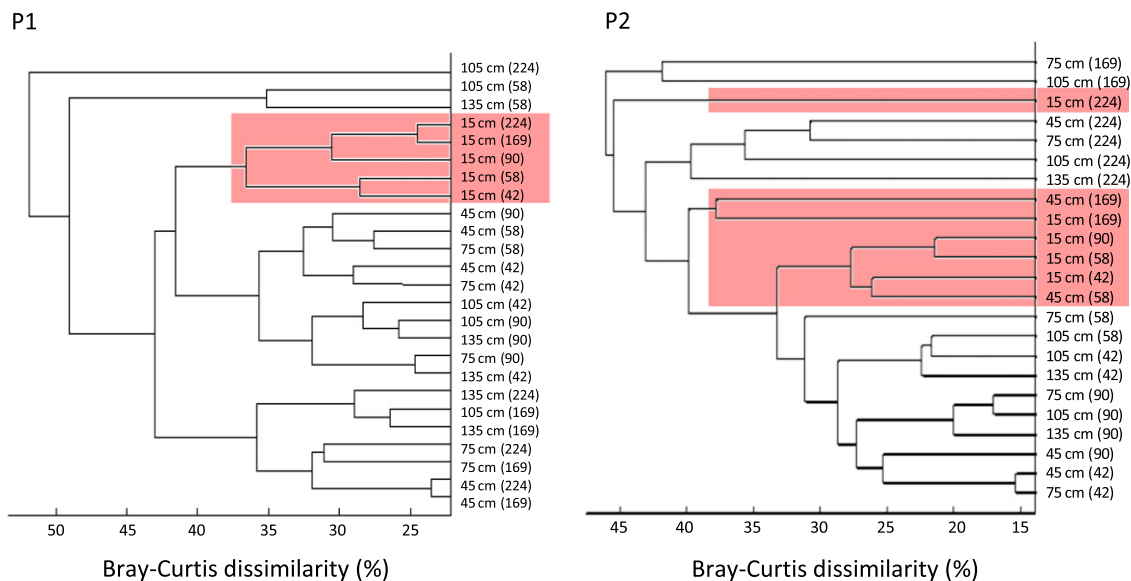
The distribution of microbial community structures along bed height in perturbed biofilters was assessed by clustering analysis based on Bray-Curtis similarity index (Fig. 5). The clustering showed the differentiation of the community structures in the layers close to the inlet (15 cm in P1, 15 and 45 cm in P2), which discriminated from the lower ones and stayed rather homogeneous along time (red boxes in Fig. 5). As each column was analyzed independently, only pseudo-replicates due to different sampling times were available and did not allow to statistically confirm this height effect. Within the lower layers, the samples were more dispersed and no stratification was observed (Fig. 5). Time effect was stronger than height effect therein: the samples clustered more by date than by height, with a separation between the first sample dates (<90 days) and the last ones (>169 days). There was therefore a threshold of disturbance intensity above which the community structure within the lower layers was influenced by the perturbations.

#### 3.4.2. Community structure in control Vs perturbed biofilters

As also reported by others (Bayle et al., 2009; Chesson et al., 2004), the impact of the shocks on the community structure was highlighted by comparing perturbed and control community structures along time, at 15 and 75 cm from the gas inlet (Fig. 6). Because a confident matrix could be constructed by comparing only a limited number of samples, the comparison of P1 and P2 communities at all sampling dates could only be done with one control community (C1), but the duplicated control communities C1 and C2 followed a highly reproducible time-trajectory, as previously evidenced (Cabrol et al., 2012). In the inlet section (15 cm), perturbed and control biofilter communities could not be discriminated at the dissimilarity threshold of 45% and no treatment nor time effect were observed (Fig. 6A, with the exception of P2 samples after day 224 which had a dissimilarity of 50%). In contrast, in the mid-height layer (75 cm), the results revealed treatment and time effects (Fig. 6B): (i) the structures of the communities sampled before day 121 in perturbed biofilters clustered together and got separated from the structures of the perturbed communities sampled after day 160; (ii) these two clusters differed from the community structure cluster obtained for control biofilters before day 100; (iii) community structures observed for control biofilter after day 169 strongly differed from the community structures obtained for all the other samples.

The selective pressure imposed by the shock loads persistently shaped a specific bacterial community structure in the mid-height section (75 cm), which was similar between the two perturbed biofilters, and kept distinct from the control community structures (Fig. 6B). In contrast, the inlet (15 cm) bacterial community structure remained similar between control and perturbed biofilters throughout the experiment (Fig. 6A). Within the inlet section, bacterial communities were submitted – most of the time – to the





**Fig. 5.** Dendrograms based on 16S DGGE profiles of the communities sampled before the shocks at 15, 45, 75, 105 and 135 cm from the gas inlet in perturbed biofilters P1 and P2. The labels indicate the height level and day of sampling (e.g. 75 cm-42 corresponds to sampling at 75 cm from the gas inlet, on day 42). The red boxes indicate the clustering of inlet communities (with the exception of sample 15 cm-224 for P2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

same contaminant concentration in perturbed and control biofilters, and shock loads were not sufficient to generate major changes in community structure in the inlet section of perturbed biofilters. Concurrently, the mid-height communities were never submitted to contaminants in the control biofilters, but experienced periods of contaminants availability in perturbed biofilters that depended on the compounds considered and disturbance intensity (Fig. 2). The results from Figs. 5 and 6 indicated the existence of a disturbance threshold above which the lower-layer community structure got influenced by the shock loads, while it was not the case at the inlet. These results thus suggest that the alternation of feast/famine conditions at mid-height (75 cm) had a stronger influence on the bacterial community structure than the alternation of standard/pulse concentrations at the inlet (15 cm). In other words, lower-layer communities were strongly influenced by the temporary breakthrough of unusual substrates, whereas inlet bacterial communities were less influenced by concentration changes of their usual substrates.

### 3.4.3. Partial coupling/uncoupling between macroscopic function and bacterial community structure

In various soil, water and engineered ecosystems, the level of coupling between the structural and functional microbial characteristics is highly debated in the literature. The correlation between structural and operational and/or functional characteristics of such systems has sometimes been evidenced: the contaminant load influences the microbial community diversity (Bayle et al., 2009) and structure (Stamper et al., 2003; Fu et al., 2011), while microbial communities differing in composition can be functionally dissimilar (Gentile et al., 2007). Previous works also reported an effect of perturbation regime on population dynamics, associated with either more temporal instability (Stamper et al., 2003) or more stability (Lozada et al., 2007). In contrast, several authors concluded to the partial uncoupling of functional and community dynamics in water and soil ecosystems (Langenheder et al., 2005; Wertz et al., 2007), and the temporal fluctuations of microbial taxa diversity did not reflect functional variations in anaerobic digester (Fernandez et al., 2000) and membrane bioreactor (Stamper et al., 2003).

The present study also led to contrasted results. Under steady-state, the differentiation of bacterial community structures within

the inlet layers compared to the lower ones (Fig. 5) could be related to both higher substrate concentration and higher RE, as also observed in controls (Cabrol et al., 2012) and other biofilters (Khammar et al., 2005; Fu et al., 2011). The community specialisation can be the result of the specific environmental conditions and can also in turn influence the macroscopic function near the inlet. During the shocks, the biofilters exhibited a higher RE resistance in the inlet layer than in the lower ones for three compounds (butyraldehyde, MB and MV), as seen in Fig. 3. This functional distribution of resistance capacity can be linked to the stratification of bacterial community structures. The consistency between the structuration of inlet community and the functional stratification argues in favour of species competition mechanisms, with selection of a specialized community within the inlet layer (Juhler et al., 2009). Furthermore, the fact that both control and perturbed biofilters exhibited the same resistance and resilience capacity within the inlet layer (absence of a disturbance history effect, as seen in Figs. 3 and 4) could be related to the persistent absence of differentiation between the perturbed and control community structures in this layer (Fig. 6A).

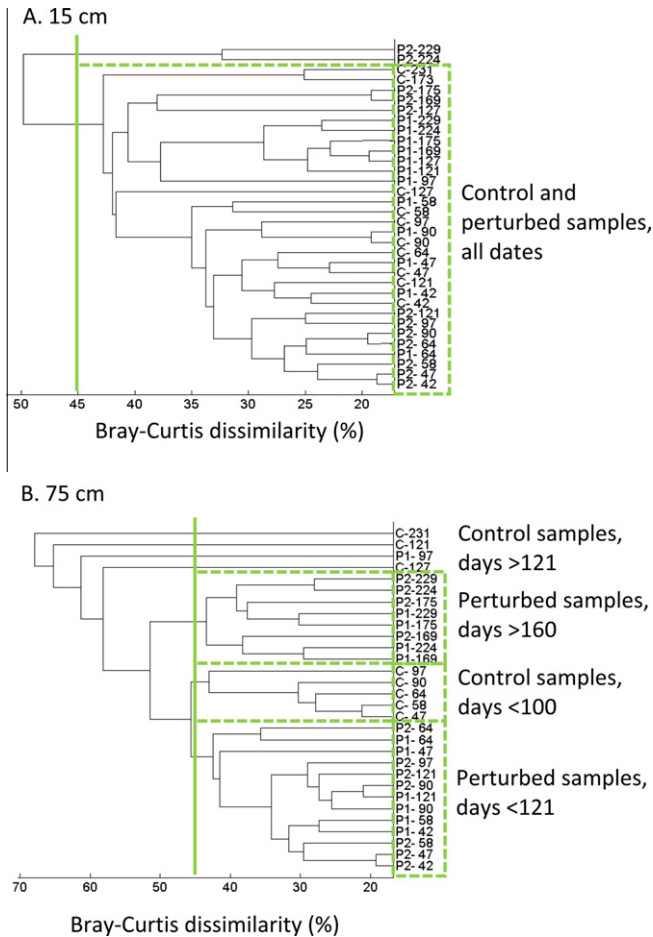
In contrast, although the bacterial community structures in the lower layers differed between perturbed and control biofilters (Fig. 6B), the resistance and resilience capacity of control and perturbed biofilters did not differ – except for ammonia – down the filters (Figs. 3 and 4). Moreover, for four compounds (acetone, MEK, ammonia and DMDS), the resistance capacity was similar in all biofilter layers, despite the longitudinal differentiation of bacterial community structure (Fig. 5). The absence of correlation between the functional and structural characteristics of biofilters could be due to the fact that the total bacterial community was targeted, which is composed not only of primary degraders but also of saprophytes not directly involved in contaminant removal (Hekmat et al., 2004). This approach may not be accurate enough to detect slight changes within a minor fraction of the community and should be improved by targeting specific functional groups or active population (Wittebolle et al., 2008). In addition, the absence of structure-function correlation likely points towards the hypothesis of competition between substrates (preferential consumption), relying on physiological regulations. In this case, the biofilter response to substrate pulses (ammonia excepted) could be explained

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2012.07.033>.

## References

- Bayle, S., Malhautier, L., Degrange, V., Godon, J.J., Fanlo, J.L., 2009. Structural and functional responses of sewage microbial communities used for the treatment of a complex mixture of volatile organic compounds (VOCs). *J. Appl. Microbiol.* 107, 85–96.
- Cabrol, L., Malhautier, L., Poly, F., Lepeuple, A.S., Fanlo, J.L., 2010. Assessing the bias linked to DNA recovery from biofiltration woodchips for microbial community investigation by fingerprinting. *Appl. Microbiol. Biotechnol.* 85, 779–790.
- Cabrol, L., Malhautier, L., 2011. Integrating microbial ecology in bioprocess understanding: the case of gas biofiltration. *Appl. Microbiol. Biotechnol.* 90 (3), 837–849.
- Cabrol, L., Malhautier, L., Poly, F., Lepeuple, A.S., Fanlo, J.L., 2012. Bacterial dynamics in steady-state biofilters: beyond functional stability. *FEMS Microbiol. Ecol.* 79 (1), 260–271.
- Carpenter, S., Walker, B., Anderies, J.M., Abel, N., 2001. From metaphor to measurement: resilience of what to what? *Ecosystems* 4, 765–781.
- Chesson, P., Gebauer, R.L.E., Schwinning, S., Huntly, N., Wiegand, K., Ernest, M.S.K., Sher, A., Novoplansky, A., Weltzin, J.F., 2004. Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia* 141, 236–253.
- Deshusses, M.A., Johnson, C.T., 2000. Development and validation of a simple protocol to rapidly determine the performance of biofilters for VOC treatment. *Environ. Sci. Technol.* 34, 461–467.
- Fernandez, A., Hashsham, S.A., Dollhopf, S.L., Raskin, L., Glagoleva, O., Dazzo, F.B., Hickey, R.F., Criddle, C., Tiedje, J.M., 2000. Flexible community structure correlates with stable community function in methanogenic bioreactor communities perturbed by glucose. *Appl. Environ. Microbiol.* 66, 4058–4067.
- Fu, Y., Shao, L., Tong, L., Liu, H., 2011. Ethylene removal efficiency and bacterial community diversity of a natural zeolite biofilter. *Bioresour. Technol.* 102, 576–584.
- Gentile, M., Jessup, C.M., Nyman, J.L., Criddle, C.S., 2007. Correlation of functional instability and community dynamics in denitrifying dispersed-growth reactors. *Appl. Environ. Microbiol.* 76, 680–690.
- Girvan, M.S., Campbell, C.D., Killham, K., Prosser, J.I., Glover, L.A., 2005. Bacterial diversity promotes community stability and functional resilience after perturbation. *Environ. Microbiol.* 7, 301–313.
- Graham, D.W., Smith, V.H., 2004. Designed ecosystem services: application of ecological principles in wastewater treatment engineering. *Front. Ecol. Environ.* 2, 199–206.
- Hashsham, S.A., Fernandez, A.S., Dollhopf, S.L., Dazzo, F.B., Hickey, R.F., Tiedje, J.M., Criddle, C.S., 2001. Parallel processing of substrate correlates with greater functional stability in methanogenic bioreactor communities perturbed by glucose. *Appl. Environ. Microbiol.* 66, 4050–4057.
- Hekmat, D., Feuchtinger, A., Stephan, M., Vortmeyer, D., 2004. Biofilm population dynamics in a trickle-bed bioreactor used for the biodegradation of aromatic hydrocarbons from waste gas under transient conditions. *Biodegrad* 15, 133–144.
- Ito, T., Miyaji, T., Nakagawa, T., Tomizuka, N., 2007. Degradation of dimethyl disulfide by *Pseudomonas fluorescens* strain 76. *Biosci. Biotechnol. Biochem.* 71, 366–370.
- Juhler, S., Revsbech, N.P., Schramm, A., Herrmann, M., Ottosen, L.D.M., Nielsen, L.P., 2009. Distribution and rate of microbial processes in an ammonia-loaded air filter biofilm. *Appl. Environ. Microbiol.* 75, 3705–3713.
- Khammar, N., Malhautier, L., Degrange, V., Lensi, R., Godon, J.J., Fanlo, J.L., 2005. Link between spatial structure of bacterial communities and degradation of a complex mixture of volatile organic compounds in peat biofilters. *J. Appl. Microbiol.* 98, 476–490.
- Kraakman, N.J.R., 2003. Robustness of a full-scale biological system treating industrial CS<sub>2</sub> emissions. *Environ. Prog.* 22, 79–85.
- Langenheder, S., Lindström, E.S., Tranvik, L.J., 2005. Weak coupling between community composition and functioning of aquatic bacteria. *Limnol. Oceanogr.* 50, 957–967.
- Lee, E.H., Cho, K.S., 2009. Effect of substrate interaction on the degradation of methyl tert-butyl ether, benzene, toluene, ethylbenzene and xylene by *Rhodococcus* sp.. *J. Hazard Mater.* 167, 669–674.
- Loisel, P., Harmand, J., Zemb, O., Latrille, E., Lobry, C., Delgenès, J.P., Godon, J.J., 2006. Denaturing gradient electrophoresis (DGE) and single-strand conformation polymorphism (SSCP) molecular fingerprintings revisited by simulation and used as a tool to measure microbial diversity. *Environ. Microbiol.* 8 (4), 720–731.
- Lozada, M., Basile, L., Erijman, L., 2007. Impact of non-ionic surfactant on the long-term development of lab-scale activated sludge bacterial communities. *Res. Microbiol.* 158, 712–717.
- Mohseni, M., Grant Allen, D., 2000. Biofiltration of mixtures of hydrophilic and hydrophobic volatile organic compounds. *Chem. Eng. Sci.* 55, 1545–1558.



**Fig. 6.** Dendrograms based on 16S DGGE profiles of the bacterial communities sampled in perturbed biofilters P1 and P2 and control biofilter C1, at (A) 15 cm and (B) 75 cm from the gas inlet. The sampling day is indicated just after the name of biofilter (e.g. P2-42 corresponds to sampling in the perturbed biofilter P2 at day 42). The green line indicates the 45%-dissimilarity threshold. The hatched boxes indicate the different clusters separated at 45%-threshold (with the exceptions of P2-224 and P2-229 samples in Fig. (A), and P1-97 sample in Fig. (B)).

by physiological responses, rather than changes in community composition.

## 4. Conclusion

The biofilters resistance and resilience capacities and memory effect depended on the contaminant considered. The total community structure was similar in the most functionally active layer, independently of the disturbance history. The structure-function relationships depended on the contaminant considered: the longitudinal distribution of resistance and bacterial composition were tightly coupled for three compounds, and uncoupled for the other four compounds. The total community structure was not sufficient to explain the functional response to repeated substrate pulses and specific behaviors emerged depending on the compounds, thus making necessary to target specific functional populations for a better understanding of structure-function relationships.

## Acknowledgements

The project funding and the PhD Grant for Léa Cabrol (CIFRE 2006/497) were supplied by Veolia Environnement Recherche et Innovation (VERI), Maisons-Laffitte, France. The authors would like to thank Janick Rocher from the Ecole des Mines d'Alès for technical collaboration.

- Muñoz, R., Díaz, L.F., Bordel, S., Villaverde, S., 2007. Inhibitory effects of catechol accumulation on benzene biodegradation in *Pseudomonas putida* F1 cultures. *Chemosphere* 68, 244–252.
- Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59, 695–700.
- Muyzer, G., Smalla, K., 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhoek* 73 (1), 127–141.
- Pimm, S.L., 1984. The complexity and stability of ecosystems. *Nature* 307, 321–326.
- Ranjard, L., Nowak, V., Echairi, A., Faloya, V., Chaussod, R., 2008. The dynamics of soil bacterial community structure in response to yearly repeated agricultural copper treatments. *Res. Microbiol.* 159, 251–254.
- Santos, P.M., Blatny, J.M., Di Bartolo, I., Valla, S., Zennaro, E., 2000. Physiological analysis of the expression of the styrene degradation gene cluster in *Pseudomonas fluorescens* ST. *Appl. Environ. Microbiol.* 66, 1305–1310.
- Sempere, F., Gabaldón, C., Martínez-Soria, V., Marzal, P., Peña-Roja, J.M., Álvarez-Hornos, F.J., 2008. Performance evaluation of a biotrickling filter treating a mixture of oxygenated VOCs during intermittent loading. *Chemosphere* 73, 1533–1539.
- Smet, E., Van Langenhove, H., Maes, K., 2000. Abatement of high concentrated ammonia loaded waste gases in compost biofilters. *Water Air Soil Poll.* 119, 177–190.
- Stamper, D.M., Walch, M., Jacobs, R.N., 2003. Bacterial population changes in a membrane bioreactor for graywater treatment monitored by denaturing gradient gel electrophoresis analysis of 16S rRNA gene fragments. *Appl. Environ. Microbiol.* 69, 852–860.
- Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Guillaumaud, N., Le Roux, X., 2007. Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. *Environ. Microbiol.* 9, 2211–2219.
- Wittebolle, L., Vervaeren, H., Verstraete, W., Boon, N., 2008. Quantifying community dynamics of nitrifiers in functionally stable reactors. *Appl. Environ. Microbiol.* 74, 286–293.
- Yang, L.H., Bastow, J.L., Spence, K.O., Wright, A.N., 2008. What can we learn from resource pulses? *Ecology* 89, 621–634.