environmental microbiology reports

Environmental Microbiology Reports (2014) **6**(2), 173–183 doi:10.1111/1758-2229.12126

Stability of soil microbial structure and activity depends on microbial diversity

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Summary

Despite the central role of microbes in soil processes, empirical evidence concerning the effect of their diversity on soil stability remains controversial. Here, we addressed the ecological insurance hypothesis by examining the stability of microbial communities along a gradient of soil microbial diversity in response to mercury pollution and heat stress. Diversity was manipulated by dilution extinction approach. Structural and functional stabilities of microbial communities were assessed from patterns of genetic structure and soil respiration after the stress. Dilution led to the establishment of a consistent diversity gradient, as revealed by 454 sequencing of ribosomal genes. Diversity stability was enhanced in species-rich communities whatever the stress whereas functional stability was improved with increasing diversity after heat stress, but not after mercury pollution. This discrepancy implies that the relevance of ecological insurance for soil microbial communities might depend on the type of stress. Our results also suggest that the significance of microbial diversity for soil functional stability might increase with available soil resources. This could have strong repercussions in the current 'global changes' context because it suggests that the combined increased frequencies of extreme climatic events, nutrient loading and biotic exploitation may amplify the functional consequences of diversity decrease.

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Introduction

Human activities and climatic changes are leading to a rapid and significant reduction of biodiversity, referred to as 'the sixth extinction' (Vitousek *et al*., 1997; Barnosky *et al*., 2011). As a consequence, understanding the effect of diversity on ecosystem functioning and stability is now a central issue in ecological sciences (Chapin *et al*., 2000; Cardinale *et al*., 2012). Most significant progress to date has been achieved through studies targeting above-ground organisms, particularly plants. Such studies revealed a greater productivity and stability of species-rich communities (Balvanera *et al*., 2006; Campbell *et al*., 2011; Cardinale *et al*., 2012; Hooper *et al*., 2012).

In contrast with plants, the diversity-functioning relationship remains largely unexplored for soil microorganisms (Balvanera *et al*., 2006; Reed and Martiny, 2007). As a consequence, the significance of microbial diversity for soil ecosystem functioning and stability remains controversial. Some studies have suggested no effect (Degens, 1998; Griffiths *et al*., 2001a; Wertz *et al*., 2006; 2007), while others have shown that the resistance and resilience of microbial processes central to carbon cycling, following disturbance, may be increased with increasing microbial diversity (Griffiths *et al*., 2000; 2001b; Degens *et al*., 2001; Girvan *et al*., 2005; Tobor-kaplon *et al*., 2005). Such controversial conclusions might result from the high functional redundancy of soil microbial communities, i.e. most soil processes are carried out by large pools of microbial species (Loreau, 2004). Consequently, biodiversity may buffer the functional shifts induced by environmental variations, hence providing a kind of ecological insurance for ecosystems (Yachi and Loreau, 1999). This hypothesis may be even more significant for microbial communities given their huge diversity. However, to date, it has not been fully validated experimentally. The few studies that have addressed this important question used different approaches to manipulate microbial diversity, but did not provide a true quantification of the resulting diversity erosion (Griffiths *et al*., 2001a; Wertz *et al*., 2006; 2007). Nor did they include parallel assessments of the structural and functional responses of microbial communities following disturbance. As a consequence, the extent to which a decrease in microbial diversity might affect the maintenance of both diversity and functioning following disturbance remains an open

question. Given the importance of microbial communities for soil ecosystem functioning, studies providing such evidence should be highly relevant to predict how much the current impact of anthropic activities and climate changes on soil microbial diversity is likely to affect soil services.

In this study, we hypothesized that: (i) the structural and functional stability of soil microbial communities would increase with increasing microbial diversity and (ii) the significance of diversity for community stability would depend on the nature of the applied disturbance. We therefore examined the impact of a decrease in soil microbial diversity on the stability (resistance and resilience) of the genetic structure and activity of microbial communities following two types of disturbance: persistent heavy metal pollution and a transient heat shock. A removal approach, involving the inoculation of sterile soil microcosms with different dilutions of a soil microbial suspension, was used to manipulate microbial diversity (Griffiths *et al*., 2004; Wertz *et al*., 2006; 2007; Baumann *et al*., 2012; Philippot *et al*., 2013) because it minimized the effects of environmental factors and focused on the microbial diversity. Briefly, soil was collected from the top 10 cm of a Cambisol near Lusignan [France: 46°25′12.91″N; 0°07′29.35″E, Long-term observatory for environmental research (ORE-ACBB, http://www.soere-acbb.com/index.php/fr/)], sieved to < 2 mm, divided into microcosms consisting in 20 g of dry weight soil placed in 150 ml hermetically sealed plasma flasks and then sterilized by γ-radiation (35 KGy; Conservatome, Dagneux, France). Sterile soil microcosms were inoculated with suspensions of the same soil, but not sterilized, as described previously (Baumann *et al*., 2012; Philippot *et al*., 2013). An initial soil suspension was prepared by mixing 100 g of soil (equivalent dry mass) with 300 ml of sterile distilled water using a Waring blender (Conair Corporation, Stamford, USA). After blending for 5 min at maximum speed, the soil suspension was serial diluted. Three levels of dilution of the soil suspension were used as inocula to create a gradient of diversity, that is, undiluted (10^0; D1), $1/10^3$ dilution (D3) and $1/10^5$ dilution (D5). For inoculation, 5.2 ml of each inocula have been poured on the soil. Because density of the non-sterile soil was 2.10^8 cells per gram of dry soil [determined by UFC (colony-forming unit) counting, data not shown], density of inoculated cells were around 3.5×10^8, 3.5×10^5 and 3.5×10^3 UFC per microcosm for D0, D3 and D5 respectively. After inoculation, the microcosms were pre-incubated for 6 weeks at 20°C to allow colonization and stabilization of the inoculated communities in terms of density and structure. During pre-incubation time, microcosms were aerated every week by flushing with air and soil water content was maintained constant. The microcosms, each containing a different diversity level, were then exposed to: (i) heat shock, (ii) mercury pollution and (iii) left undisturbed (controls). For heat stress, the microcosms were incubated at 50°C for 24 h. Mercury pollution consisted of spiking the soil with a solution of mercury (20 μg g^{-1} of dry soil). After treatment, the microcosms were maintained at 60% of the maximum water holding capacity (corresponding to 5.2 ml of H_2O for 20 g of soil) and incubated at 20°C for 80 days. In total, there were 216 microcosms (3 treatments × 3 levels of diversity × 8 dates of sacrifice × 3 replicates). After 0, 1, 3, 5, 10, 20, 40 and 80 days of incubation, triplicate microcosms for each treatment and diversity level were sacrificed for molecular analysis. At T0, the magnitude of microbial diversity loss (bacterial and fungal) was quantified by high-throughput pyrosequencing of ribosomal genes [16S- and 18S-ribosomal DNA (rDNA) for bacteria and fungi respectively]. Microbial biomass was followed in each treatment and diversity level through quantification of DNA extracted from soil. Marstop and colleagues (2000) and Widmer and colleagues (2006) indeed demonstrated a high correlation between soil DNA content and microbial biomass estimated by chloroform fumigation, and they concluded that these two methods were equivalent to measuring soil microbial biomass. The response of microbial communities to the applied stress was investigated by monitoring genetic structure by Automated Ribosomal Intergenic Spacer Analysis (ARISA) fingerprinting and soil respiration over time.

Results

Microbial density

Similar abundances of bacterial and fungal communities were observed at T0, whatever the diversity level (Table 1). Thus, the 16S- and 18S-rDNA gene copy numbers per gram of dry soil were on average 1.4×10^{11} and 1.1×10^9 gene copies g^{-1} in all microcosms. Microbial molecular biomass at T0 also showed similar values between the three diversity levels (Fig. 1). After application of the stress, no difference of biomass was observed according to the diversity level whatever the time of incubation or the treatment considered. During the incubation, microbial biomass in the mercury treatment was similar to control, whereas values tended to be lower in the heat treatment.

Microbial diversity at T0

Totals of 181 448 and 225 339 sequences of 16S- and 18S-rDNA genes, respectively, were generated from the nine samples by pyrosequencing. For bacterial communities, the average Operational Taxonomic Units (OTU) richness ranged from 889 (D0) to 606 (D3) and 248 (D5), evidencing a significant decrease in species OTU

Table 1. Diversity and density parameters of the microbial communities (bacteria and fungi) for each level of diversity before the application of stress (T0) values are averages from three replicates for the index of diversity and nine replicates for density ± standard deviation.

Communities	Level of diversity	Sequences after equalization and filtering steps	Index of diversity			Density
			Richness[a]	Shannon[b]	Evenness[c]	Log genes copies g^{-1} of dry soil
Bacterial (16S rDNA)	D0	8885	889 ± 139.1 (a)	4.49 ± 0.19 (a)	0.66 ± 0.01 (a)	$1.20 \times 10^{11} \pm 9.61 \times 10^{10}$ (a)
	D3	7487	606 ± 44.8 (ab)	4.10 ± 0.06 (ab)	0.64 ± 0.02 (ab)	$1.54 \times 10^{11} \pm 5.59 \times 10^{10}$ (a)
	D5	8066	248 ± 15.3 (b)	1.98 ± 0.13 (b)	0.36 ± 0.02 (b)	$1.50 \times 10^{11} \pm 7.17 \times 10^{10}$ (a)
Fungal (18S rDNA)	D0	15573	480 ± 17.4 (a)	3.25 ± 0.02 (a)	0.53 ± 0.00 (a)	$9.11 \times 10^{8} \pm 2.38 \times 10^{8}$ (a)
	D3	16198	327 ± 50.8 (a)	2.07 ± 0.69 (a)	0.36 ± 0.11 (a)	$2.16 \times 10^{9} \pm 2.18 \times 10^{9}$ (a)
	D5	14942	316 ± 93.5 (a)	2.55 ± 0.83 (a)	0.44 ± 0.12 (a)	$1.79 \times 10^{8} \pm 1.44 \times 10^{8}$ (b)

a. Richness refers to the number of OTU defined at the genus level.
b. Shannon diversity index. A higher number represents more diversity.
c. Evenness is a measure of the relative abundance of the different species making up the richness.
For each level of diversity, values with different letters differ significantly ($P < 0.05$).

richness ($P < 0.05$) with increasing dilution (Table 1). Shannon and evenness indexes showed the same trend with a considerable decrease for the low diversity treatment D5 (1.98) compared with D0 (4.49) and D3 (4.10). Concerning fungal diversity, average species richness tended to decrease according to the dilution [average OTU number ranged from 480 (D0) to 327 (D3) and 316 (D5)], but differences between the three diversity levels were not significant (Table 1). In the same way, the Shannon index also indicated a reduction of diversity in the diluted treatments (D3 and D5) compared with the undiluted soil D0. Index values were in agreement with rarefaction curves built from pyrosequencing data, which also highlighted a decrease of diversity along the gradient D0 > D3 > D5 (Fig. S1). The analysis of taxonomic composition evidenced significant differences between the three diversity levels (Fig. S2), mainly due to an increase in the relative abundance of *Proteobacteria* and *Ascomycota* groups with increasing dilution ($P < 0.05$) (Fig. 2).

Response of bacterial community structure to heat and mercury stress

Heat shock impacted bacterial community structure whatever the diversity level, as evidenced by the Euclidian distances calculated at T1, corresponding to the end of the 24 h stress application (Fig. 3A). However, the amplitude of the modifications depended on the diversity level, with major changes observed when diversity was low ($P < 0.05$). Two days after heat stress (i.e. T3), the bacterial genetic structure showed resilience at all three diversity levels, which decreased as the dilution increased (Fig. 3A). After heat shock and up to 40 days incubation, two patterns of community response could be distinguished between the lowest diversity D5 treatment, which displayed significantly higher modifications than the D0 and D3 treatments, which were more resilient and not significantly different from each other. At the end of incubation, the genetic structure of the D0 bacterial communities was much closer to the corresponding

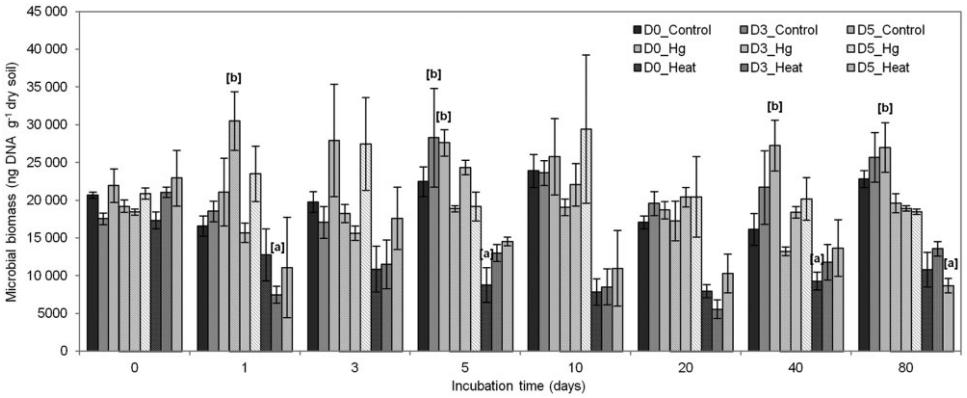

Fig. 1. Soil microbial biomass (ng DNA g^{-1} of dry soil) for each treatment and level of diversity during the incubation. Letters in bracket indicate a significantly different biomass (Kruskal–Wallis test; $P < 0.05$). Error bars denote standard deviation of biological replicates ($n = 3$).

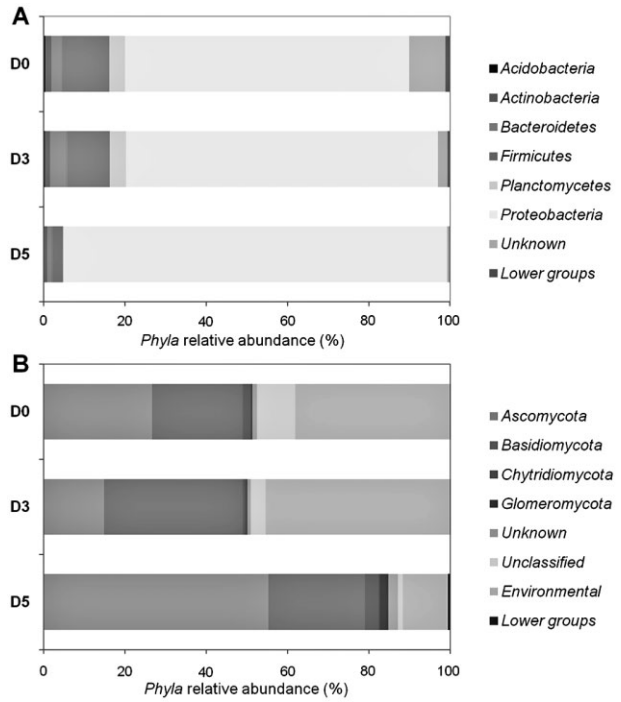

Fig. 2. Relative abundances of both (A) bacterial and (B) fungal *phyla* of microbial communtity composition for each level of diversity at T0. Lower groups represent the phylogenetic groups (*phyla*) with a relative abundance < 1%.

control and consequently more resilient than the other diversity levels (D3 and D5).

Spiking soil with mercury induced immediate changes in the genetic structure of bacterial communities in the D3 and D5 treatments (Fig. 3B), but not in D0. At T1, there was a clear gradient of community modifications (D5 > D3 > D0) (Fig. 3B). Two distinct patterns of community response to heavy metal stress were then apparent depending on the level of diversity. The first pattern, represented by D0, was characterized by a resistant phase up to 10 days of incubation (i.e. no change in community structure) followed by a second phase during which gradual changes occurred in the D0 bacterial structure, leading to a very different genetic structure from the control at the end of incubation. The second pattern, observed in the D3 and D5 treatments, was characterized by large fluctuations in community changes throughout incubation, the magnitude of the changes increasing as the diversity decreased.

Response of fungal community structure to heat and mercury stress

Heat shock resulted in large and early changes in the structure of fungal communities in D3 and D5, but not in D0 (Fig. 4A), as evidenced by the Euclidian distances

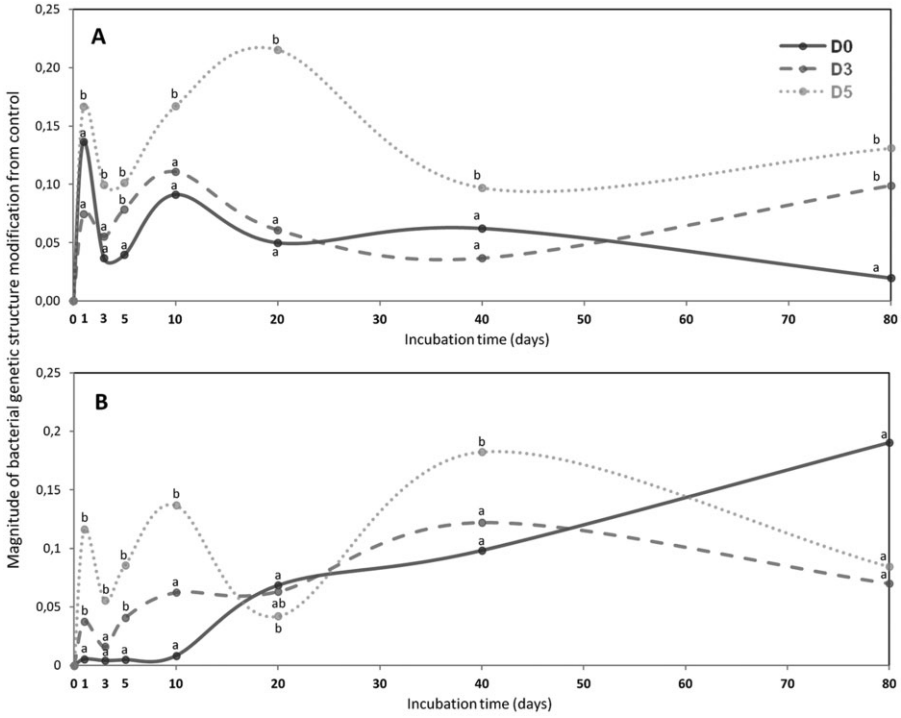

Fig. 3. Magnitude of the bacterial genetic structure modifications during 80 days after (A) heat stress and (B) mercury pollution for the three levels of diversity; D0 (black full line), D3 (grey dotted line) and D5 (light grey dashed line). Magnitude was determined by calculating the Euclidean distance between disturbed and control microcosms for each incubation time. Notation indicates a significantly different response in terms of genetic structure change between the three levels of diversity (Kruska–Wallis test; $P < 0.05$).

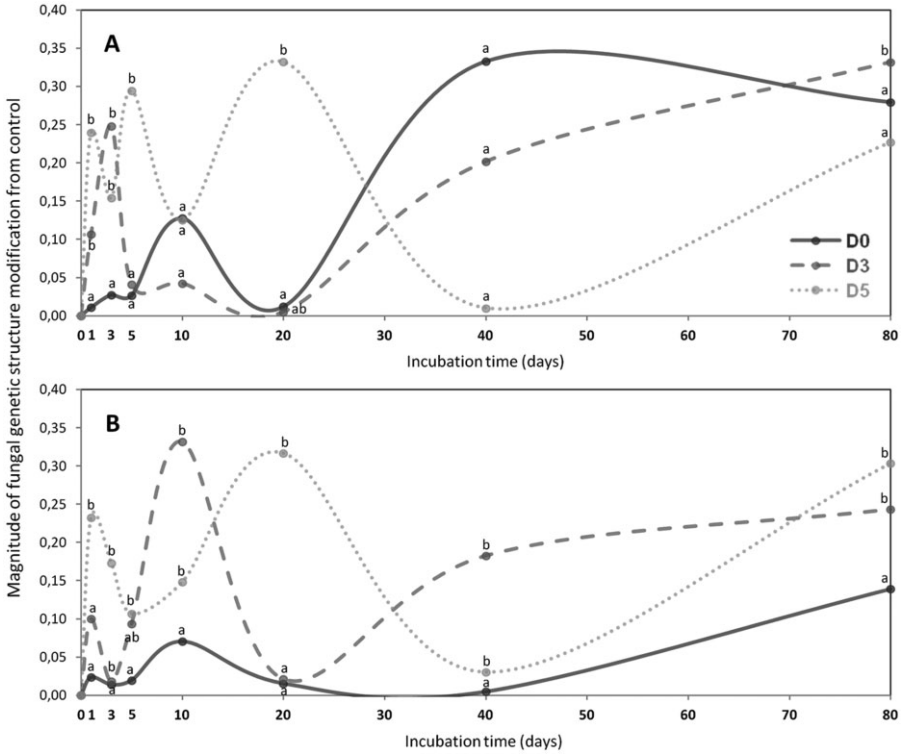

Fig. 4. Magnitude of the fungal genetic structure modifications during 80 days after (A) heat stress and (B) mercury pollution for the three levels of diversity; D0 (black full line), D3 (grey dotted line) and D5 (light grey dashed line). Magnitude was determined by calculating the Euclidean distance between disturbed and control microcosms for each incubation time. Notation indicates a significantly different response in terms of genetic structure change between the three levels of diversity (Kruskal–Wallis test; $P < 0.05$).

calculated at T1 (corresponding to the end of the 24 h stress application). The magnitude of the community modifications was dependent on the level of diversity and exhibited the following gradient D0 < D3 < D5 ($P < 0.05$). After heating, the short-term community response was also dependent on the diversity level, with no change occurring in D0 up to 4 days after heat stress (i.e. T5, Fig. 4A), whereas large changes were observed in D3 and D5 during the same time. Considerable modifications occurred after this short-term response regardless of the diversity level. However, two patterns could be distinguished, with D0 and D3 following similar dynamics, different to D5. At the end of incubation, the structure of the fungal communities was very different from the control microcosms for all three levels of diversity.

Spiking soil with mercury induced large early changes (after 1 day) in the structure of fungal communities in D3 and D5 (Fig. 4B), but not in D0 where diversity was high. As for the bacterial communities, two patterns could be distinguished between: (i) D3 and D5, which displayed large fluctuations in genetic structure throughout the incubation; and (ii) the high diversity treatment D0 that showed slight changes during the same period.

Response of soil respiration to heat and mercury stress

Soil respiration in the control microcosms did not differ significantly between the three diversity levels at any time during incubation ($P = 0.497$). At the end of incubation, the amount of CO_2 released ranged from 322 ± 6 for D5 to 343 ± 9 μg CO_2 g^{-1} of dry soil for D0.

A strong increase in soil respiration occurred during the heat stress, whatever the level of diversity, the intensity decreasing with the decrease of diversity (Fig. 5A) ($P < 0.01$). After heat stress, respiration decreased significantly ($P < 0.01$) at all three diversity levels, to reach significantly lower values than the respective controls up to 60 days of incubation. D5 was significantly more impacted than D0 and D3 up to 50 days of incubation ($P < 0.05$), as evidenced by the higher delta values recorded (Fig. 5B). From T50 until the end of the incubation, resilience of soil respiration was observed for the three levels of diversity. At the end of incubation, the amount of CO_2 released by the heat-stressed microcosms was significantly higher in D0 and D3 (259 ± 15, and 257 ± 3 μg CO_2 g^{-1} of dry soil) than in D5 (218 ± 29 μg CO_2 g^{-1} of dry soil) ($P < 0.05$).

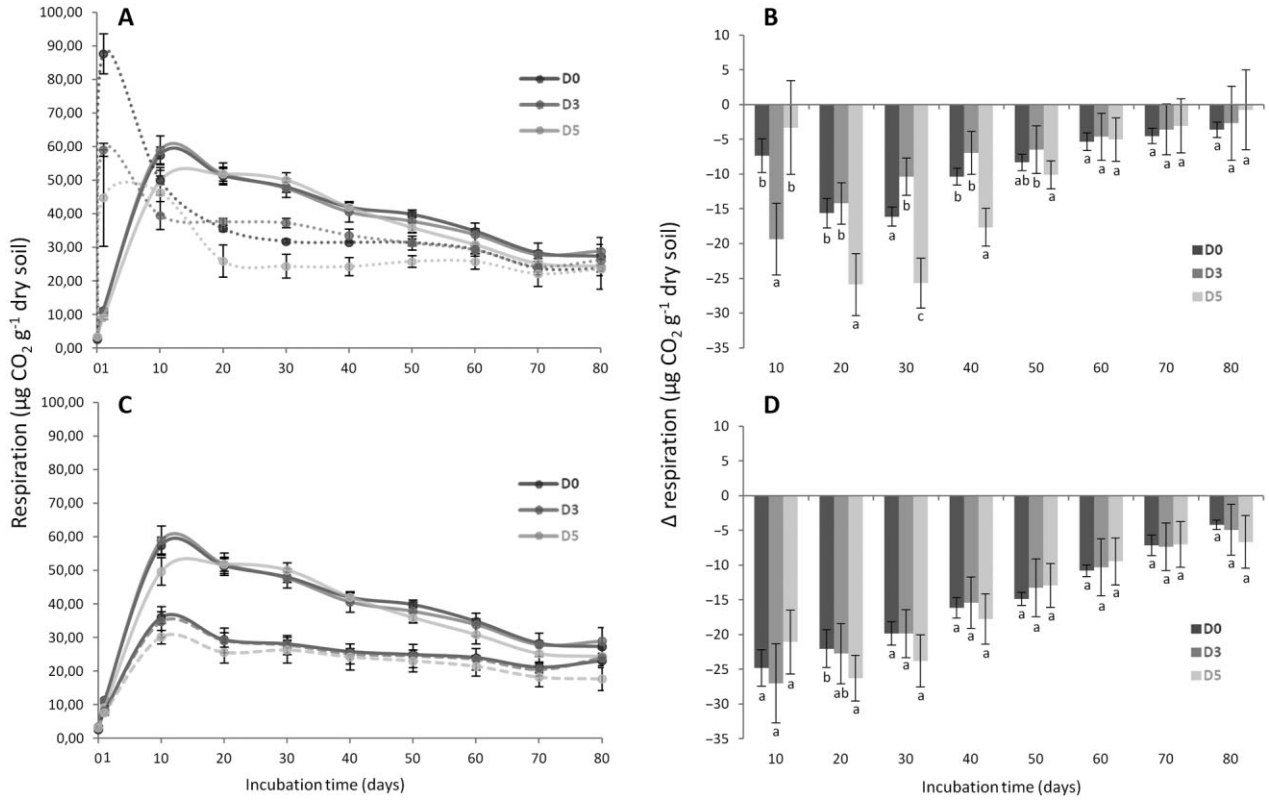

Fig. 5. Soil respiration (µg CO_2 g^{-1} of dry soil) over 80 days after (A) heat stress and (C) mercury pollution for the three levels of diversity with D0 (black), D3 (Grey) and D5 (light grey). Full lines represent control microcosms and dashed lines represent perturbed microcosms (mercury or heat stress). The difference between respiration (Δ µg CO_2 g^{-1} of dry soil) of control and disturbed microcosms after (B) heat stress and (D) mercury pollution are shown for the three levels of diversity (D0: black, D3: grey and D5: light grey). For each incubation time and level of diversity, values with different letters differ significantly ($P < 0.05$).

Spiking soil with mercury resulted in a significant decrease in soil respiration ($P < 0.01$) for all diversity levels compared to the control microcosms (Fig. 5C). At the end of incubation, the average amount of CO_2 released in the mercury-polluted treatments (213 ± 21 µg CO_2 g^{-1} of dry soil) was significantly lower ($P < 0.05$) than in the control treatments (336 ± 17 µg CO_2 g^{-1} of dry soil). However, the impact of mercury on soil respiration did not vary according to the level of diversity (Fig. 5D). As for heat stress, a resilience of soil respiration was observed at the end of incubation whatever the diversity level.

Discussion

The aim of our study was to see how microbial diversity might relate to the functional and structural stability of soil communities following stress application. To this end, our first challenge was to create a diversity gradient, i.e. prepare different diversity treatments consisting of microcosms of the same soil accommodating the same microbial abundance, but different communities in terms of taxonomic richness and composition. For this, we used a dilution to extinction approach that has already proved successful (Wertz *et al*., 2006; Baumann *et al*., 2012; Philippot *et al*., 2013). To obtain the same density in all three diversity levels, we relied on the fact that following the inoculation into a sterile soil, microbial communities are known to develop until reaching the soil carrying capacity (Wertz *et al*., 2006; Baumann *et al*., 2012). At T0 (i.e. end of the 6 weeks pre-incubation period), the bacterial and fungal densities as well as the microbial biomass did not differ between the three diversity treatments. In addition, whatever the diversity level microbial biomass in control treatment did not differ significantly with time, indicating that the 6 weeks of pre-incubation truly allowed reaching the soil carrying capacity. For each treatment, no difference of biomass according to the diversity level was observed whatever the incubation time. We were therefore able to exclude that any further observed decrease in respiration rates with dilution would be due to a lower community biomass.

Dilution and pre-incubation led to the establishment of communities with contrasting structure and diversity, as

evidenced by the ARISA and pyrosequencing results obtained at T0. These changes might reflect a loss of microbial populations because of the actual dilution process, as suggested by the simplified ARISA profiles (data not shown) and the overall decrease in diversity index observed along the diversity gradient. However, our results also evidenced that the composition of the communities in the inoculated microcosms did not simply result from the arithmetic loss of microbial populations due to dilution (Franklin *et al*., 2001). In particular, *Acidobacteria*, which represented 10% of the indigenous community (data not shown), were not retrieved in any of the diversity treatments whereas the abundance of *Proteobacteria*, which represented 47% of the indigenous community, increased along the diversity gradient to attain up to 95% of the sequences in D5. *Acidobacteria* are cosmopolitan soil bacteria (Barns *et al*., 1999; Youssef and Elshahed, 2009) described as slow-growing oligotrophs (Cleveland *et al*., 2007; Davis *et al*., 2011). Consequently, these organisms might be mainly k-strategists, and hence harbour poor competitiveness and colonization ability, which could explain their absence in the inoculated microcosms. Contrastingly, *Proteobacteria* are described as fast-growing copiotrophs (Aneja *et al*., 2006; Bernard *et al*., 2007; Jenkins *et al*., 2010; Pascault *et al*., 2013). As such, they would be mainly r-strategists, harbouring strong competitiveness and colonization abilities, which could explain their preferential selection in the inoculated microcosms with increasing dilution.

Altogether these observations indicated that the differences in community composition at each diversity level resulted, in addition to the loss of populations with dilution, from the selection of r-strategist populations against the slower-growing k-strategist organisms during soil recolonization. It is noticeable that, because of the dilution to extinction approach used, this selection has probably also been increased by the difference of microbial density among the three suspensions used for inoculation (D1 > D2 > D3). In our study, it is likely that this could have conferred communities with a higher reactivity to the applied stresses.

Diversity affects population dynamics and functional response of microbial communities to heat shock

During the heat shock, soil respiration increased significantly whatever the diversity level, in agreement with other studies (Bárcenas-Moreno and Bååth, 2009; Bérard *et al*., 2011; Hamdi *et al*., 2011). Higher C substrate availability corresponding to the recycling of cells killed by heating (Bérard *et al*., 2011; Hamdi *et al*., 2011), and improved soil enzymes activities may both have contributed to this respiration increase (Davidson and Janssens, 2006). The shift in bacterial and fungal community structure observed at T1 might reflect the killing of part of the community by heating. In other respects, no change in microbial biomass due to heating was recorded, thereby excluding the hypothesis that the increased respiration as well as differences between diversity treatments could be ascribed to an increase in microbial biomass and/or diversity-dependent sensitivity of the microbial communities to temperature, leading to the release of different amounts of necromass. However, the stimulation of soil respiration increased with soil diversity (D1 > D3 > D5), highlighting that other mechanisms, driven by diversity, might also have been involved. For example, the complementarity function niche hypothesis (Salles *et al*., 2009) states that species-rich communities can use more of the overall available resource because different species will colonize different habitats and use slightly different resources (Bell *et al*., 2005). Facilitative interactions (i.e. decomposition products of one species acting as nutrient source for another species), which might be enhanced in a species-rich community, could also have contributed to the observed differences among the three diversity treatments (Bell *et al*., 2005).

After heat shock, soil respiration decreased and did not recover the level of the controls in any of the diversity treatments, up to 60 days of incubation. This lower respiration could be ascribed to nutrient depletion that occurred during the heating event due to increased microbial activity, hence leading to limiting conditions for the microbial communities (Kirschbaum, 2006; Lützow and Kögel-Knabner, 2009; Hamdi *et al*., 2011). In addition, the slight but not significant decrease of microbial biomass compared with control may also have contributed to the overall decrease of respiration observed. Interestingly, respiration in D5 remained lower than in D0 and D3, which no longer differed. This would indicate a greater resilience in species-rich communities. Again, the two diversity-driven mechanisms mentioned above might explain these results. Such mechanisms might confer, to species-rich communities, greater abilities to decompose the more recalcitrant C-substrates remaining after heating, in agreement with Baumann and colleagues (2012) who recently demonstrated that lignin decomposition in soil increases with microbial diversity. Interestingly, the higher functional resilience in D0 and D3 was also associated with similar and more resilient patterns in the structural response of bacterial and fungal communities, as compared with D5, which displayed higher fluctuations and consequently lower resistance and resilience abilities. This highlights the increased structural stability with increased diversity and suggests that microbial diversity exerts a buffering effect on changes in soil carbon mineralization induced by temperature stress, in agreement with the insurance hypothesis (Loreau *et al*., 2001).

In addition, our results evidence the existence of a diversity decrease threshold, between D3 and D5, which shifted the structural and functional community response to soil heat (Scheffer *et al*., 2001).

The buffering effect of microbial diversity seems to be time limited because, after incubation for 60 days, D5 finally reached the same level as in D0 and D3. It is likely that this functional resilience, which occurred after 60 days and uniformized the three diversity levels, does not involve diversity-driven mechanisms. We suggest that it might instead be ascribed to nutrient depletion during the incubation, which also occurred in the controls, and therefore led to similar limiting conditions in both control and stressed treatments. Such depletion of available substrates indeed is classically reported under laboratory conditions (Kirschbaum, 2006; Hamdi *et al*., 2011). This hypothesis could explain why there was no difference in respiration rate between D0 and D3 after heating, probably because of greater nutrient depletion during heat stress (Kirschbaum, 2006). A similar shift in the functional significance of diversity in relation to soil functioning by soil resource level has also been recently reported for processes and communities associated with denitrification in the nitrogen cycle (Philippot *et al*., 2013). In other respects, availability of readily decomposable substrates has also been demonstrated to determine the sensibility of soil respiration to temperature increase (Kirschbaum, 2006; Hamdi *et al*., 2011). In this context, our results suggest that soil nutrient availability might be an important parameter regulating the significance of microbial diversity in the response of soil functioning to heat stress.

Diversity affects population dynamics but not the functional response of microbial communities to heavy metal pollution

Spiking soil with mercury led to a significant decrease of soil carbon mineralization, in agreement with other studies (Müller *et al*., 2002; Harris-Hellal *et al*., 2009). Mercury is a highly toxic pollutant that binds to soil organic matter and therefore has a close relationship with this component (Ranjard *et al*., 1997; Grigal, 2003; Akerblom *et al*., 2010). As a result, mercury might represent a kind of chemical protection, reducing the accessibility of soil organic matter to microbial decomposition. In addition, mercury has been shown to reduce the enzymatic activities involved in carbon mineralization (Harris-Hellal *et al*., 2009). Amplitude of the respiration decrease did not vary with microbial diversity. This is not in agreement with other studies that demonstrated a positive relationship between microbial diversity and the resistance of soil respiration following residual stress caused by heavy metals such as copper (Griffiths *et al*., 2000; 2001a; Degens *et al*., 2001; Girvan *et al*., 2005) or lead (Tobor-kaplon *et al*., 2005). However, in these studies, the pollutants were added together with organic matter such as plant residues. It is therefore likely that the discrepancy between our results and those in the literature can be attributed to the nutrient availability in soil, which, in these studies, was high whereas no C-substrate was added in our experiment. This hypothesis is in agreement with the functional response to heat stress discussed above and suggests that the effect of microbial diversity on the response of soil functioning to heavy metal pollution is dependent on soil nutrient availability.

In contrast with the functional data, microbial diversity strongly influenced the structural response of bacterial and fungal communities to mercury. This discrepancy between functional and structural response emphasized the functional redundancy of soil microbial communities for carbon transformations in soil (Maron *et al*., 2011). Similarly, Müller and colleagues (2001) reported that long-term exposure to constant mercury stress shaped microbial diversity, but had only a slight effect on the functional potential of the community measured in terms of sole carbon source utilization. As observed for heat stress, a threshold of diversity decrease was observed, occurring right from the first dilution, which shifted the structural response of microbial communities to mercury pollution (Scheffer *et al*., 2001). Again, structural stability was increased in species-rich communities, with a lag phase in the community response to mercury occurring only in D0 (up to 10 and 5 days for bacterial and fungal communities, respectively) whereas D3 and D5 displayed highly fluctuating dynamics throughout the incubation. The observed threshold, which was lower than in the heat treatment, might be due to the fact that mercury resistant/tolerant microorganisms are naturally very scarce in soil (Ranjard *et al*., 1997; Rasmussen and Sørensen, 2001). Consequently, it is likely that these populations were lost as soon as the community was diluted. This is in agreement with the appearance of much more intense bands on the B-ARISA profiles (Fig. S3) that occurred only in D0 after 10 days incubation. These bands could be attributed to the development of populations that initially shared resistant/tolerant mercury traits or acquired them via mechanisms of gene transfer (Smit *et al*., 1998; Rasmussen and Sørensen, 2001).

In conclusion, our study shows that the functional and structural stability of microbial diversity increase with microbial diversity when soil undergoes disturbances, in agreement with the insurance hypothesis. Our results also suggest that a decrease of diversity might not be associated with gradual shifts in the functional and structural patterns of community response to heating and mercury pollution. Thus, thresholds of diversity loss were demonstrated, the levels being dependent on the type of

stress applied (mercury pollution < heating). Another important end-product of this study concerns the significance of diversity for the functional response of microbial communities to stress, this apparently being related to the amount of available soil resources. This latter finding could have important consequences in the context of global changes, which are assumed to lead to increased frequency of extreme climatic events such as heat waves, but also to enhanced primary productivity and consequently nutrient loading of the soil due to increased atmospheric CO_2 concentrations (Bardgett *et al*., 2008). Our results suggest that this could, in turn, amplify the functional significance of the decrease/modification of microbial diversity induced by human activities.

Acknowledgements

This work was supported by the Agence Nationale de Recherche (ANR) under the framework of the ANR Systerra project DIMIMOS (Link between microbial diversity and organic matter decomposition in soil), by a grant from the Regional Council of Burgundy, and by the European Commission within EcoFINDERS project (FP7–264465). This work benefited from the technical facilities of the GenoSol platform of the infrastructure ANAEE-Services [ANAlysis and Experimentations on Ecosystems, ANR programme 'Investments for the Future' (reference ANR-11-INBS-0001)], as well as of the ORE-ACBB (http://www.soere-acbb.com/index.php/fr/). Authors have no conflict of interest regarding to declare.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Rarefaction curves determined by pyrosequencing of the (A) 16S- and (B) 18S-rDNA genes obtained for three

replicates of each level of diversity, based on randomly selected and filtered sequences. Rarefaction curves were determined using clustering at 95% of similarity.

Fig. S2. Factorial map of: (A) bacterial and (B) fungal phylogenetic community composition (*phyla*) at T0. Monte Carlo tests were performed with 1000 permutations to confirm the significance of the discriminated clusters.

Fig. S3. Bacterial-ARISA profiles obtained after 10, 20, 40 and 80 days of incubation for the level of diversity D0. For each sampling date, three replicates of control soils (**Ct**) and soils spiked with mercury (**Hg**) were prepared. Arrows indicate significant modifications between profiles (e.g. common bands with different relative abundance).

Appendix S1. Experimental procedures.