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Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance

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Summary

Analysing the consequences of the decrease in biodiversity for ecosystem functioning and stability has been a major concern in ecology. However, the impact of decline in soil microbial diversity on ecosystem sustainability remains largely unknown. This has been assessed for decomposition, which is insured by a large proportion of the soil microbial community, but not for more specialized and less diverse microbial groups. We determined the impact of a decrease in soil microbial diversity on the stability (i.e. resistance and resilience following disturbance) of two more specialized bacterial functional groups: denitrifiers and nitrite oxidizers. Soil microbial diversity was reduced using serial dilutions of a suspension obtained from a non-sterile soil that led to loss of species with low cell abundance, inoculation of microcosms of the same sterile soil with these serial dilutions, and subsequent incubation to enable establishment of similar cell abundances between treatments. The structure, cell abundance and activity of denitrifying and nitrite-oxidizing communities were characterized after incubation. Increasing dilution led to a progressive decrease in community diversity as assessed by the number of denaturating gradient gel electrophoresis (DGGE) bands, while community functioning was not impaired when cell abundance recovered after incubation. The microcosms were then subjected to a model disturbance: heating to 42°C for 24 h. Abundance, structure and activity of each community were measured 3 h after completion of the disturbance to assess resistance, and after incubation of microcosms for 1 month to assess resilience. Resistance and resilience to the disturbance differed between the two communities, nitrite oxidizers being more affected. However, reducing the diversity of the two microbial functional groups did not impair either their resistance or their resilience following the disturbance. These results demonstrate the low sensitivity of the resistance and resilience of both microbial groups to diversity decline provided that cell abundance is similar between treatments.

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Introduction

Anthropogenic disturbances, such as land-use change and climate change, have led to an important decline in global biodiversity (Chapin *et al.*, 2000). The impact of biodiversity decrease on ecosystem functioning, and more particularly ecosystem stability following disturbance, have thus been evaluated in many studies (e.g. McNaughton, 1977; Tilman, 1996; van der Heijden *et al.*, 1998). Ecosystem stability following disturbance comprises both resistance (i.e. the ability of the system to withstand disturbance) and resilience (the ability of the system to recover after disturbance) (Holling, 1973; Grimm and Wissel, 1997). Biodiversity (usually considered as species richness) may have an important role in the stability of ecosystem functioning. Indeed, the presence of a large number of species that can respond differently to disturbances may provide insurance that minimizes the likelihood of large changes in ecosystem functioning after disturbances (Yachi and Loreau, 1999; Ives *et al.*, 2000; McCann, 2000).

Diversity–stability (D–S) relationships have been studied mainly in plant systems (e.g. McNaughton *et al.*, 1977; Tilman, 1996; Wardle *et al.*, 2000) and to a lesser extent in aquatic microbial communities (McGrady-Steed *et al.*, 1997; Naem and Li, 1997; Petchey *et al.*, 2002). However, the importance of the diversity of microbial

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Table 1. Abundances of culturable denitrifying and nitrite-oxidizing bacteria in sterile soil microcosms inoculated with a non-sterile soil suspension, giving final dilutions of 10^{-1}–10^{-10} of the non-sterile soil, after incubation for 8 months.

Dilution treatment	Denitrifiers	Nitrite oxidizers
10^{-1}	7.93×10^6 (1.64×10^6) a	2.21×10^4 (1.12×10^4) ab
10^{-3}	9.4×10^6 (2.52×10^6) a	6.94×10^3 (1.6×10^3) a
10^{-4}	6.17×10^6 (2.32×10^6) a	5.45×10^3 (1.51×10^3) a
10^{-5}	6.66×10^6 (1.82×10^6) a	3.16×10^2 (1.39×10^2) b
10^{-6}	5.79×10^6 (2.88×10^6) ab	–
10^{-8}	3.5×10^2 (3.50×10^2) b	–
10^{-10}	–	–

Means ($n = 5$) are presented with standard errors. For each community, values with different letters differ significantly ($P < 0.05$). –, cell number below detection limit.

communities for the stability of soil ecosystem functioning following disturbances remains largely unknown. Previous D–S studies for soil microorganisms have focused on the heterotrophic community and on general functions, such as soil respiration or litter decomposition (Degens, 1998; Griffiths *et al.*, 2000; 2001; Muller *et al.*, 2002). Because most microorganisms are heterotrophs and have a potential role in the many pathways involved in the breakdown of soil organic matter, functional redundancy is likely to be very high in that case. Equivalent D–S studies have not been carried out for soil ecosystem functions performed by more specialized microbial functional groups.

The aim of this study was therefore to determine the impact of decreasing soil microbial diversity on the resistance and resilience of two more specialized microbial functional groups, denitrifiers and nitrite oxidizers, following a model disturbance imposed on soil (soil heating). Presently characterized denitrifiers belong to approximately 50 genera (Zumft, 1997) so that this functionally specialized group is relatively diverse, while presently characterized nitrite oxidizers belong to only four genera (*Nitrobacter*, *Nitrospira*, *Nitrococcus* and *Nitrospina*) (Teske *et al.*, 1994), although the actual diversity of this group remains unknown. Soil biodiversity decrease was induced by a removal approach in which less abundant species were removed first, which is a realistic scenario of decrease in soil microbial diversity (Gans *et al.*, 2005). This was achieved by inoculation of sterile soil microcosms with serial dilutions of a suspension obtained from the same non-sterile soil and incubation to enable establishment of similar cell abundances between treatments. This approach was designed to cause a progressive loss of taxa, preferentially those with low abundances, in the inoculum (Franklin and Mills, 2006, Wertz *et al.*, 2006) and a progressive decrease in the diversity of denitrifying and nitrite-oxidizing communities. Resistance and resilience of denitrifying and nitrite-oxidizing communities with different initial diversity levels were quantified following soil heating, and results highlight the high level of functional redundancy of these soil microbial functional groups.

Results

Cell abundance, diversity and activity of each functional group before disturbance

After incubation for 8 months, the abundance of denitrifying bacteria was similar among the sterile soil microcosms inoculated with the non-sterile soil suspension giving final dilutions of 10^{-1}–10^{-6} of the original soil suspension (dilution treatments 10^{-1}–10^{-6}) (Table 1). Lower cell abundance was observed in microcosms inoculated with higher dilutions. The abundance of nitrite-oxidizing bacteria after incubation was similar in microcosms inoculated with dilutions 10^{-1}–10^{-4}, and was lower in microcosms inoculated with higher dilutions (Table 1).

For treatments with similar cell abundance after incubation for 8 months, the diversity of each functional group, assessed as the number of denaturating gradient gel electrophoresis (DGGE) bands (Fig. 1) and from the Shannon index computed from the DGGE data matrix (Fig. S1), decreased progressively from microcosms inoculated with low soil dilutions to those inoculated with high dilutions. In addition, the structure of each community was highly variable between replicates of a given dilution treatment (dissimilarity between replicates around 60%, data not shown). Denitrification activity did not differ significantly between dilution treatments (i.e. treatments 10^{-1}–10^{-6}) for which the denitrifier abundance was similar. Similarly, nitrite oxidation did not vary significantly between the dilution treatments 10^{-1}–10^{-4} (Fig. 1). For each functional group, analyses of resistance and resilience following heat disturbance were restricted to the dilution treatments leading to similar cell abundances after incubation.

Resistance and resilience of the denitrifying community following heat disturbance

The effect of disturbance on denitrification, measured 3 h after heating, was significant, with no dilution*disturbance interaction effect. Denitrification in disturbed soils was reduced to approximately 60% of the activity in the control, non-disturbed soils independently of dilution treat-

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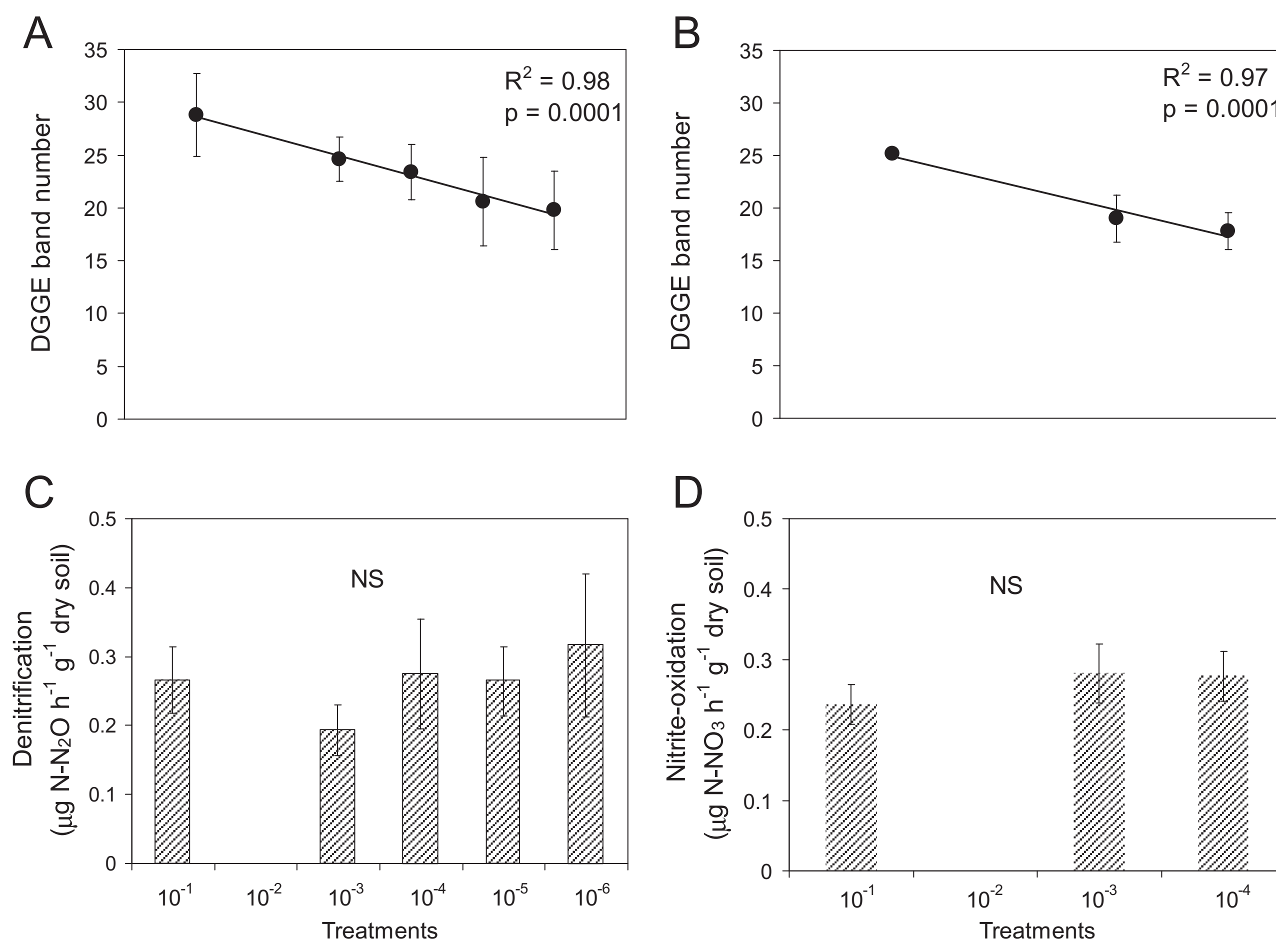

Fig. 1. A and B. Linear relationships observed between community diversity, assessed as the number of DGGE bands, and dilution treatment (10 – x) for (A) the denitrifying community ($y = -1.85x + 30.5$) and (B) the nitrite-oxidizing community ($y = -2.56x + 2.75$). For both communities, one-way analysis of variance revealed differences in number of bands between dilution treatments ($P = 0.0034$ and $P = 0.0001$ for denitrifying and nitrite-oxidizing communities respectively).
C and D. Activity levels for each dilution treatment for (C) the denitrifying community and (D) the nitrite-oxidizing community. For each community, only dilution treatments with similar cell abundance after incubation for 8 months are considered. Bars represent standard errors ($n = 5$). NS, not significantly different (ANOVA at level $P < 0.05$).

ment (Fig. 2). One month after disturbance, disturbance and dilution*disturbance effects were not significant, demonstrating the resilience of denitrifying activity for all dilution treatments (Fig. 2). It should be noted that 3 h after disturbance, a dilution treatment effect on denitrification was observed, whereas no effect was observed at 1 month (Fig. 2). This was due to higher denitrification levels in 10^{-1} and 10^{-6} treatments compared with 10^{-3}, 10^{-4} and 10^{-5} treatments only in control soils (one-way ANOVA result not significant for perturbed soils). No significant correlation was observed by linear regression analysis between denitrification activity and dilution at 3 h. The disturbance and dilution*disturbance effects on the abundance of culturable denitrifiers 3 h and 1 month after disturbance were not significant (data not shown). The DGGE band number for the denitrifying community was not modified by heating for all dilution treatments (no disturbance and dilution*disturbance effects) (Fig. 3). In addition, there was no detectable effect of heating on denitrifier community structure (e.g. Fig. S2 for dilution treatment 10^{-4}) but variation in DGGE patterns between replicates of a particular treatment (i.e. perturbed or control soils at 3 h or 1 month after disturbance) was high.

Resistance and resilience of the nitrite-oxidizing community following heat disturbance

The effect of disturbance on nitrite oxidation, measured 3 h after disturbance, was significant, with no dilution*disturbance interaction effect. Nitrite oxidation in perturbed soils was reduced to approximately 45% of that in control soils (Fig. 4) and increased during subsequent incubation. The effect of disturbance was still significant (without dilution*disturbance interaction effect)

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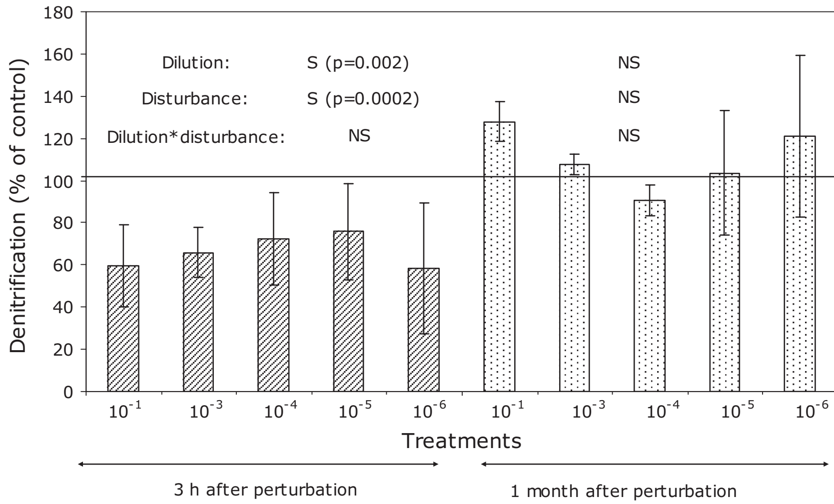

Fig. 2. Influence of inoculum dilution treatment on resistance and resilience of denitrification following heating of soil. Activity in perturbed soils is expressed as percentage of that in control soils. Bars represent standard errors ($n = 4$). For each date, results of two-way analysis of variance testing the dilution (initial level of diversity decline), perturbation and dilution*perturbation effects on denitrification are indicated.

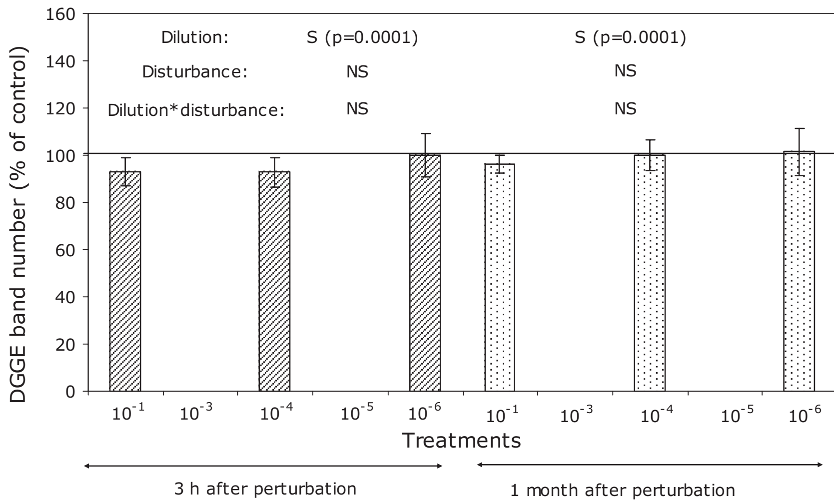

Fig. 3. Influence of inoculum dilution treatment on resistance and resilience of the diversity of the denitrifying community, assessed as number of DGGE bands, following heating of soil. Values in perturbed soils are expressed as percentage of those in control soils. Bars represent standard errors ($n = 4$). For each date, results of two-way analysis of variance testing the dilution, perturbation and dilution*perturbation effects are indicated. ND: not determined.

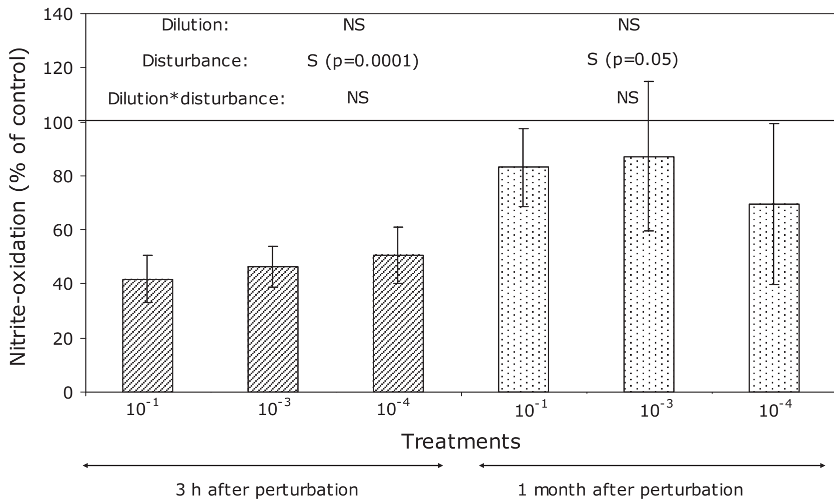

Fig. 4. Influence of inoculum dilution treatment on resistance and resilience of nitrite oxidation following the heating disturbance. Levels of activity in perturbed soils are expressed as percentage of those in control soils. Bars represent standard errors ($n = 4$). For each date, results of two-way analysis of variance testing the dilution, perturbation and dilution*perturbation effects on nitrite oxidation are indicated.

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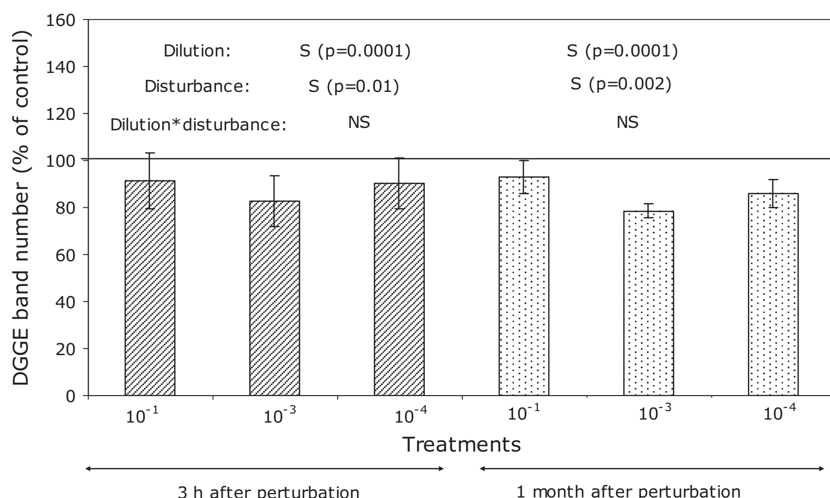

Fig. 5. Influence of inoculum dilution treatment on the resistance and resilience of the diversity of the nitrite-oxidizing community, assessed as number of DGGE bands, following the heating disturbance. Values in perturbed soils are expressed as percentage of those in control soils. Bars represent standard errors ($n = 4$). For each date, results of two-way analysis of variance testing the dilution, perturbation and dilution*perturbation effects are indicated.

1 month after disturbance, when nitrite oxidation in perturbed microcosms was approximately 80% of that in control microcosms (Fig. 4). Neither resistance nor resilience of nitrite oxidation varied significantly between dilution treatments (Fig. 4). The abundance of nitrite oxidizers was significantly reduced 3 h after heating (significant disturbance effect but no dilution*disturbance interaction effect) to a small percentage of control values, independently of dilution treatments (data not shown). Nitrite oxidizer abundances in disturbed soils increased with subsequent incubation and reached approximately 50% of values observed in control soils 1 month after disturbance, but no effect of dilution treatments was observed (data not shown). Heat disturbance had a significant effect on the DGGE band number for the nitrite-oxidizing community, without a dilution*disturbance interaction effect. A moderate decrease in the number of DGGE bands to 80–90% of control values was observed in all dilution treatments 3 h and 1 month following disturbance (Fig. 5). No detectable effect of heating on the structure of the nitrite-oxidizing community was observed (e.g. Fig. 6 for the dilution treatment 10^{-4}) but high variability in the structure of nitrite-oxidizing community was detected between replicates of similar treatments (i.e. perturbed or control soils at 3 h or 1 month after disturbance).

Discussion

Previous studies have shown that the activity of microbial functional groups such as decomposers, denitrifiers and nitrifiers under constant environmental conditions is not sensitive to decline in soil microbial diversity (Griffiths *et al.*, 2001; Wertz *et al.*, 2006). However, the level of diversity within functional groups is likely to be particularly important when ecosystems experience disturbance, with a prediction that more diverse communities will be more resistant and resilient (Ives *et al.*, 2000; McCann, 2000). Degens and colleagues (2001) and Griffiths and colleagues (2000; 2001) have assessed the impact of reducing soil microbial diversity on the stability of the decomposition process. Their studies revealed either a negative effect or no consistent effect of soil microbial diversity decline on stability of decomposition. However, the impact of decreasing soil microbial diversity on the stability of more specialized communities has never been assessed. The aim of the present study was therefore to determine the impact of a decrease in microbial diversity on the resistance and resilience of more specialized soil microbial functional groups following a model disturbance: soil heating. Two soil microbial functional groups were studied as models: denitrifying bacteria and nitrite-oxidizing bacteria. Within these two functional groups, different species may respond differently to heat disturbance (Buchanan and Gibbons, 1974; Harms *et al.*, 1976, e.g. for nitrite oxidizers).

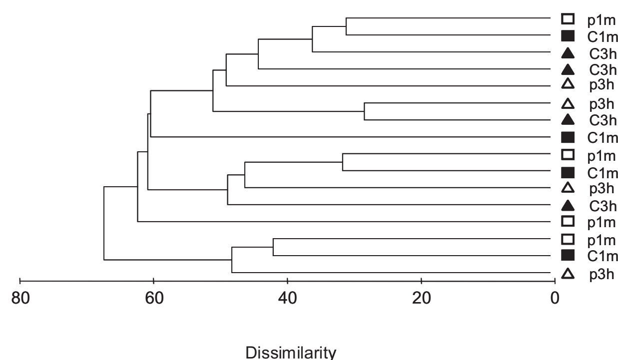

Fig. 6. Comparison of the structures of the nitrite-oxidizing community in control and perturbed soils for the dilution treatment 10^{-4}. C3h (▲): control soils, and p3h (△): perturbed soils assessed 3 h after disturbance. C1m (■): control soils, and p1m (□): perturbed soils assessed 1 month after disturbance.

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Soil microbial diversity was reduced using a removal approach already used by Wertz and colleagues (2006), in which sterile soil microcosms were inoculated with serial dilutions of a suspension of the same non-sterile soil. This approach removes less abundant species first, and communities were then allowed to recover their original cell abundances by incubating for a period of 8 months. As reported by Gans and colleagues (2005) for a pristine soil following pollution by heavy metal, the preferential removal of less abundant species is a realistic scenario of microbial diversity decline. For each functional group, the number of bands and the Shannon diversity index computed from functional gene fingerprints was progressively decreased from microcosms inoculated with low soil dilutions to those inoculated with high soil dilutions. Denaturating gradient gel electrophoresis band number was used here to demonstrate that the suspension/dilution approach led to a decline in microbial diversity, and not to quantify accurately actual microbial diversity, which is not possible in this type of study. As noted by Loisel and colleagues (2006), the number of bands in genetic fingerprints is largely insensitive to community richness for species-rich communities such as those in soil; however, the number of bands is sensitive to richness for communities with lower species richness (Fromin *et al.*, 2002; Loisel *et al.*, 2006) and the decrease in the number of DGGE bands observed in our study therefore implies a decrease in the number of bacterial taxa, particularly those with abundance high enough to be detectable by DGGE. This approach does not provide information on taxa with low abundance, below the detection level, but mathematical simulation (Wertz *et al.*, 2006) demonstrates a considerable decrease in soil bacterial diversity using this soil suspension/dilution approach. For example, a decrease of > 86% and > 99.5% of the number of bacterial taxa in the inoculum is predicted for the 10^{-4} and 10^{-6} dilutions respectively. Although the level of diversity decline in the inoculum could not be estimated accurately due to the lack of data on richness and abundance distribution of taxa for soil denitrifiers and nitrite oxidizers, the considerable decrease in soil bacterial diversity estimated by Wertz and colleagues (2006) and the decrease in DGGE band number and Shannon index observed here strongly suggest considerable decreases in the diversity of denitrifying and nitrite-oxidizing communities.

Characterization of the diversity of nitrite reducers using *nirS* in addition to *nirK* could be valuable for this study. However, the effect of decrease in microbial diversity induced by our suspension/dilution approach has been characterized for the same soil (in our previous study: Wertz *et al.*, 2006, and in this study) for four different marker genes specific of four different microbial communities: (i) ribosomal intergenic sequences for the bacterial community, (ii) 16S rRNA gene sequences specific for soil ammonia-oxidizing bacteria, (iii) the *nirK* gene sequences, targeting nitrite reducers, and (iv) *norA* genes sequences specific for nitrite oxidizers. For all these markers, the suspension/dilution approach induced consistent patterns in diversity decline, i.e. significant decreases in band number and diversity indices observed from fingerprints. Although all the possible marker genes of the different communities, in particular *nirS* for nitrite reducers, have not been assessed, the consistent patterns of microbial diversity decline obtained for these four gene makers provide evidence of the generality of the effect of our suspension/dilution approach on microbial diversity.

For a given dilution treatment, differences in molecular fingerprints were observed between replicates. Given the good repeatability of the amplification/fingerprinting procedure used (i.e. the same profile was obtained when a given sample was run twice), this was likely due to the complexity of soil microbial communities that does not allow achievement of exact replication of community structure, and to stochastic effects associated with species removal and initial stages of recolonization. This has already been observed by Griffiths and colleagues (2001) for the soil bacterial community and by Wertz and colleagues (2006) for the denitrifying and ammonia oxidizing communities.

Comparison of disturbance effects between denitrifying and nitrite-oxidizing communities

Nitrite-oxidizing bacteria were more sensitive to heat disturbance than denitrifiers. Similarly, Sierra and Marban (2000) showed that nitrification was more sensitive to high temperatures than N mineralization and respiration, functions insured by heterotrophic bacteria. In our study, heating had no significant effect on the abundance of denitrifiers and led to a moderate, short-term reduction in denitrification activity. In addition, no variation in diversity was observed and no modification of the structure of the denitrifying community could be detected following heat disturbance. Reduction in denitrification following heating was thus likely due to physiological effects such as denaturation and inactivation of denitrifying enzymes or other damage to different cellular components (i.e. outer cell layers, cytoplasmic membrane, ribosomal RNA, proteins and enzymes and DNA; Russell, 2003).

In contrast, the observed reduction in nitrite oxidation activity immediately after heating was associated with a strong reduction in the abundance of nitrite oxidizers and a slight decrease in diversity of the nitrite-oxidizing community (with no detectable modifications to community structure). These results suggest that modification in nitrite oxidizer abundance rather than reversible physi-

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ological effects was a major factor influencing the response of nitrite-oxidizing activity following heat disturbance. The incomplete recovery of the abundance of nitrite oxidizers and nitrite-oxidizing activity 1 month following disturbance can be explained by the low energy yield of this process and the low growth rates of these organisms (Prosser, 1989).

Resistance and resilience of microbial communities with decreased levels of diversity

Our results show that a reduction in the diversity of denitrifying and nitrite-oxidizing communities did not affect their resistance and resilience following heat disturbance, provided that cell abundance recovered. Indeed, for each functional group, effects of heat were similar for all dilution treatments. This could be expected for denitrifiers, as this process is carried out by a wide range of bacteria and archaea (Tiedje, 1988; Zumft, 1997), and functional diversity with respect to temperature activity optima is likely to be large. This is more surprising for nitrite oxidizers, which are restricted to a smaller range of phylogenetic groups (Prosser, 1989). Several hypotheses can be proposed to explain this result. For example, different taxa may not differ significantly in their response to heat disturbance. However, some studies have shown that strains within phylogenetic groups of cultivated organisms, defined as species, differ in their sensitivity to heat (for nitrite oxidizers, see Buchanan and Gibbons, 1974; Harms *et al.*, 1976). Thus, considering the high diversity of soil microorganisms, the more likely hypothesis is that the diversity of both microbial functional groups following soil diversity decline may remain high enough to maintain their stability.

Experimental procedure

Soil microcosms

Soil microcosms were prepared using the approach described by Wertz and colleagues (2006). Soil characteristics are given by Le Roux and colleagues (2003). Sterility of soil was determined by enumeration of heterotrophic bacteria by the most probable number technique (Alexander, 1982). Serial dilutions of the non-sterile soil were used to inoculate the sterile soil microcosms according to Wertz and colleagues (2006), giving final dilutions from 10^{-1} to 10^{-10} g of non-sterile soil per gram of sterile soil. For each dilution level, 21 replicate microcosms were established and several microcosms containing sterile soil were also established as controls of sterility during the experiment. After incubation for 8 months at 20°C, five replicate microcosms per dilution level were sampled for assessment of activity, cell abundance and structure of the denitrifying and nitrite-oxidizing communities before application of the disturbance.

Disturbance treatment

Half of the remaining microcosms (i.e. eight per dilution level) were incubated at 42°C for 24 h and then incubated at 20°C. The temperature of heat disturbance was calibrated following a preliminary experiment testing the short-term impact (3 h to 6 days) of a range of temperatures (from 40°C to 47.5°C) on the functioning of the two communities (data not shown). A temperature of 42°C was chosen following these experiments because it (i) resulted in significant effects on both denitrification and nitrite oxidation without eliminating nitrite oxidation completely, and (ii) allowed study of the resilience of both functions. Such a temperature is in the highest range of temperatures experienced at the dry soil surface in areas unshaded by vegetation during the summer at the study site. Incubation of the control microcosms (i.e. eight per dilution level) was continued at 20°C. The activity, cell abundance and genetic structure of the denitrifying and nitrite-oxidizing communities were monitored for each dilution treatment in four replicates of perturbed and four replicates of control (non-perturbed) soils microcosms 3 h after disturbance, and 1 month after disturbance.

Enumeration of bacteria within each functional group

Culturable denitrifying and nitrite-oxidizing bacteria were enumerated by the most probable number technique as described by Patra and colleagues (2005), except that 4 g of soil samples and fivefold serial dilutions were used. Nutrient broth medium and double strength mineral salts medium (Schmidt and Belser, 1982), containing 0.34 mg ml^{-1} $NaNO_2$, were used for the culture of denitrifiers and nitrite oxidizers respectively. The lack of contamination during incubation was verified by enumeration of culturable heterotrophic, denitrifying and nitrite-oxidizing bacteria in control sterile soils.

Community structure of each functional group

To determine the structure of denitrifying and nitrite-oxidizing communities, DNA was extracted from 0.5 g of homogenized soil using the fast DNA SPIN Kit for soil (BIO 101 Systems; Qbiogene, Carlsbad, CA, USA). The structure of the denitrifying community was characterized by DGGE analysis of the *nirK* gene (coding for Cu-nitrite reductase) as described by Wertz and colleagues (2006). The structure of the denitrifying community before disturbance was characterized for dilution treatments 10^{-1}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. At the two sampling times after disturbance (3 h and 1 month after heating disturbance), the structure of the denitrifying community was characterized only for the dilution treatments 10^{-1}, 10^{-4} and 10^{-6}. An example of DGGE profiles for the denitrifying community is presented in Fig. S3. At the same sampling times, the structure of the nitrite-oxidizing community was characterized for treatments 10^{-1}, 10^{-3} and 10^{-4} by PCR-DGGE analysis of the *nor*A gene (coding for nitrite oxidoreductase). Amplification conditions and DGGE analysis are as presented by Wertz and colleagues (S. Wertz, F. Poly, X. Le Roux and V. Degrange, submitted). All gels were stained, and photographed using BiocaptMW (Vilber-Lourmat, France). An example of DGGE profiles for the nitrite-oxidizing community is presented in Fig. S3.

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Activity of each functional group

The method used for denitrifying activity measurements was as described by Patra and colleagues (2005), except that 2 g of soil samples was used. Briefly, the denitrifying activity was determined as the rate of production of N_2O during short-term incubation under anaerobiosis (8 h) using a gas chromatograph (Agilent P200, USA). Because C_2H_2 was used to inhibit nitrous oxide reductase activity, and no accumulation of nitric oxide was detected during these enzyme activity assays, possible truncated denitrification pathways were not likely to have an impact when comparing activities and community structure. KNO_3 (200 μg of NO_3^--N per gram of dry soil), glucose (0.5 mg of C per gram of dry soil) and glutamic acid (0.5 mg of C per gram of dry soil) were added to the soil samples and the soil moisture was brought to 100% water holding capacity. A linear rate of N_2O production was always observed. Nitrite-oxidizing activity was determined using a modified version of the method described by Smorczewski and Schmidt (1991). Samples of 3 g of soil (equivalent dry mass) were placed in 100 ml Erlenmeyer flasks containing 30 ml of a solution of $NaNO_2$ (50 μg of N-NO_2^- per gram of dry soil). Flasks were sealed with parafilm and incubated at 28°C for 48 h with shaking. After incubation for 0, 24, 30 and 48 h, 1.5 ml of the suspensions (soil + solution) was centrifuged (5000 r.p.m. for 2 min). The supernatants were then filtered (0.2 μm pore size) and analysed for NO_2^- concentration on a spectrophotometer (Uvikon 800) at 520 nm using Griess reagent. A linear rate of NO_2^- consumption was always observed during the 48-h period.

Data analysis

Denaturating gradient gel electrophoresis banding profiles for each bacterial functional group were analysed as follows. Data matrices consisting of the intensity and relative position of each DNA band in all samples were constructed from banding profiles using Gel ComparII software (Applied Maths, Kortrijk, Belgium). The total band intensity for each sample was normalized among samples. For each sample, the number of bands in the profile was used as a measure of community diversity. Similarity matrices were calculated from the data matrices of all samples using PRIMER-E Ldt software (Plymouth, UK). Statistical differences between samples were evaluated by one-way ANOSIM (analysis of similarity) tests. The percentage dissimilarity in DGGE profiles within replicates of each treatment was computed. Cluster analysis (visualized as dendrograms) was also performed from similarity matrices using PRIMER-E Ldt software.

The resistance and resilience of the activity, cell abundance and number of DGGE bands for each functional group were assessed by expressing their values in perturbed soils as percentages of those in control soils. For each functional group, one-way analysis of variance was performed to test if cell abundance, number of DGGE bands and activity differed between dilution treatments before application of the disturbance. For each functional group and for each sampling time following disturbance (3 h and 1 month), two-way analysis of variance was performed to test for dilution, perturbation and dilution*perturbation effects on cell abundance, band number and activity.

Acknowledgements

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Supplementary material

The following supplementary material is available for this article online:

Fig. S1. Variation in the Shannon diversity index computed from the DGGE data matrix as a function of the dilution treatment, for (top) the nitrite-reducing community and (bottom) the nitrite-oxidizing community before the disturbance.

Fig. S2. Comparison of the structure of the denitrifier community for control and perturbed soils for the dilution treatment 10^{-4}. C3h (▲): control soils, and p3h (△): perturbed soils assessed 3 h after disturbance. C1m (■): control soils, and p1m (□): perturbed soils assessed 1 month after disturbance.

Fig. S3. A. Example of genetic profiles of the denitrifier community obtained for soil microcosms of the different dilution treatments just before heat disturbance (one replicate microcosm per dilution treatment is presented on the gel).

B. Example of genetic profiles of the nitrite oxidizer community obtained for control and perturbed soil microcosms for the dilution treatments 10^{-1}, 10^{-3} and 10^{-4}. C1m: control soils, and p1m: perturbed soils, 1 month after disturbance.

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