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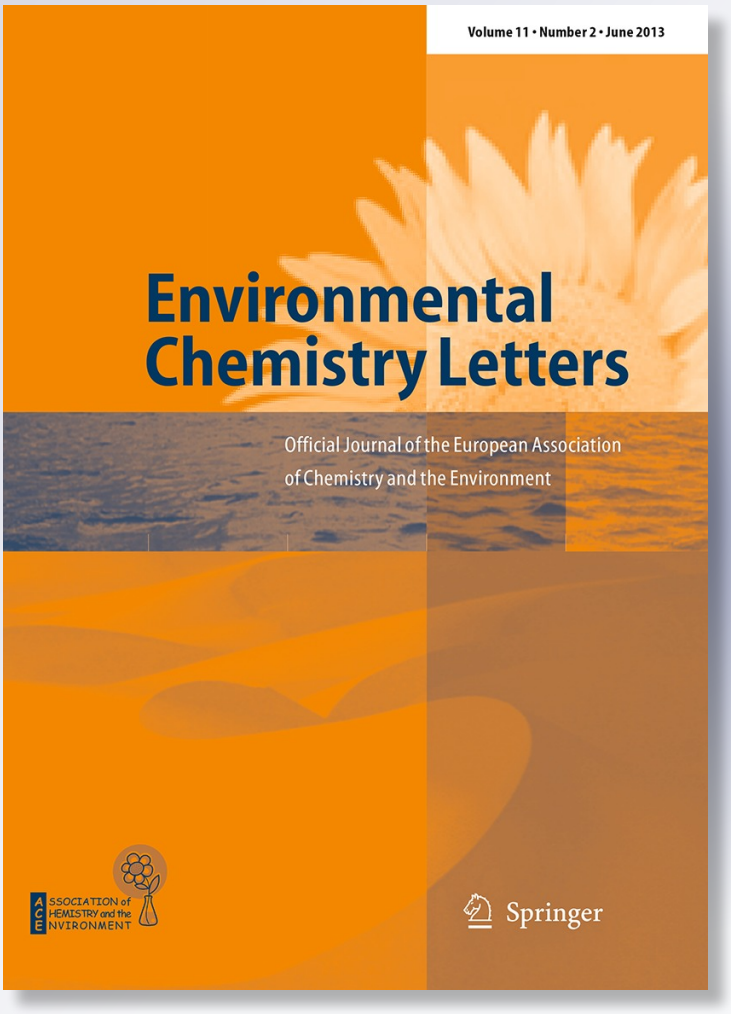

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ORIGINAL PAPER

Unexpected similar stability of soil microbial CO_2 respiration in 20-year manured and in unmanured tropical soils

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Abstract Soil respiration is one of the main CO_2 sources from terrestrial ecosystems. Soil respiration is therefore a major source of greenhouse gas. Knowledge of the impact of agronomic practices such as manuring on the stability, for example resistance and resilience, of heterotrophic C–CO_2 respiration to disturbance is scarce. Here, we studied the stability of soil microbial heterotrophic respiration of two tropical soils from plots annually enriched or not with manure applications during more than 20 years. Stability was quantified after heating soils artificially. We hypothesized that field manuring would change the stability of the microbial community. Additionally, the impact of both manured and unmanured soils to addition of an organic cocktail was assessed under controlled conditions in order to discriminate the metabolic capacity of the microbial community, and to link the metabolic capacity up with the microbial heterotrophic soil respiration. Our results show that total respiration was not significantly different in manured and unmanured pots. Moreover, contrary to our hypothesis, manure amendment did not affect the stability (resistance, resilience) of the microbial abundance or the basal metabolism, in our experimental conditions. By contrast, the diversity of the bacterial community in heated soils was different from that in unheated soils. After heating, surviving microorganisms showed different carbon utilization efficiency, manuring stimulating the growth of different resistant communities, that is, *r*-strategist or *K*-strategist. Microbial community of manured soils developed in the presence of the organic cocktail was less resistant to heating than microbial community of unmanured plots.

Keywords Stability · Heat disturbance · Microbial community · Manure · CO_2 respiration

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Introduction

Soils play key roles for many ecosystem services such as nutrient cycling, primary production and carbon sequestration. Sustainable soil management needs to better understand how these functions could withstand any natural and/or anthropogenic changes that might affect their functioning. Soils are exposed to a variety of stresses or disturbances. The ability of soils to face these perturbations is defined as stability. It encompasses two components: resistances, that is, the ability of a system to withstand the stress or disturbance, and resilience which refers to the rate and degree to which the system recovers from the perturbation (Pimm 1984). Soil

resilience is recognized as a fundamental component of soil quality (Seybold et al. 1999). Several indices have been developed to assess the resistance and the resilience of soil functions (see Orwin and Wardle 2004).

Soil respiration is one of the main sources of CO_2 from terrestrial ecosystems, producing more CO_2 per year than fossil fuel combustion (IPCC 2007). Small variations in heterotrophic respiration owing to variations in climate can have major consequences on future C–CO_2 concentrations in the air. Much research is currently being carried out into the relationship between temperature and heterotrophic respiration (Fang et al. 2005). Most of these studies entail long-term experiments, exposing the soils, in laboratory or the field conditions, to increased temperatures ranging from a few degrees to several tens of degrees (Bradford et al. 2008; Steinweg et al. 2008).

This study aimed at quantifying the resistance and the resilience of soil microbial heterotrophic respiration to an artificial disturbance by extreme pulse events (heat). Since organic inputs are known to positively affect soil microbial properties (Freschet et al. 2008; Kamaa et al. 2011; Mando et al. 2005), it explored the possibility of these amendments to affect the resistance and the resilience of microbial community responsible for CO_2 respiration. This study is based on two field stations set up in Kenya and Madagascar. These trials enable the comparison of plots that had received annual input of manure since 1976 and 1991, respectively, to unmanured plots. The effect of the disturbance was quantified by measuring the basal metabolism of the soil communities of manured and unmanured plots as well as the decomposition of an organic cocktail, used to discriminate the metabolic capacity of the microbial community (Degens and Harris 1997). It was then assumed that the response to the organic cocktail would be due essentially to the activity of the soil microbial communities (Sall et al. 2006).

Experimental

Sites and soil sampling procedures

Soil samples were taken from two different sites:

- *Kenya* field trial located at Kabete (36°46′ E and 01°15′S), where the Kenya Agricultural Research Institute (KARI) set up in 1976 a trial using a randomized complete block design with eighteen treatments replicated four times (Kamaa et al. 2011). Of the various field treatments, this study compared plots treated annually with 10 t ha^{-1} of farmyard manure with unmanured soil. The crop was maize (*Zea mays L.*). The soil type is classified as Andic Dystrustept. The organic C concentration[1] was 18.3 and 20.1 mg g^{-1} soil, for unmanured and manured soils, respectively, difference being not significant. The total *N* was 1.4 and 1.6 mg g^{-1} soil, for unmanured and manured soils, respectively, difference being not significant.
- *Madagascar* field trial located at Bemasoandro (19°46′S and 47°06′ E), where the NGO TAFA (Tany sy Fampandrosoana) had set up in 1991 a trial meant at comparing the effect manure amendment (5 t ha^{-1}) vs. unmanured treatment on soil properties and yield (Rabary et al. 2008). The cropping system used a rotation of soybean (*Glycine max L.*) and rice using direct seeding through crop residues. Each treatment was replicated 6 times. The soil type is classified as Humic Nitisol. The organic C concentration was 35.7 and 34.9 mg g^{-1} soil, for unmanured and manured soils, respectively, difference being not significant. The total *N* was 2.7 mg g^{-1} soil for both unmanured and manured soils.

For both sites, manured and unmanured soils were sampled from 3 replicate plots 6 weeks after sowing (e.g. 05 May 2007 in Kabete and 24 January 2007 in Bemasoandro). For each replicate plot, 6 subsamples were cored from the 0- to 10-cm soil layer. The subsamples from each plot were pooled, air dried, sieved <2 mm and stored at room temperature.

Microcosms and sampling strategy

Each soil subsample (e.g. 30 g soil equivalent dry soil) was divided into two aliquots: one was kept at a constant temperature (30 °C) (control) and the other was heated for 16 h at 60 °C. Soil subsamples were wetted to 50 % of the water holding capacity (WHC).

When the samples had been heated for 16 h, the temperature was lowered to 30 °C, temperature reached 1 h after having stopped heating. Half of the replicates of the unheated and heated soils were enriched with a substrate formed of a cocktail of asparagine, mannose, α-ketoglutaric acid and glucosamine established as being an accurate means for discriminating the functional diversity of soil microorganisms (Degens and Harris 1997). The substrates were added to the soil in solution to adjust the soil moisture content to 100 % of the WHC and give a concentration of 5 % (i.e. 500 μgC g^{-1} of dry weight soil). For the replicates without substrate, the moisture content was adjusted by the addition of distilled water. The soil moisture was kept constant at 100 % of the water holding capacity for each sample throughout the incubation period by weighing each vessel every other day. The vessels (250 ml glass jars) were sealed and incubated at 30 °C in the dark for 21 days.

[1] Analyses performed by the ISO 9001(2008) LAMA Laboratory, Dakar, US Imago, IRD.

Samples were analysed just as temperature cooled down to 30 °C (e.g. for enriched subsamples just after the addition of the substrates) (t_0), then after 8 h ($t_{8\ h}$), 48 h ($t_{48\ h}$) and 21 days ($t_{21\ days}$). At each sampling date, 3 replicates were analysed for each combination of treatments (e.g. [manured vs. unmanured soils] × [heated vs. unheated subsamples] × [subsamples enriched with the organic cocktail vs. subsamples without the organic cocktail]).

Microbial CO_2 respiration

CO_2 respiration was measured every hour for the first day and then every day for the rest of the incubation period. Measurements were taken by direct injection into a micro GC Analytical Instruments SRA (MTI P200, Microsensor Technology, Fremont, Calif., USA) with a thermal conductivity detector (TCD), using helium as the carrier gas and ambient air as a control. After each measurement, the headspaces were flushed with fresh air. The headspaces of the other vessels were also flushed to avoid any effect from CO_2 accumulation.

The cumulative respiration was measured for the whole period (21 days) of incubation and expressed in μg C g^{-1} soil as a mean of 3 replicates. C–CO_2 fluxes were measured for each period (i.e. 0–1 h, 1–8 h, 8–48 h, 48 h–21 days) and expressed as a mean of 3 replicates in μg C g^{-1} soil h^{-1}.

The response of the microbial community to the addition of the organic cocktail was calculated from the C–CO_2 respired by soil enriched with the organic cocktail minus the C–CO_2 respired by soil in the absence of the cocktail, assuming that there was no priming effect. The response was calculated for the whole period of incubation (0–21 days) and for each period (i.e. 0–1 h, 1–8 h, 8–48 h, 48 h–21 days).

Microbial biomass

The microbial biomass was estimated by measuring the DNA on soil subsamples taken from each replicate vessel at each sampling date. Soils were immediately frozen and stored at −20 °C until DNA analysis. The DNA was extracted from quadruplicates of 0.5 g soil per replicate, using the method described by Martin-Laurent et al. (2001). After extraction, the DNA from the four microtubes was pooled in a total volume of 30 μl. A supplementary purification step using the Wizard purification system (Promega, 2800 Woods Hollow Road, Madison, USA) was performed. The genomic DNA was then quantified by comparison with standards (calf thymus DNA Sigma-Aldrich) using a 1 % agarose gel electrophoresis and ethidium bromide staining. The DNA concentration of samples was determined by densitometry with reference to the calibrated standards using TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK).

The microbial biomass was assayed for each treatment at each sampling date (i.e. $t_{0\ h}$ at $t_{8\ h}$, at $t_{48\ h}$ and at $t_{21\ days}$).

Genetic structure of total bacterial community

The genetic structure of the total bacterial community was assayed for each treatment at $t_{0\ h}$ and $t_{21\ days}$.

Five nanogram of DNA was used as a template for polymerase chain reactions (PCR). The reaction mixtures contained the illustra^{TM} PureTaq Ready-To-Go PCR Beads (GE Healthcare, Little Chalfont Buckinghamshire, HP7 UK), 0.5 μM of each of the eubacterial primers 338f-GC (Olsson et al. 1996) and 518r (Muyzer et al. 1993) targeting the bacterial 16S rDNA, in a final volume of 25 μl. Amplifications were performed in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, USA) with an initial denaturation of 5 min at 94 °C, followed by 20 touchdown PCR cycles of 1 min at 94 °C (denaturation), 1 min at 65 °C with a 1 °C decrease every second cycle and 1 min at 72 °C (elongation). This was followed by 10 cycles with a constant annealing temperature of 55 °C and a final elongation step for 10 min at 72 °C. PCR products were then separated by electrophoresis in a 1.5 % (w/v) agarose gel and stained for 30 min with ethidium bromide (1 mg L^{-1}). A standard DNA ladder (Eurogentec) loaded on the same gel was used for quantification of the amplicons. A standard quantity of 700 ng of amplified DNA was loaded for all DGGE assays.

The denaturing gradient gel electrophoresis (DGGE) gels were 8 % acrylamide (acrylamide–bisacrylamide 40 %, 37.5:1, Sigma-Aldrich, St. Quentin Fallavier, France), with a 45–70 % gradient of chemical denaturant (100 % denaturant corresponding to 7 M urea and 40 % v:v formamide; Muyzer et al. 1993). The DGGE was run for 18 h at 100 V in a 1 X TAE buffer at a constant temperature of 60 °C using an INGENY phorUelectrophoresis system (Ingeny International, Goes, The Netherlands). The gels were stained in a 1:20000 Sybr Green (Bioproducts) solution for 20 min under agitation. The gels were rinsed in distilled water for 5 min and the bands were quantified under UV using a CCD camera and Biocapt software (Vilber Lourmat).

Resistance (RS), Resilience indices (RL)

The indices proposed by Orwin and Wardle (2004) were used to express the resistance and resilience of the microbial community to artificial heating.

The resistance (RS) is:

$$RS = 1 - 2\ I D_0 I / (C_0 + I D_0 I)$$

where D_0 is the difference between the control (C_0) and the disturbed soil after 1 h.

The resilience (RL) at time *tx* is

$$\text{RL } tx = [2\ \text{I}D_0\text{I}/(\text{I}D_0\text{I} + \text{I}D_x\text{I})] - 1$$

where D_0 is as above and D_x is the difference between the control (C_x) and the disturbed soil at time *tx*.

The resistance and resilience indices were calculated to estimate the resistance and the resilience of the microbial biomass, its basal metabolism and the response of the microbial community to the addition of the exogenous cocktail.

Statistical analysis

The C–CO_2 respiration data were analyzed using the Fisher LSD test (XLSTAT version 6.1.9, Addinsoft, 40, rue Damrémont 75018 Paris, France), with $p < 0.05$ indicating that differences were significant. The effects of the treatments and interactions were tested using one-way and two-way ANOVA.

For the denaturing gradient gel electrophoresis data, the complex banding patterns of the bacterial communities were analyzed using Totallab TL120 V 2006 (Nonlinear Dynamics, USA). For each plot, at each time, bands in the heated soils were compared with those of the controls. To illustrate the overall changes of the genetic structure, principal component analysis (PCA) was performed on the data matrix of normalized band intensities (individual band intensities expressed as a fraction of the sum of the intensities of all bands in a lane) using the XLSTAT PCA option.

Results and discussion

Cumulative basal C–CO_2 respiration

Cumulative basal C–CO_2 was calculated for the whole period of incubation. For both soils (e.g. from Kabete and Bemasoandro), the cumulative C–CO_2 respired by unheated soils was not significantly different in the manured and unmanured plots (Table 1). For Kabete, C–CO_2 respired corresponded to 5.1 % and to 5.3 % of the total carbon content of unmanured and manured plots, respectively. For Bemasoandro, it represented 6.5 % and 7.7 % of the total carbon content of unmanured and manured plots, respectively. Similarly, the C–CO_2 fluxes measured during the various incubation periods (0–1 h, 1–8 h, 8–48 h, 48 h–21 days) were not significantly different in unmanured vs. manured plots for both soils (data not shown). These results might be explained by similar carbon content in manured and unmanured plots for each soil.

Nevertheless, the comparison of the effect on heating revealed significant differences between manured and unmanured plots. For Kabete, the effect of heating on the basal metabolism of the soil microbial communities was only significant for the manured plots: the cumulative C–CO_2 respired after heating the soil was significantly greater than that respired by the unheated soil, for example, 179.50 and 110.70 μg C–CO_2 g^{-1}soil, respectively. Contrastingly, heating the unmanured soil from Bemasoandro significantly increased the cumulative C–CO_2 respired, while no difference was observed after heating the manured plots. Although long-term application of manure did not significantly affect the total carbon content, the quality of organic compounds seemed to be different. Heating is known to solubilize organic compounds metabolized by the microbial community (Agren and Wetterstedt 2007). For Kabete, these compounds seemed to be in higher quantity in the manured plot, compared with that in unmanured plot, while for Bemasoandro, they seemed to be more abundant in the manured plot compared with that in the unmanured one.

Table 1 Cumulative C–CO_2 (μg C g^{-1} soil) respired during the whole incubation (21 days)

	C–CO_2					
	Kabete			Bemasoandro		
	μg g^{-1} soil			μg g^{-1} soil		
Basal C–CO_2 respiration						
Unmanured plots						
Unheated soil	95.20	a	A	233.80	a	A
Heated soil	128.60		A	249.0		B
Manured plots						
Unheated soil	110.70	a	A	267.70	a	A
Heated soil	179.50		B	289.4		A
Induced C–CO_2 respiration						
Unmanured plots						
Unheated soil	319.56	a	A	276.94	a	A
Heated soil	352.98		A	376.81		B
Manured plots						
Unheated soil	280.95	a	A	341.57	a	A
Heated soil	313.25		A	383.46		A

Different lower case letter indicated difference when comparing unmanured vs. manured soil for each situation ($n = 3$, $p < 0.05$)

Different upper case letter indicated difference when comparing unheated vs. heat soil for unmanured and manured treatments for each situation ($n = 3$, $p < 0.05$)

Note that the cumulative C–CO2 respired by unheated soils was not significantly different in the manured and unmanured plots

Stability of the microbial biomass and of the basal $C–CO_2$ respiration

The stability of a system, consisting of two components (resistance and resilience) (Pimm 1984), describes its ability to continue to function under changing conditions. The stability of the microbial communities affects its turnover and therefore the functions driven by them (Wardle and Parkinson 1990). Orwin and Wardle (2004) proposed two indices to estimate the resistance (RS) and the resilience (RL) of microbial processes, showing several advantages compared with other indices.

For unheated, no significant difference was observed between microbial biomass extracted from manured and unmanured plots at $t_{0\ h}$. Thus, for Kabete, DNA contents were 260 and 277 ng DNA g^{-1} soil, for the unmanured and the manured plot, respectively. For Bemasoandro, these were 917 and 600 ng DNA g^{-1} soil for the unmanured and the manured plots, respectively. Heating soils (e.g. Kabete, Bemasoandro) resulted in a decrease in the DNA content of the soil. Resistance indices of microbial biomass amounted to 0.13 and 0.12 in unmanured and manured plots of Kabete, and 0.15 and 0.12 in unmanured and manured soil of Bemasoandro, respectively (Table 2). For each soil, adding manure for more than 20 years did not modify the resistance of the microbial community, resistance indices (RS) being not significantly different when comparing manured vs. unmanured plot of a same soil. Similarly, no significant differences were noted when comparing the genetic structure of the total bacteria community of manured and unmanured soils (data not shown). Nevertheless, the impact of heating on the structure of this community was clearly observed immediately after heating (Fig. 1). There was a clear shift in the microbial community structure in the soil from both sites, heated soils and unheated soils being separated along the principal axis PC1. The impact of heating was also recorded for the basal $C–CO_2$ respired, quantities being reduced after heating the soils. However, this reduction was of similar magnitude in manured and unmanured soils, resistance indices showing no significant difference between manured and unmanured plot either for Kabete or for Bemasoandro (Table 3). Resistance indices calculated for Bemasoandro showed lower values than those for Kabete, indicative of a lower resistance to heat of the basal $C–CO_2$ respiration.

Monitoring the resilience indices of the estimated microbial biomass and basal respiration during incubation did not reveal any significant difference between manured and unmanured plots (Tables 2 and 3). Contrastingly, Wada and Toyota (2007) showed that soil amended with manure for many years was more resistant to the action of disinfectants than unamended soil. This discrepancy between our results and those of the literature could be explained by many factors such as different agents (temperature *vs.* chemicals) (Kuan et al. 2007) and intensity (Banning and Murphy 2008). At the end of the incubation, microbial biomass from Kabete seemed to better recover from heating than microbial biomass form Bemasoandro, since resilience indices were closer to 1 in Kabete than those for Bemasoandro (Table 3). During incubation, the basal $C–CO_2$ respiration in the heated soils recovered up to the previous level very close to that of soils that had not been heated (values of resilience indices tending towards 1). For Kabete, the basal $C–CO_2$ respiration in unmanured plot recovered earlier after heating (48 h) than that observed in manured plot. For this soil, resistance indices calculated at 48 h amounted to 0.58 in the unmanured plot, while it was almost equal to 0 for the manured plot at the same period. Similar pattern was observed for Bemasoandro. Higher resilience indices were calculated compared with those for Kabete towards the end of the incubation. However, it is important to note that the recovery of the basal metabolism cannot be attributed to the development of a microbial community that was identical to the initial

Table 2 Resistance and resilience of soil microbial biomass

	Kabete								Bemasoandro							
	Resistance (RS)		Resilience (RL)						Resistance (RS)		Resilience (RL)					
			$t_{8\ h}$		$t_{48\ h}$		$t_{21\ days}$				$t_{8\ h}$		$t_{48\ h}$		$t_{21\ days}$	
In the absence of the organic cocktail																
Unmanured plots	0.13	A	0.09	A	0.14	A	0.73	A	0.25	A	0.09	A	−0.02	A	0.41	A
Manured plots	0.12	A	0.03	A	0.11	A	0.66	A	0.16	A	−0.11	A	−0.21	A	0.25	A
In the presence of the organic cocktail																
Unmanured plots	0.15	A	−0.23	A	−0.07	A	0.76	A	0.31	A	−0.05	A	−0.06	A	0.62	A
Manured plots	0.12	A	0.10	A	0.16	A	0.61	A	0.21	A	−0.11	A	−0.11	A	0.25	A

Different letters indicated difference when comparing unmanured vs. manured plots for each period of incubation ($n = 3$, $p < 0.05$)

In the absence of the organic cocktail adding manure for more than 20 years did not modify the resistance than the resilience indices of the microbial community

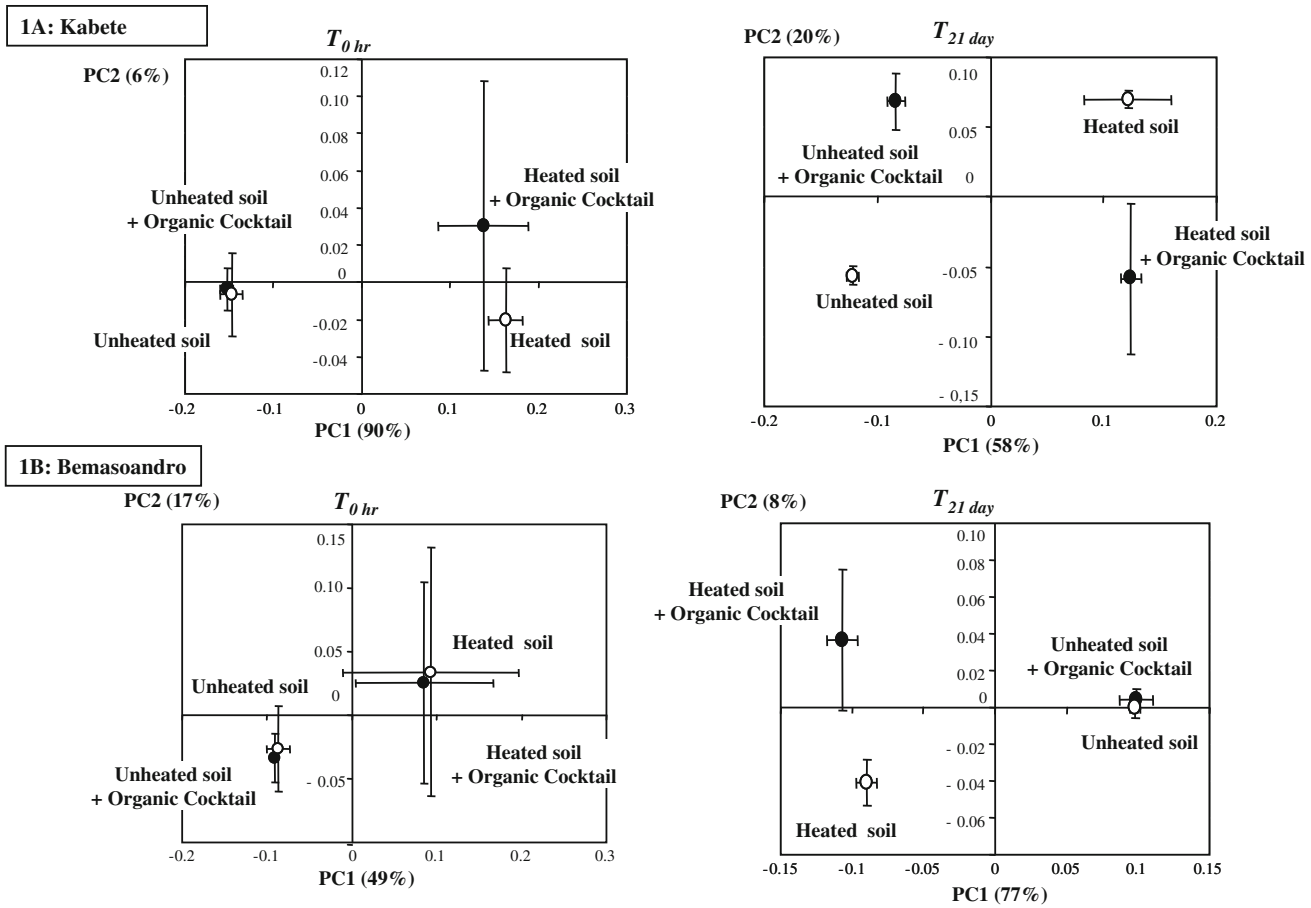

Fig. 1 Principal component analysis ordinations obtained from denaturing gradient gel electrophoresis fingerprints, illustrating the evolution within time ($T_{0\ h}$, $T_{21\ days}$) of the genetic structure of the total bacteria community in unheated soil and heated soil in the presence of the organic cocktail, and in its absence. *Symbols* represent the mean of coordinates of triplicates samples scores with associated standard errors

Table 3 Resistance and resilience of C–CO_2 respiration

	Kabete								Bemasoandro							
	Resistance (RS)		Resilience (RL)						Resistance (RS)		Resilience (RL)					
			$t_{8\ h}$		$t_{48\ h}$		$t_{21\ days}$				$t_{8\ h}$	$t_{48\ h}$		$t_{21\ days}$		
Basal C–CO2 respiration																
Unmanured plots	0.41	a	0.11	a	0.58	a	0.86	a	0.08	a	nd	0.74	a	0.98	a	
Manured plots	0.49	a	−0.30	a	0.03	b	0.86	a	0.31	a	0.21	0.30	a	0.94	a	
Induced C–CO2 respiration																
Unmanured plots	0.26	a	−0.24	a	0.41	a	0.99	a	0.28	a	nd	−0.16	a	0.59	a	
Manured plots	0.19	b	−0.06	b	0.60	a	0.99	a	0.03	b	0.12	−0.002	a	0.92	a	

Different letters indicated difference when comparing unmanured vs. manured plots for each period of incubation ($n = 3$, $p < 0.05$)

Note that resistance indices for basal respiration for each soil did not show any significant difference between manured and unmanured plots, by contrast, the resistance of the induced C–CO2 respiration recorded for both soils was significantly lower in manured plots compared with that in unmanured plots

community. As a matter of fact, although the resilience indices of the microbial biomass increased during incubation, it was still far below 1 in all cases. Moreover, the analysis of the DNA-based diversity of the bacterial communities clearly showed that the community that developed after heating was different from that in the soils before

heating (Fig. 1). Some studies have shown that heating reduces the microbial biomass (Neary et al. 1999) and that bacteria appear to be more tolerant than fungi (Hart et al. 2005). Our study showed that there was no relation between the microbial biomass and the C–CO_2 fluxes. C–CO_2 respired depends on the efficiency of the substrate, for example, the fraction used for maintaining the microbial biomass, the fraction used for the synthesis of new organisms and the fraction of the energy that returns to the soil in the form of their metabolic products. This efficiency can be characterized by the specific emission of CO_2 per unit of microbial biomass per unit of time, defined as the metabolic quotient (Anderson and Domsch 1993). It is usually accepted that nearly 35–60 % of the carbon decomposed by the microorganisms is used for growth and maintenance (Shields et al. 1973), and that fungi have a higher substrate utilization efficiency than bacteria (Paul and Clark 1996). However, the metabolic quotient is also determined by the metabolic demand of microorganisms (dormant or active) (McGill et al. 1981) and the nature of the substrate (Herman et al. 1997). Therefore, our results may be explained by the carbon utilization efficiency being lower (more CO_2 respired per unit of biomass) for the communities that withstood the heating and grew after heating. The slow recovery of the microbial communities was not due to a lack of substrate but to the fact that these communities had a low growth rate. In a study on the stability of denitrifying communities, Wertz et al. (2007) also showed that their low resilience was due to the communities that withstood a pulse exposure to heat having a different cellular metabolism. For the soil amended with manure from Kabete and the unmanured from Bemasoandro, heating did not affect the global C–CO_2 respired, even though the microbial communities in the soil were different. Despite major changes in the microbial community after heating, the survivors (genetically different and less abundant) had the same C–CO_2 respiration as those in unheated soils, showing that the heterotrophic respiration function has considerable redundancy.

Stability of the induced C–CO_2 respiration

In addition to the approach based on studying the basal C–CO_2 respired by microbial communities in response to disturbance by heating, our study measured the respiration in response to the addition of an organic cocktail, meant to discriminate the functional diversity of soil microorganisms (Degens and Harris 1997). The cumulative-induced respiration recorded for unheated soil was not affected by amendment with manure, similar amounts being measured in unmanured and manured both for Kabete and Bemasoandro (Table 2). Heating the soil had no effect on the induced C–CO_2 respired from unmanured and manured plots for Kabete, it stimulated the cumulative-induced respiration of the unmanured soil of Bemasoandro (Table 2).

In both soils, the resistance (RS) to heating of the microbial biomass developed in the presence of the organic cocktail was not modified by the application of manure (Table 2). By contrast, the resistance (RS) of the induced C–CO_2 respiration recorded for both soils was significantly lower in manured plots compared with that in unmanured plots (Table 3). Heating had a greater effect on the functional community involved in the immediate decomposition of the added substrate. *R*-strategists involved in the decomposition of added high-energy substrates (Fontaine et al. 2003) are likely to be more sensitive to disturbance by heating. Moreover, the repeated application of manure seems to favour the growth of these microorganisms, as the manure is added in easily decomposable substrates. Towards the end of incubation, the resilience indices of the microbial biomass developed in the presence of the organic cocktail increased, but were lower than 1, indicating that microbial biomass did not fully recovered after heating. Moreover, the genetic structure of the bacterial community confirmed that at the end of the incubation, microorganisms surviving the heat and growing after were different from those present at the beginning of the experiment (Fig. 1). Although microbial biomass did not fully recovered, the resilience of the induced C–CO_2 respiration tended to 1 at the end of the incubation. This confirmed the fact that microbial community surviving the heat has a different metabolic quotient compared with community present before this disturbance.

Conclusion

Manuring did not affect the stability (resistance, resilience) of the microbial abundance or the basal C–CO_2 respiration. However, sensitivity to disturbance by heating of manured soils was different from that of unmanured one. The type of soil affected sensitivity and heating stimulated the basal metabolism of the microbial community of the manured soil in Kabete and the unmanured soil in Bemasoandro. Our results indicated that the microorganisms that survived heating might have different carbon utilization efficiency and that amendment using manure might produce different resistant communities. After the addition of an organic cocktail, manured soil appeared to produce *r*-strategist communities that were less resistant to disturbance by heating than other microbial communities (*K*-strategist).

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