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Impact of atmospheric CO₂ and plant life forms on soil microbial activities

Gilles Pinay^{a,b,*}, Patricia Barbera^a, Alba Carreras-Palou^a, Nathalie Fromin^a, Laurette Sonié^a, Marie Madeleine Couteaux^a, Jacques Roy^a, Laurent Philippot^c, Robert Lensi^a

^aCEFE, CNRS, 1919 route de Mende, F34293 Montpellier cedex 5, France

^bDepartment of Limnology & Hydrobotany, University of Vienna, Althanstrasse 14, A1090 Vienna Austria

^cINRA 17 rue Sully, BP 86510, 21065 Dijon, France

Abstract

From the global change perspective, increase of atmospheric CO₂ and land cover transformation are among the major impacts caused by human activities. In this study, we are addressing the combined issues of the effect of CO₂ concentration increase and plant type on soil microbial activities by asking how annual and perennial plant groups affect soil microbial processes under elevated CO₂. The experimental design used a mix of species of different growth forms for both annuals and perennials. Our objective was: (1) to determine how two years of annual or perennial plant cover and CO₂ enrichment could affect Mediterranean soil microbial processes; (2) to test the resistance and the resilience of these soil functional processes after a natural perturbation. We determined the effects of 2 years atmospheric CO₂ enrichment on soil potential respiration (SIR), denitrification (DEA) and nitrification (NEA) activities. We could not find any significant effect of CO₂ increase on SIR, DEA and NEA. However, we found a strong effect of the plant cover type, i.e. annuals versus perennials, on the potential microbial activity related to N cycling. DEA and NEA were significantly higher in soil under annual plants while SIR was not significantly different. To determine whether these changes would survive a natural perturbation, we carried out a rain event experiment once the experimental treatments (i.e. different plant cover and atmospheric CO₂ concentration) were stopped. The soil potential respiration, as expressed by the SIR, was not affected and remained stable. DEA rates converged rapidly under annuals and perennials after the rain event. Under both annuals and perennials NEA increased significantly after the rain event but remained significantly higher in the soil with annual plants. The relative change of the soil microbial processes induced by annual and perennial plants was inversely related to the density and the diversity of the corresponding microbial functional groups.

Keywords: Denitrification; Nitrification; Soil respiration; Atmospheric CO₂ enrichment

1. Introduction

The increase of atmospheric CO₂ is one of the major contributor to global changes caused by human activities. Many studies have focused on describing how the primary responses to CO₂ will develop in future ecosystems, especially through the analysis of plant responses to the future atmosphere composition. More recently, indirect effects of CO₂ via plants on soil microbial activities have

also been investigated and led to the development of a conceptual model describing the consequences of atmospheric CO₂ increase on soil microbial activities via direct and indirect plant effects (Hungate et al., 1996, 1997a, b). Elevated atmospheric CO₂ should increase carbon allocation to roots (Norby, 1994) which would in turn increase nitrogen immobilization in soils and reduce nitrification. On the other hand, organic carbon availability in the root zone coupled with an increase in soil moisture due to reduced plant transpiration would increase denitrification. This model has been partly confirmed by recent studies. For instance, in two annual grasslands, elevated CO₂ increased soil moisture, due to decreased plant

*Corresponding author. CEFE, CNRS, 1919 route de Mende, F34293 Montpellier cedex 5, France.

E-mail address: gilles.pinay@cefe.cnrs.fr (G. Pinay).

transpiration (Hungate et al., 1997b). Similarly, using natural Delta ^{15}N of soil nitrate in planted and unplanted soils with double CO_2 , Robinson and Conroy (1999) showed that plants can increase denitrification under elevated CO_2 probably by reducing evapo-transpiration and maintaining soil moisture at higher values. Baggs et al. (2003) found also that denitrification was increased under elevated $p\text{CO}_2$ in *Lolium perenne* swards and they hypothesized that an increase of belowground C allocation under elevated $p\text{CO}_2$ provides the energy for denitrification. Arnone and Bohlen (1998), Ineson et al. (1998), Baggs and Blum (2004) and Carnol et al. (2002) measured higher N_2O emission rates under elevated $p\text{CO}_2$. They assumed that enhanced root-derived available soil C acts as an energy source for denitrification. Yet, this increase of denitrification activity forecasted in the general model is not always verified because several other authors did not find any significant change in N_2O fluxes (Mosier et al., 2002, 2003) or potential denitrification activity (Barnard et al., 2004a) under increased CO_2 . In a recent review of the potential effects of global change on nitrification and denitrification Barnard et al. (2005) found that the response of potential denitrification activity to CO_2 increase was mostly negative. They suggested that the CO_2 increase was generally associated with a decrease in soil nitrate, which might reduce the availability of electron acceptors for denitrification thus reducing its activity. Similar results have been obtained by Deiglmayr et al. (2004) who reported a decreased nitrate reductase activity in the rhizosphere of *Lolium perenne* and *Trifolium repens* under elevated CO_2 .

Most of these studies coupled the direct or indirect effect of plant on soil microbial activity under increased CO_2 concentration. But they hardly addressed the role of plant type on soil microbial activity under increased CO_2 concentration. Yet, change in community composition is the other major effect of global change caused by human activities (Chapin et al., 2000). A few experiments addressed this combined issue of the effect of CO_2 concentration increase and plant species composition on soil microbial activities and their results do not present any general pattern. Barnard et al. (2004b) found that potential nitrification was strongly decreased at elevated CO_2 under *Holcus lanatus* but was not affected under *Festuca rubra*, two C3 perennial grasses. In another study dealing with elevated CO_2 concentration, Smart and Penuelas (2005) measured a greater increase of soil respiration after simulated precipitation under a ruderal perennial species (*Festuca pratensis*) than under a woody perennial subshrub (*Artemisia absinthium*). Other studies showed that plant species composition partly determines the direction of changes in soil N cycling (Hungate et al., 1996) and soil CO_2 flux (Craine et al., 2001) in response to elevated CO_2 .

These above-mentioned studies underline that there is no general pattern in the combined effects of CO_2 increase and plant community composition on soil microbial activities. This apparent absence of a general pattern might be due to the fact that these studies have mixed different functional

plant groups. In this study we address this issue by asking how functional plant groups affect soil microbial processes under elevated CO_2 . Our objectives were: (1) to determine if two years of annual versus perennial plant cover and CO_2 enrichment could affect microbial processes in a Mediterranean soil. This was performed by measuring the potential microbial respiration, nitrification and denitrification at the end of the second growing season of perennial and annual plants under CO_2 enrichment. (2) To test the resistance and the resilience of these soil functional processes after a natural perturbation. Once the experimental treatments were stopped, i.e. harvest of different plant cover and atmospheric CO_2 enrichment were harvested, we simulated a rain event to trigger soil microbial processes and monitored the soil potential microbial activities patterns for two months.

2. Materials and methods

2.1. Experimental design

Thirty two intact soil monoliths ($0.71 \times 0.71 \text{ m}$; 0.28 m deep) were excavated from an old-field at the CNRS campus in Montpellier (France, lat. $43^\circ 38' \text{E}$, long. $3^\circ 52'$) and inserted in plastic coated containers. Herbaceous vegetation had been maintained in this old-field by occasional tillage for two decades. Original vegetation was removed from the monoliths before the experiment. The soil is a clay loam (Chromic Cambisol, FAO classification) with low levels of organic matter (1.8–2.5%) and a pH of 8.2. Eight monoliths were randomly assigned to each of four greenhouses with climate control. For two years, two greenhouses were run at $350 \mu\text{mol CO}_2 \text{ mol}^{-1}$ (375 during the three winter months) and two at $700 \mu\text{mol CO}_2 \text{ mol}^{-1}$ (750 during the three winter months). At the beginning (October 2000) and then every 2–3 months during the growing period for two years, the two CO_2 concentrations were randomly assigned to the four greenhouses and the monoliths incubated at a given CO_2 concentration were randomly assigned to one of the two greenhouses run at that concentration. Temperature and CO_2 were sampled every 2 s in each greenhouse and in the adjacent experimental field. The mean annual temperature was 15.1 and 14.6°C for the 1st and 2nd year, respectively. The soil moisture was maintained by watering, following the outside precipitation: 630 and 512 mm for the 1st and 2nd year, respectively.

Genera from three common growth forms in Mediterranean old-fields (legumes, grasses and composites) were chosen so that annual and perennial species could be found in the same genera. Annual plants comprised *Bromus madritensis*, *Lolium rigidum*, *Centaurea solstitialis*, *Urospermum pteroides*, *Medicago minima*, *M. orbicularis*, *Conyza Blakei*, *C. bonariensis*. The perennial plants comprised *Bromus erectus*, *Lolium perenne*, *Centaurea aspera*, *Urospermum delechampii*, *Medicago sativa*, *M. arborea*, *Dichanthium ischaemum*, *D. Saccharoides*, *Senecio*

inaequideus, *S. pterophorus*. This combination of species provides a clear comparison of life-forms, controlled for phylogenetic variability. Seeds were collected in the vicinity of Montpellier except the wild population of *Medicago sativa* provided by INRA (Mauguio, France) and *Medicago arborea* provided by the Montpellier botanical garden. Young individuals of the latter species were assimilated to herbaceous plants. The six annual species were planted (40 individuals per species) as a mixed community in four of the eight monoliths of each greenhouse and the six perennial species were planted together in the remaining four monoliths. The planting was done in October 2000 and again in October 2001 for the annual community. A hexagonal planting pattern with 4 cm interspaces between plants provided a density of 700 plants m^{-2} . Therefore the experimental design offered 32 monoliths in four different modalities, i.e. annual and perennial herbaceous species, under either 375 or 700 $\mu\text{mol CO}_2 \text{mol}^{-1}$. Average above-ground biomass was not significantly different, neither between annuals (1574 g m^{-2} ; SE = 24) and perennials (1204 g m^{-2} ; SE = 16) nor between the 350 (1290 g m^{-2} ; SE = 25) and 700 $\mu\text{mol CO}_2 \text{mol}^{-1}$ (1496 g m^{-2} ; SE = 31) after the first year of experiment. Yet, after the second year, both decreased significantly and the annual aboveground biomass (794 g m^{-2} ; SE = 16) was more than twice than what was measured in the perennial mesocosms (340 g m^{-2} ; SE = 6). The aboveground biomass of legumes did not significantly differ with treatment and averaged ca. 40% of the total biomass. Unfortunately, belowground biomass was not measured.

2.2. Sampling strategy

Our first objective was to determine if two years of annual or perennial plant cover and CO_2 enrichment could affect Mediterranean soil microbial activities. This was performed by measuring the potential microbial respiration, nitrification and denitrification at the end of the second growing season of perennial and annual plants under CO_2 enrichment. The end of the growing period corresponds to very dry soil conditions under Mediterranean climate (less than 10% of WFP). Therefore, we sampled the soils under these dry conditions and after the plants were harvested to measure potential respiration (SIR), denitrification (DEA) and nitrification (NEA) activities.

Our second objective was to test the resistance and the resilience of these soil functional processes after a natural perturbation. Three months after the experimental treatments were stopped, i.e. atmospheric CO_2 enrichment and harvest of different plant cover, we simulated a typical Mediterranean autumn storm event by adding 70 mm of water on each monolith to trigger soil microbial processes and followed the soil potential microbial activities patterns for two months.

The samples collected before the rain event are considered to represent the residual effects of the two year

experimental protocol described above. Soil respiration and N_2O emissions were monitored 10 times during the two months following the rain event. At the same times soil samples were taken to measure the evolution of the potential microbial activities mentioned above.

2.3. Methods

Soil bulk density (D_b) was measured by oven drying a given volume of soil (251 cm^3) at 105 °C for 24 h. The bulk density was then calculated using the formula: $D_b = \text{dry mass of soil} / \text{total volume of soil}$. Soil porosity was calculated by the formula:

$S_t = 100(1 - D_b/P_p)$, where P_p , the particle density, was assumed to be equal to 2.65 g cm^{-3} (Vomocil, 1965). Soil subsamples were oven-dried for 24 h at 105 °C in order to determine dry mass and percent moisture content (P_w). Water filled pore space (WFPS) was calculated using the formula: % WFPS = P_w/S_t .

Extracts of the soil leaching after the rain event were filtered and analyzed for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ using a Technicon auto analyzer (Technicon, 1976). Nitrate was analyzed colorimetrically by the Griess-Ilosvay method (Keeney and Nelson, 1982) after reduction by percolation on copperized cadmium column. Ammonia was measured following the Indophenol Blue Method (Keeney and Nelson, 1982). DOC was measured using a Shimadzu apparatus (Shimadzu, 5000).

Soil C/N was measured using an automatic CHN analyser (Thermo-Finnagan EA 1112 NC soil analyzer).

In situ soil respiration was measured during all the sampling periods after the rewetting using a close chamber of 10 cm in diameter and 8 cm in height connected to a Li-Cor Inc., model LI6400, Lincoln, NE, USA.

Natural N_2O and CO_2 emissions were measured during all the sampling periods after the rewetting using a close chamber of 10 cm in diameter and 10 cm in height. Gas samples were collected in vacutainers after 2 h and then analyzed, using a gas chromatograph equipped with an electron capture detector (Varian Star 3800, Varian, Walnut Creek, CA, USA) for N_2O and a microcatharometer for CO_2 (Varian GC4900, Varian, Walnut Creek, California, USA).

2.3.1. Nitrifying enzyme activity measurements

This activity is equivalent to the concentration of specific nitrifying enzyme(s) present at a given moment and in a given sample where no other factor is limiting, and without de novo enzyme synthesis and cell multiplication (Tiedje, 1982). The enzymatic potential of nitrification was measured according to Lensi et al. (1986): the NO_3^- produced during an aerobic incubation was deduced from N_2O measurements after an anaerobic incubation in the presence of C_2H_2 . From each soil sample, 15 g subsamples ($n = 6$) were placed in 150 ml plasma flasks. Three subsamples were used to estimate the initial NO_3^- content: they were supplied with 6 ml of a suspension

of a denitrifying organism (*Pseudomonas fluorescens*, $OD_{580} = 2$) in a solution containing glucose and glutamic acid (final soil C content for each compound: 0.5 mg C g^{-1} dry soil). This procedure ensures high denitrifying potential and electron donors in excess. The flasks were sealed with rubber stoppers and the atmosphere of each flask was replaced by a $\text{He-C}_2\text{H}_2$ mixture (90:10) to ensure anaerobic conditions and N_2O -reductase inhibition. The N_2O accumulation was followed until a constant value (i.e. a total conversion of soil NO_3^- content into N_2O) was reached. The three other subsamples were used to determine the kinetics of NO_3^- accumulation: they were enriched with 2 ml of a $(\text{NH}_4)_2\text{SO}_4$ solution (final soil N content: 0.2 mg g^{-1} dry soil) in order to ensure a moisture content equivalent to 80% of the water holding capacity and no limitation by ammonium (presence of NH_4^+ was also supposed to limit NO_3^- assimilation by microorganisms). Then, the flasks were sealed with parafilm[®] (which prevents soil from drying but allows gas exchange) and incubated at 27°C for 48 h in a horizontal position to ensure optimal and homogeneous aeration of the soil. After this aerobic incubation which allows nitrate to accumulate, the soil samples were enriched with 6 ml of a *P. fluorescens* suspension ($OD_{580} = 2$) in a solution containing glucose and glutamic acid (in concentrations adequate to achieve the same final soil C content as above). Anaerobiosis and N_2O inhibition were obtained in the flasks as described above and the N_2O accumulation was followed until a constant value was reached. We ascertained that neither nitrate nor nitrite remained in the *Pseudomonas* suspensions before they were added to the soil: (1) by colorimetry procedure using Morgan's and Griess-Ilosway's reagents (Alexander and Clark, 1965); and (2) by anaerobic incubation of suspension aliquots (with carbon and C_2H_2 supplies) in order to verify that no N_2O was produced.

N_2O was analyzed on a gas chromatograph equipped with an electron capture detector (Varian).

The enzymatic potential of nitrification was computed by subtracting the nitrate initially present in the soil from the nitrate accumulated after aerobic incubation and expressed as $\text{ng NO}_3^- \text{-N}$ produced by per gram of dry soil and per hour.

2.3.2. Denitrification enzyme activity

Denitrification enzyme activity (DEA) was measured at each sampling period using the procedure of Smith and Tiedje (1979). From each monolith 10 g of dry soil was amended with C+N ($10 \mu\text{g NO}_3^- \text{-N g}^{-1}$ and $4 \text{ mg C-glucose g}^{-1}$ soil fresh weight basis). Samples were made anoxic by flushing with helium; acetylene was added and incubated for 4 h at 20°C . N_2O was analyzed on a gas chromatograph equipped with an electron capture detector (Varian).

2.3.3. Potential respiration (SIR)

Substrate induced respiration (SIR) was measured according to Beare et al. (1990) after incubating in a

125 ml serum flask 20 g dry mass of soil at 80% water holding capacity with $1.5 \text{ mg C-glucose g}^{-1}$ dry soil. CO_2 concentration was measured after 4 h at 25°C and in the dark using a microcatharometer (Varian GC4900, Varian, Walnut Creek, CA, USA).

2.4. Statistics

Since CO_2 levels of the greenhouses were frequently randomly assigned and the monoliths of each life-form cultivated at a given CO_2 level randomly assigned to one of the two greenhouses with that CO_2 level for the considered period, data were analyzed using two-way ANOVAs (StatBoxPro 5, Grimmersoft, France) with atmospheric CO_2 level and life-form as factors. All significances are reported at 95% significance level or more.

3. Results

3.1. CO_2 enrichment versus functional plant type

Soil C/N ratio under annuals or perennial plants treatment did not differ significantly after two years of experiment (C/N = 13.09 and 13.16 for soils under annuals and perennials, respectively). Yet, under 700 ppm CO_2 , soil C/N ratio (C/N = 13.28) was significantly higher ($p < 0.01$) than under 350 ppm CO_2 (C/N = 12.90) for both life forms.

After plant senescence and summer drought, soil organic carbon, nitrogen and potential microbial activities, i.e. respiration (SIR), denitrification (DEA) and nitrification (NEA) were measured on the 32 monoliths. DEA rates were one order of magnitude higher than NEA. Whatever the microbial process considered, no significant differences could be found between the 350 and 700 ppm treatments (Fig. 1A). However, analysis of the effect of annual versus perennial plants on the potential microbial activities showed some significant differences (Fig. 1B). In fact, NEA ($p < 0.001$) and DEA ($p < 0.01$) were significantly higher in soil from the annual plant treatment than from the perennial plant treatment ones yet, the most significant difference was for NEA. On the contrary SIR was not significantly different under annual or perennial cover.

3.2. Simulation of rain fall

The simulated rain event increased the percentage of soil moisture, expressed as percentage of water filled porosity, from 9% to 50% of WFP immediately after wetting (Fig. 2). The rain event triggered some soil leaching for several hours. The volume of leachate measured from the annuals (5.11) was significantly lower than the volume measured under the perennials (6.31). Nitrate-N leaching was 10–200 times higher than ammonia-N (Table 1). No significant effect of the CO_2 treatment could be noticed. However, the plant cover had a significant effect on the amount of mineral N and dissolved organic carbon (DOC)

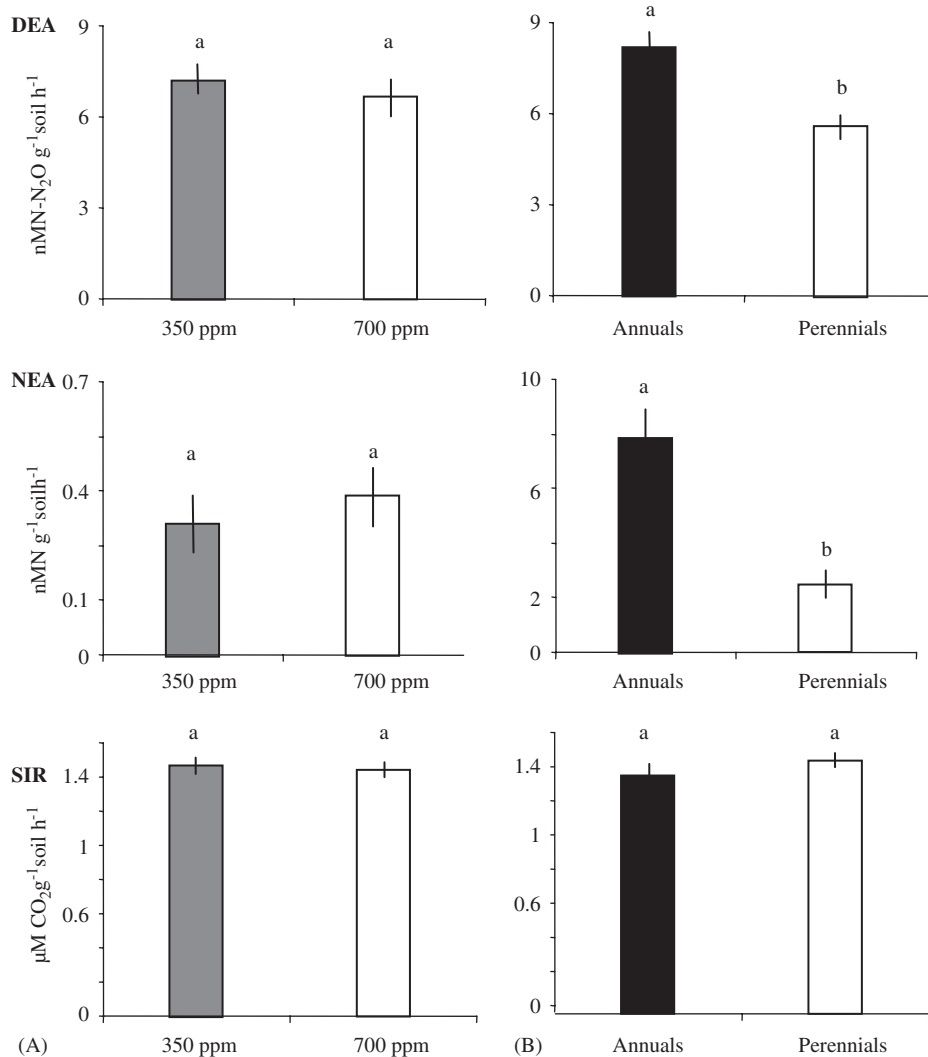


Fig. 1. Average rates of soil potential nitrification (NEA), respiration (SIR) and denitrification (DEA) measured before the rain fall simulation. (A) comparison between CO₂ atmospheric concentration with 350 and 700 ppm; (B) comparison between annual and perennial plant cover. Different small letters present significant differences between averages.

leaching the soils. Nitrate leaching was significantly higher ($p < 0.001$) under annuals than perennials while leaching of ammonia ($p < 0.002$) and DOC ($p < 0.04$) were significantly higher under perennials (Table 1). Soil moisture then declined to 30% WFP after one month and to 16% WFP after two months (Fig. 2). The soil moisture content decreased faster in soils under perennials than annuals plants; but the difference was not significant. High rates of nitrous oxide emissions were measured 1 day after the rain event in all the monoliths and sharply decreased afterwards (Fig. 3). No significant difference could be detected between treatments. *In situ* soil respiration was significantly higher ($p < 0.01$) in the soils under perennial plants than those under annual ones during the whole period following the rain event (Fig. 4); but no significant differences could be found between 350 and 700 ppm CO₂ treatments. Ten days after the rain event, SIR rates became higher in soils under perennial plants than in soils under annual ones

(Fig. 5) and remained higher until the end of the experiment, i.e. 66 days after the rainfall. However, these differences were not significant. DEA exhibited a different pattern. It was significantly higher in soils under annual plants before the rain event but then, rates did not differ significantly between soils under annual and perennial plants (Fig. 6). DEA rates fluctuated around an average value of $7 \text{ nMN-N}_2\text{O g}^{-1} \text{ h}^{-1}$. The ratio DEA/SIR was calculated using the stoichiometric relationship between the numbers of moles of nitrate reduced by denitrifying bacteria during organic carbon respiration. Since we used acetylene which blocks the last reduction reaction, the molar N/C ratio is equal to 1. Therefore, we approximated that 1 mole of N-N₂O produced was equivalent to one mole of C-CO₂ produced. Potential denitrification rate represented about 1/100 of the total potential heterotrophic respiration. The DEA/SIR ratio was significantly higher in soil under annual plants before the rain event. Then it

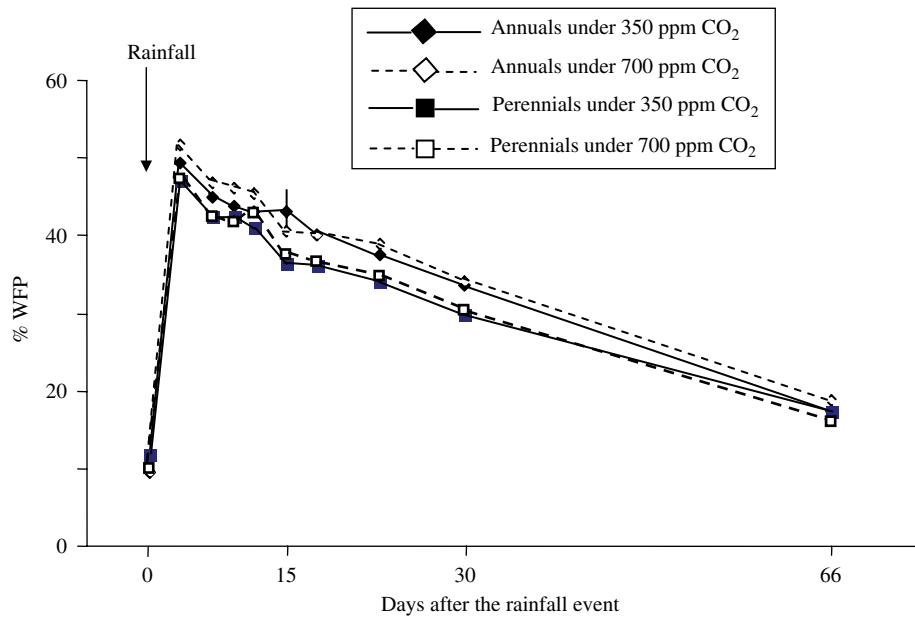


Fig. 2. Evolution of the average percentage of soil water filled porosity after the rain fall simulation in the eight monoliths of each modality, i.e. annual plants under 350 or 700 ppm of CO₂, perennial plants under 350 or 700 ppm of CO₂.

Table 1
Nutrients and DOC leaching from soils under perennials and annuals after the rain event

	Annuals average & s.e.	Perennials average & s.e.
Ammonium (mg N-NH ₄)		
350 ppm	1.58 (0.41)	5.84 (1.98)
700 ppm	0.81 (0.21)	5.27 (1.11)
Nitrate (mg N-NO ₃)		
350 ppm	183.4 (15.6)	113.3 (34.4)
700 ppm	184.0 (14.1)	61.3 (13.5)
Dissolved organic carbon (mg DOC)		
350 ppm	124.6 (13.5)	174.7 (22.7)
700 ppm	125.9 (14.9)	177.6 (34.7)

The average volume of water leaching from soils planted with annuals was 5.11 and 6.31 from soils planted with perennials.

followed a similar pattern as DEA with no significant differences between annual and perennial treatments.

3.3. Resilience of microbial processes

The comparison of the three potential microbial processes, before the rainfall and two months after the event showed different patterns (Fig. 7). Soil SIR did not change, neither under annual nor perennial plants. DEA decreased in soils under annual plants while it increased in soils under perennial ones. As a result, two months after the rain event, DEA rates in soil under annual and perennial plants did not differ significantly anymore. NEA increased significantly two months after the rain event both in soil under annual and perennial plants. The significantly

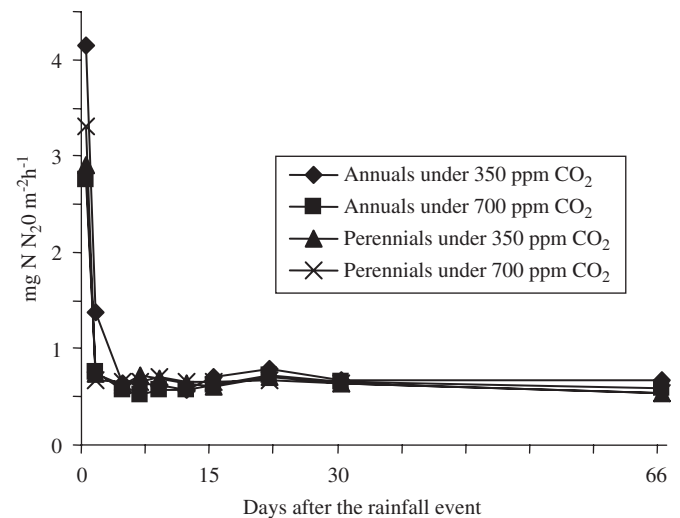


Fig. 3. Evolution of the average in situ N₂O emission after the rain fall simulation in the eight monoliths of each modality, i.e. annual plants under 350 or 700 ppm of CO₂, perennial plants under 350 or 700 ppm of CO₂.

higher rates under annual plants versus perennial plants persisted and even became more significant two months after the rain event.

4. Discussion

We first determined whether two years of atmospheric CO₂ enrichment could affect potential respiration (SIR), denitrification (DEA) and nitrification (NEA) activities in a Mediterranean soil. We could not find any significant effect of CO₂ increase on SIR, DEA and NEA (Fig. 1A)

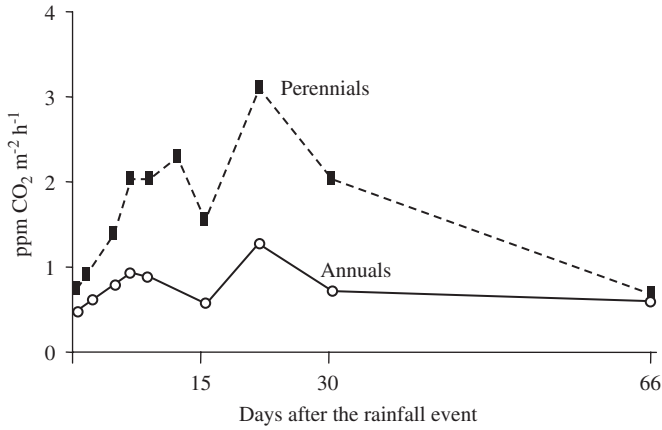


Fig. 4. Evolution of the average *in situ* respiration of the 16 monolith soils under annuals and perennials plant cover after the rain fall simulation.

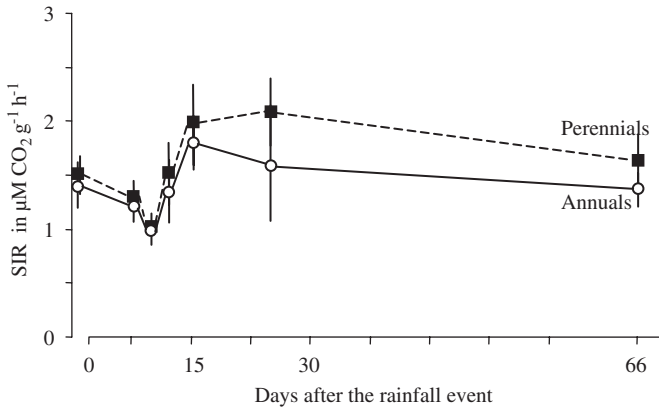


Fig. 5. Evolution of the average and standard error (se) of the potential respiration (SIR) of the 16 monoliths under annuals (open circle) and perennials (black square) plant cover after the rain fall simulation. The first two points on the left-hand side of the graph are the average values measured before the rain fall event.

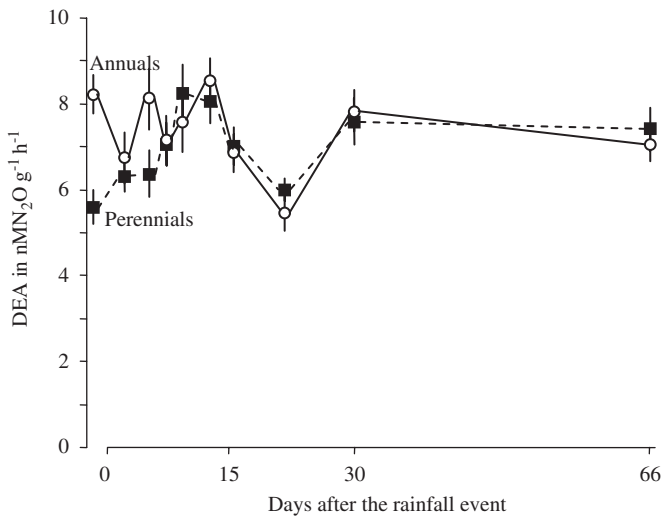
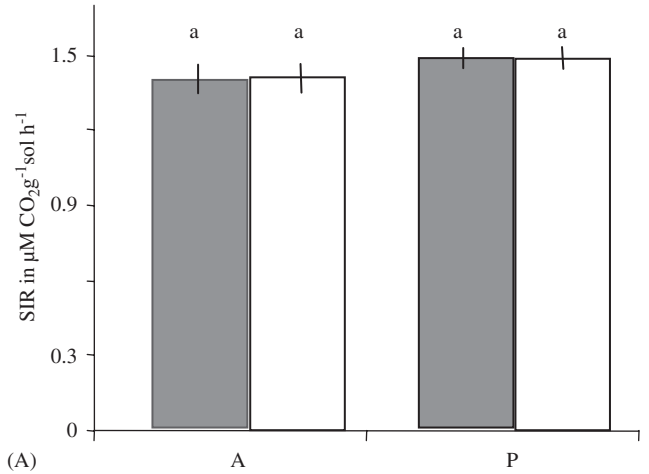
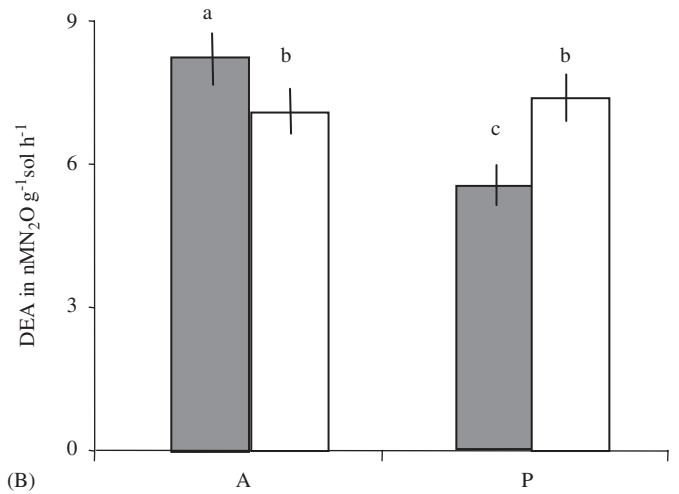


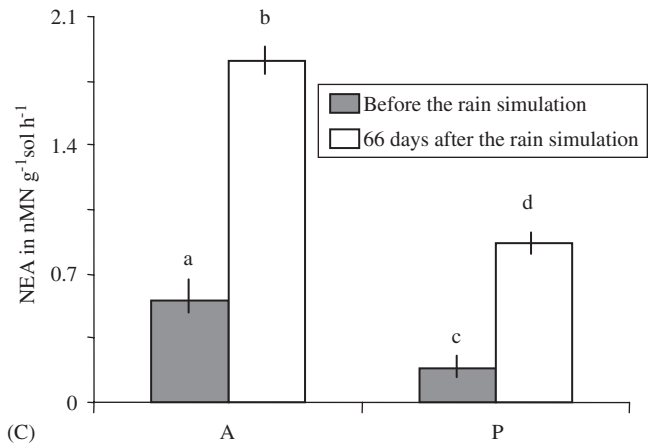
Fig. 6. Evolution of the average and standard error (se) of the potential denitrification (DEA) of the 16 monoliths under annuals (open circle) and perennials (black square) plant cover after the rain fall simulation. The first two points on the left-hand side of the graph are the average values measured before the rain fall event.



(A)



(B)



(C)

Fig. 7. Comparison of (A) the average potential respiration (SIR), (B) the average potential denitrification (DEA), and (C) the average potential nitrification (NEA) in soil under annual and perennials plant cover. Dark bars represent averages of these microbial activity potentials before the rain fall event and the white bars represent the averages 66 days after the rain fall event. Different small letters (a,b,c,d) present significant differences between averages.

despite a higher C/N ratio under 700 ppm CO₂. This absence of difference in potential microbial activities is in accordance with Mosier et al. (2003) who could not find any significant alteration of CO₂, NO_x and N₂O fluxes on

weekly measurements throughout the year during 4.5 years of CO₂ increase. This was also mentioned by [Barnard et al. \(2004a\)](#) who did not find any significant change in NEA and DEA under elevated CO₂ in four different grasslands.

4.1. Effect of plant cover type

We found a strong effect of the plant cover type, i.e. annuals versus perennials, on the potential microbial activity related to N cycling ([Fig. 1B](#)). In fact, DEA and NEA were significantly higher in soil under annual plants while SIR was not significantly different. NEA and DEA are both controlled by soil water content, readily available organic matter and inorganic nitrogen ([Knowles, 1982](#)). These factors are affected indirectly by CO₂ and vegetation type ([Barnard et al., 2005](#)). Yet, the vegetation cover was the main factor explaining these differences in potential activities. Two complementary reasons might explain this higher potential nitrification and denitrification under annual plants. Soil organic matter turn over under annual plants may be faster due to a faster decay of the root system ([Mun and Whitford, 1998](#); [Silver and Miya, 2001](#)). This would provide larger organic nitrogen to be mineralized. Eventually a part of the produced nitrate can be respired by denitrifiers using available C from the roots as a source of energy. Soil organic carbon under annuals is probably more rapidly used than under perennials; this could explain the significantly larger leakage of DOC after the rain event under perennials ([Table 1](#)). The second reason which could explain the higher DEA and NEA under annuals is that perennials maintain a root system which affects the soil structure by forming preferential water flow paths, provoking a better drainage system ([Yunusa and Newton, 2003](#); [Cresswell and Kirkegaard, 1995](#); [Chan et al., 2001](#)). In fact, soil moisture declined faster under the perennials than under the annuals after the rain event ([Fig. 2](#)); although not statistically significant at $p < 0.05$, this difference in drainage pattern could allow the maintenance of wetter soil conditions under annuals which are more favourable to sustain both nitrification and denitrification activity in dry Mediterranean conditions. Absence of significant differences in potential respiration (SIR), both between CO₂ and plant treatments suggests that this soil process is very resistant to perturbation. A large range of organisms is indeed capable of decomposing organic matter. Therefore, a change in the atmospheric CO₂ content, plant cover or a slight change in soil moisture content would not affect whole groups of organisms responsible for the soil fluxes of CO₂ ([Schimel and Gullege, 1998](#)). On the contrary, denitrifiers — and nitrifiers to an even larger extend — represent a much smaller diversity and size of community in soils ([Gamble et al., 1977](#); [Tiedje, 1988](#)). Thus, environmental changes such as those manipulated in the experiment are more likely to affect their community structure and through this, their functional activity. The ratio of potential respiration due to denitrifiers compared to total respiration was significantly

higher in soils under annuals than under perennials; it was calculated at about 1.5% of the total potential respiration. The two year experiment with annual and perennial plants seems to have established different soil heterotrophic community structures, annual plants favouring denitrifiers as compared to perennial ones.

4.2. Consequences of the rain fall event on soil microbial processes

The rain event experiment was performed to determine whether these functional changes could persist or converge after a natural perturbation, once the experimental treatments (i.e. different plant cover and atmospheric CO₂ concentration) were stopped. The rain event revealed different patterns among the three microbial activities. SIR often presented higher rates in soils under perennial plants but not significantly different than those measured in soils under annual plants ([Fig. 5](#)). Yet, *in situ* respiration rates were significantly higher under perennials ([Fig. 4](#)). However, the heterotrophic respiration functionality, as expressed by the SIR, is not significantly affected and remains rather stable. DEA rates converged rapidly under annuals and perennials after the rain event ([Fig. 6](#)). This result reveals that the functional differences in DEA triggered directly (organic carbon supply) and indirectly (soil moisture) by plant cover are not resistant to perturbation. Once the vegetation cover is harvested, denitrification functionality converged to an average rate. It would be interesting to determine whether this convergence is solely due to the convergence of environmental conditions or to the convergence of functional denitrifying communities. NEA followed a different pattern. Under both annuals and perennials, NEA increased significantly after the rain event ([Fig. 7](#)). The moisture increase triggered organic matter decomposition which was revealed by an increase of *in situ* respiration ([Fig. 4](#)). This organic matter mineralization has entailed an increase of ammonification and nitrification. Yet, the functional differences between annuals and perennials measured after the two-year treatment, remained after the rain event ([Fig. 7](#)). We could not determine in this experiment whether this functional difference was due to a difference in the nitrifying microbial community structure and or density in response to differences in environmental conditions. Yet, this difference seems robust. High nitrate leaching after the rain event under the annuals ([Table 1](#)) reveals faster N cycling in soil under annuals versus perennials. Nitrification is a narrow physiological process ([Kowalchuk and Stephen, 2001](#)). Nitrification is produced by bacteria species in few genera. Therefore, this process is under the control of a very distinct bacteria community which may not allow much functional redundancy. It is possible that environmental differences such as perennial versus annual plant covers, would have triggered a drastic change in the composition of the nitrifying functional community. The second possibility is that it might have changed nitrification

dynamics. Indeed, some total nitrification inhibition by savanna plant species (*Hyparrhenia diplandra*) has been reported (Lata et al., 1999), but there is no indication of such inhibitory effect among the six perennial species planted in this experiment. Another argument in favour of a change in nitrifying community density rather than a change in the structure is that potential nitrification increased at the same rate (doubling in 60 days) under both annuals and perennials. Therefore, we hypothesize that the differences in environmental conditions, i.e. annuals versus perennials, have affected the density rather than the community structure of nitrifiers.

5. Conclusion

Soil microbial activities measured by SIR, DEA and NEA were not sensitive to an increase of atmospheric CO₂ but some of these functions were significantly altered by the type of plant cover, i.e. annuals versus perennials. The relative change of the soil microbial processes induced by annual and perennial plants was inversely related to the density and the diversity of the corresponding functional bacterial groups, i.e. change in nitrification (NEA) > change in denitrification (DEA) > change in respiration (SIR). In other words, the functional community with the least diversity and density, i.e. nitrification, was the most affected by the plant cover type and these changes remained after the rain event. On the contrary, the respiration process, under the control of a wide diversity and density of micro-organisms, did not present any significant change. Denitrification presented an intermediate pattern with significant rate differences after the two-year experiment, but functionally converged two months after the rain event.

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