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# Impact of soil matric potential on the fine-scale spatial distribution and activity of specific microbial degrader communities

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[Correction added after online publication 24 May 2012: All author forenames and surnames corrected]

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## Keywords

2,4-D; relative abundance of degraders; water content; glucose; organic substrate mineralization; spatial variability.

## Introduction

Soil is made up of a huge diversity of microhabitats with a range of different properties, such as variable O<sub>2</sub> levels, moisture content and pH (Sextone *et al.*, 1985; Nunan *et al.*, 2006; Young *et al.*, 2008; Franklin & Mills, 2009). This results in highly heterogeneous distributions of soil microorganisms and microbial activity at very fine scales (Parkin, 1993; Ranjard *et al.*, 2000; Nunan *et al.*, 2002; Gonod *et al.*, 2003). The type of spatial distribution (aggregated, homogeneous and random) of microorganisms is thought to affect ecosystem functioning. For example, it has been shown that even distributions of microbial degraders result in higher

## Abstract

The impact of the soil matric potential on the relationship between the relative abundance of degraders and their activity and on the spatial distribution of both at fine scales was determined to understand the role of environmental conditions in the degradation of organic substrates. The mineralization of <sup>13</sup>C-glucose and <sup>13</sup>C-2,4-dichlorophenoxyacetic acid (2,4-D) was measured at different matric potentials (−0.001, −0.01 and −0.316 MPa) in 6 × 6 × 6 mm<sup>3</sup> cubes excised from soil cores. At the end of the incubation, total bacterial and 2,4-D degrader abundances were determined by quantifying the 16S rRNA and the *tfdA* genes, respectively. The mineralization of 2,4-D was more sensitive to changes in matric potential than was that of glucose. The amount and spatial structure of 2,4-D mineralization decreased with matric potential, whilst the spatial variability increased. On the other hand, the spatial variation of glucose mineralization was less affected by changes in matric potential. The relationship between the relative abundance of 2,4-D degraders and 2,4-D mineralization was significantly affected by matric potential: the relative abundance of *tfdA* needed to be higher to reach a given level of 2,4-D mineralization in dryer than in moister conditions. The data show how microbial interactions with their microhabitat can have an impact on soil processes at larger scales.

2,4-dichlorophenoxyacetic acid (2,4-D) degradation rates than aggregated distributions (Dechesne *et al.*, 2010). The physical structure of soil regulates the diffusion and the availability of substrates and metabolites to and from microbial cells (Strong *et al.*, 1997; Chenu & Stotzky, 2001). Therefore, the spatial localization of microorganisms in the three-dimensional structure may play an important role in microbial processes and the persistence and turnover of organic compounds in soil (Foster, 1988; Strong *et al.*, 1998), by affecting the probability of encounter between degraders and substrate (Pallud *et al.*, 2004).

The degradation of organic substrates depends not only of the presence of degraders but also on environmental

factors, particularly soil water content. Linn & Doran (1984) showed that aerobic microbial activity increases with soil water content up until a maximum point ( $-0.01$  MPa) before decreasing. Schroll *et al.* (2006) demonstrated that the optimal matric potential for the mineralization of a number of pesticides (isoproturon, benazolin-ethyl, and glyphosate) was  $-0.015$  MPa and that, when the soil moisture was close to water-holding capacity, pesticide mineralization was considerably reduced. Variations in soil moisture affect the diffusion of soluble substrates, the motility of microorganisms and the diffusion of oxygen which, in turn, affect soil microbial activities (Skopp *et al.*, 1990; Cattaneo *et al.*, 1997; Treves *et al.*, 2003; Or *et al.*, 2007; Dechesne *et al.*, 2010). By acting on the physiological status of bacteria, soil moisture content affects their subsequent substrate utilization response (Harris, 1981; Griffiths *et al.*, 2003). Moreover, higher moisture contents create connections between different microhabitats, resulting in modifications in interactions between microorganisms and competition among cells. Changes in soil moisture content therefore sustain high level of functional diversity (Or *et al.*, 2007; Young *et al.*, 2008).

A consequence of the heterogeneous nature of soil is that microbial 'perception' of macro-environmental conditions is highly dependent on both location within the soil pore network and spatial distribution of the microorganisms. For example, at certain matric potentials bacteria in large pores will not have sufficient water for activity, whilst those in small pores will experience optimal  $O_2$  and moisture levels (Young *et al.*, 2008). Widely and evenly spread microorganisms will, on average, experience conditions that are very similar to the macro-conditions, but this will not be the case for aggregated microorganisms, which will be very dependent on their location within the pore network (in large or small pores for example). From a functional standpoint, the spatial distribution of a given functional group may affect the response of the function to changes in environmental conditions. Degradation carried out by aggregated degraders may be more sensitive to changes in environmental conditions as they are likely confined to specific microhabitats.

The aims of the present study were threefold: (1) to determine the effect of changes in matric potential ( $-0.001$ ,  $-0.01$  and  $-0.316$  MPa corresponding to 0.40, 0.25 and 0.16 g of water per g dry soil, respectively) on glucose and 2,4-D mineralization rates and on the spatial variability of both of these processes; (2) to determine the influence of matric potential on total and degrading soil bacterial populations and (3) to determine how the relationship between the relative abundance of 2,4-D degraders and mineralization was affected by variations

in environmental conditions, in this case moisture levels. Glucose and 2,4-D were chosen as model substrates. Glucose is a readily metabolized substrate used in different metabolic pathways (White, 2007). It is assimilated by many soil microorganisms and shows little spatial variation at fine scales. Glucose mineralization can therefore be considered to have a wide niche breadth and to be present in many microhabitats in soil. 2,4-D, on the other hand, is a complex substrate degraded either by specific microorganisms harbouring the genes encoding for the degradation or by co-metabolism (Soulas, 1993). 2,4-D mineralization may be carried out by one microorganism possessing all the enzymes necessary to degrade 2,4-D or by a consortium of microbial species acting together (Ou & Thomas, 1994; Vallaey *et al.*, 1997). The more limited number of organisms involved in 2,4-D mineralization suggests that the niche breadth is narrower than that of glucose degraders, and therefore, the spatial distribution is more aggregated and confined to specific microhabitats (Gonod *et al.*, 2003; Hybholt *et al.*, 2011). It was hypothesized that average glucose degradation would be less affected by variations in matric potential than average 2,4-D degradation owing to the widespread and less aggregated nature of microorganisms able to use glucose as a substrate. It was also hypothesized that the spatial distribution of 2,4-D degradation would be more sensitive to changes in matric potential than that of glucose.

The microplate system recently developed by Monard *et al.* (2010), in which the mineralization of  $^{13}C$ -labelled substrates can be measured in small soil samples ( $216\text{ mm}^3$ ), was used. At the end of the incubations, total bacterial and 2,4-D degrader abundances were estimated by quantifying the 16S rRNA gene and the first gene involved in 2,4-D biodegradation and leading to 2,4-dichlorophenol, *tfdA*, on the same samples as used to measure mineralization.

## Materials and methods

### Soil

Undisturbed soil cores (diameter 80 mm, height 100 mm) were sampled in the Spring of 2009 from the top 0–30 cm horizon at an INRA (Institut National de Recherche Agronomique) field experiment in the park of the Palace of Versailles. The experimental plots from which samples were taken had been cropped to wheat for the previous 16 years and had never been treated with 2,4-D during this period. The soil was a silt loam (30% sand, 53% silt and 17% clay) with a pH of 6.8. Soil cores were stored at  $4\text{ }^\circ\text{C}$  before use. The sampling water content was of  $0.15\text{ g g}^{-1}$  soil.

## Preparation of soil samples

To compare substrate mineralization in soil cubes, we determined whether it was better to work on soil slices sampled at the same depth in different soil cores or on soil slices sampled at different depths between 0 and 80 mm in the same soil core. The total soil microbial biomass was thus measured by fumigation–extraction (Jenkinson & Powlson, 1976; Vance *et al.*, 1987) in slices sampled at eight different depths for five soil cores, and the results showed that intra soil core variability was less important than that among cores (coefficients of variation of 12% vs. 27%). On this basis, all measurements were performed using cubes from a single soil core. One entire slice was used per substrate and matric potential to allow visual assessment of the spatial distribution of the activity measured.

Soil cubes were excised from the soil core as described by Gonod *et al.* (2003). Briefly, the soil core was sectioned into consecutive slices (thickness 6 mm), and each slice was divided into 74 undisturbed soil cubes ( $6 \times 6 \times 6 \text{ mm}^3$ ;  $418 \pm 39 \text{ mg}$  dry soil). The relative location of each cube on the slice was recorded.

## Substrate mineralization

Under  $\text{CO}_2$ -free atmosphere, soil cubes ( $n = 74$  per substrate and per matric potential) were amended with solutions of either  $^{13}\text{C}$ -glucose ( $\delta^{13}\text{C}_{\text{glucose}} = 4527, 4414$  or  $4014\text{‰}$ ; Euriso-Top, France) or  $^{13}\text{C}$ -2,4-D ( $\delta^{13}\text{C}_{2,4\text{-D}} = 5027, 4778$  or  $5054\text{‰}$ ; Dislab'system, France) to bring them to a matric potential  $\Psi$  of  $-0.001$  (pF 1),  $-0.01$  (pF 2) and  $-0.316$  MPa (pF 3.5) corresponding to 0.40, 0.25 and 0.16 g of water per g dry soil, respectively. Therefore, the substrates were added to pores with a maximum pore neck diameter of 300, 30 and 1  $\mu\text{m}$ , respectively. The loading rate was  $13 \mu\text{g C g}^{-1}$  dry soil for all cubes, corresponding to  $30 \mu\text{g 2,4-D g}^{-1}$  dry soil and  $32.6 \mu\text{g glucose g}^{-1}$  dry soil. Substrate mineralization in each soil cube was measured using the experimental device developed by Monard *et al.* (2010). This device consisted of 24-well microtitre plates into which were placed the soil cubes, one per well. A glass microfiber filter (Prat-Dumas, France) impregnated with a solution of sodium hydroxide (0.2 N; Titrisol<sup>®</sup>, Merck) was then placed on cylindrical plastic supports in each well to trap the  $^{12}\text{C}$ - and  $^{13}\text{C}$ -labelled  $\text{CO}_2$ . The microtitre plate was covered with a seal made of Viton<sup>®</sup> and closed with a clamp designed to apply a homogeneous pressure over the whole microtitre plate, ensuring that each well was closed with an airtight seal. Samples were incubated at  $20 \text{ °C}$  in the dark during 48 and 96 h for glucose and 2,4-D amended samples, respectively. These two durations

corresponded to the middle of the exponential phase of the mineralization curves (data not shown).

At the end of the incubation, the microtitre plates were opened under  $\text{CO}_2$ -free atmosphere and the filters transferred to 10-mL glass vials with Teflon<sup>®</sup> septa sealed on with crimped aluminium seals. Phosphoric acid (500  $\mu\text{L}$ , 85%, Merck) was subsequently added through the septum with a syringe to release the  $\text{CO}_2$  from the filters. The filters were left to react with the acid for 24 h at  $50 \text{ °C}$ , and the resulting  $\text{CO}_2$  was quantified with a micro-GC (Agilent 3000A, Qplot column). The isotopic signature of the C– $\text{CO}_2$  was determined using a GC (Hewlett-Packard 5890) coupled to an isotopic ratio mass spectrometer (Isochrom Optima, Micromass). The analytical variability of these instruments was always less than 1%. The cubes of soil were stored at  $-20 \text{ °C}$  for subsequent DNA extraction.

## DNA extraction

DNA was extracted from all cubes that had been incubated with 2,4-D at  $\Psi = -0.001$  MPa ( $n = 74$ ) and from 20 and 16 cubes incubated with 2,4-D at  $\Psi = -0.316$  and  $-0.01$  MPa, respectively. In the latter two cases, the soil cubes were chosen to have a range of 2,4-D mineralization activities. DNA extraction was performed using 250 mg of soil according to the ISO standard 11063 (Petric *et al.*, 2011) derived from Martin-Laurent *et al.* (2001). DNA was quantified at 260 nm using a Biophotometer (Eppendorf, Hamburg, Germany).

## Quantitative PCR assays

Absolute quantifications of 16S rRNA and *tfdA* genes were performed in triplicate using standard curves generated with known copy numbers of the gene of interest as described by Monard *et al.* (2008). Prior to quantification, the absence of inhibitors was determined by comparing the quantification of standard plasmid to that of soil DNA spiked with known amount standard plasmid.

Quantitative PCR was carried out in an ABI Prism 7900HT (Applied Biosystems) apparatus. The 20- $\mu\text{L}$  reaction mixtures contained 10  $\mu\text{L}$  of ABsolute<sup>™</sup> qPCR SYBR<sup>®</sup> Green Mix (Thermo Scientific ABgene, UK), 0.3  $\mu\text{M}$  of 341f (Muyzer *et al.*, 1993) and 515r (Xia *et al.*, 2000) for amplification of the 16S rRNA gene or TfdAr (Baelum *et al.*, 2006) and TfdAr (Baelum *et al.*, 2008) for amplification of the *tfdA* gene, 2  $\mu\text{L}$  of 10 times diluted DNA, 1  $\mu\text{L}$  of T4 gp 32 (QBiogene) and 3.5  $\mu\text{L}$  of ultrapure water. The thermal cycling conditions for the 16S rRNA sequence amplification consisted of an initial step of 15 min at  $95 \text{ °C}$  followed by 30 cycles at  $95 \text{ °C}$  for 15 s,  $60 \text{ °C}$  for 30 s,  $72 \text{ °C}$  for 30 s and  $80 \text{ °C}$  for 30 s.

Additional cycles of 95 °C for 15 s, 80 °C for 15 s and 95 °C for 15 s were performed to obtain specific dissociation curves for the targeted sequence and thereby to check the purity of the qPCR. Identical thermal cycling conditions were used to quantify *tfDA* except that 40 cycles were performed instead of 30 with an annealing temperature of 64 °C.

The calibration curves were as follows:

$$C_t = -3.2 \times (\text{number of 16S rRNA sequences}) + 37.82 \quad (R^2 = 0.99)$$

$$C_t = -3.4 \times (\text{number of } tfDA \text{ sequences}) + 36.78 \quad (R^2 = 0.99)$$

### Calculations

The amount of substrate mineralized was determined as described by Monard *et al.* (2010) using the following equation:

$$C_{\text{Substrate}} = C_{\text{Respired}} \times \left( \frac{\delta^{13}\text{C}_{\text{Respired}} - \delta^{13}\text{C}_{\text{SOM}}}{\delta^{13}\text{C}_{\text{Substrate}} - \delta^{13}\text{C}_{\text{SOM}}} \right) \quad (1)$$

where  $C_{\text{Substrate}}$  is the amount of added substrate C mineralized,  $C_{\text{Respired}}$  is the amount of C derived from the mineralization of both soil organic matter (SOM) and substrate,  $\delta^{13}\text{C}_{\text{Substrate}}$  and  $\delta^{13}\text{C}_{\text{SOM}}$  are the  $^{13}\text{C}$  isotopic signatures of the substrate and the SOM C, respectively.  $\delta^{13}\text{C}_{\text{SOM}}$  was determined from control samples incubated without substrate addition and was equal to  $-35.5\text{‰}$ .

### Data and statistical analysis

The extent of spatial autocorrelation was determined using the SPDEP package (SPDEP, spatial dependence: weighting schemes, statistics and models, version 0.5-26; Bivand, 2009) in R (R: A Language and Environment for Statistical Computing, 2010; <http://www.R-project.org/>). Moran's  $I$  coefficient was computed over multiple distances, and a correlogram was constructed by plotting the coefficient against neighbour distances.

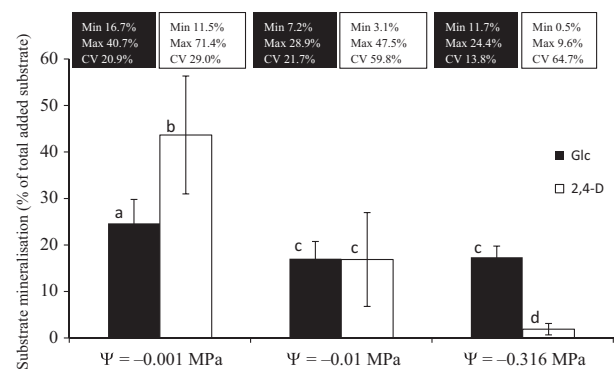
Treatment effects were tested by analysis of variance. When testing for treatment effects, the occurrence of spatial autocorrelation had to be accounted for. To achieve this, the Type I error rate,  $\alpha$ , was adjusted to a more conservative value,  $\alpha' = \alpha/5$ . With positive spatial autocorrelation, there is a lack of independence among sample neighbours. This reduces within-group variability, artificially increasing the relative treatment variance (Legendre & Legendre, 1998). The problem is essentially a reduction in the effective sample size (Dale & Fortin, 2002). A 1%

significance level was used rather than 5% because the critical value for 1% with  $\nu = 70$  (the degrees of freedom) in these experiments had all samples been independent is close to the critical value for 5% with  $\nu = 6$ . This means that even if the effective sample size was reduced by an order of magnitude owing to spatial autocorrelation, a Type I error rate of  $< 0.05$  was certain and any detected significance could be trusted (Dale & Fortin, 2002).

## Results

### Impact of matric potential on substrate mineralization and its spatial heterogeneity

Mean glucose mineralization reached 24.7%, 17.1% and 17.4% at  $\Psi = -0.001$ ,  $-0.01$  and  $-0.316$  MPa, respectively. It was significantly higher in the wettest system ( $P < 0.001$ ; Fig. 1). The variability of glucose mineralization also changed with moisture content, with coefficients of variation of 20.9% and 21.7% at  $\Psi = -0.001$  and  $-0.01$  MPa, respectively, but only of 13.8% at  $\Psi = -0.316$  MPa. 2,4-D mineralization was significantly different at all the matric potentials tested ( $P < 0.001$ ) and was higher at  $\Psi = -0.001$  MPa (43.7%) than at  $\Psi = -0.316$  MPa (1.9%) with an intermediate value at  $\Psi = -0.01$  MPa (16.7%). The coefficient of variation of 2,4-D mineralization ranged between 29% and 65%. The soil water content had a significant impact on both glucose and 2,4-D mineralization ( $F = 88.8$ ,  $P < 0.01$  and  $F = 365$ ,  $P < 0.01$ , respectively, Fig. 1), but the mineralization of 2,4-D was far more sensitive to changes in matric potential than was the mineralization of glucose. There was a 23-fold increase in the mineralization of 2,4-D between matric potentials  $\Psi$  of  $-0.316$  and



**Fig. 1.** Mineralisation of glucose (black) and 2,4-D (white) in 74 soil cubes of one soil core slice at  $\Psi = -0.001$ ,  $-0.01$  and  $-0.316$  MPa. Error bars indicate standard deviations. Minimum and maximum values and coefficients of variation are indicated.

−0.001 MPa whilst glucose mineralization only increased by a factor of 1.4 (Fig. 1). Furthermore, glucose mineralization only showed a significant difference between the matric potential  $\Psi = -0.001$  MPa and the other matric potentials. The coefficients of variation of glucose and 2,4-D mineralizations displayed opposite relationships with matric potential. As the matric potential decreased, the variability of 2,4-D mineralization increased whilst that of glucose was less variable and more stable before decreasing (Fig. 1). In the dryer system, the mineralization of glucose was higher than that of 2,4-D, but the contrary was observed at  $\Psi = -0.001$  MPa. The mineralization of the two substrates was similar at the intermediate matric potential.

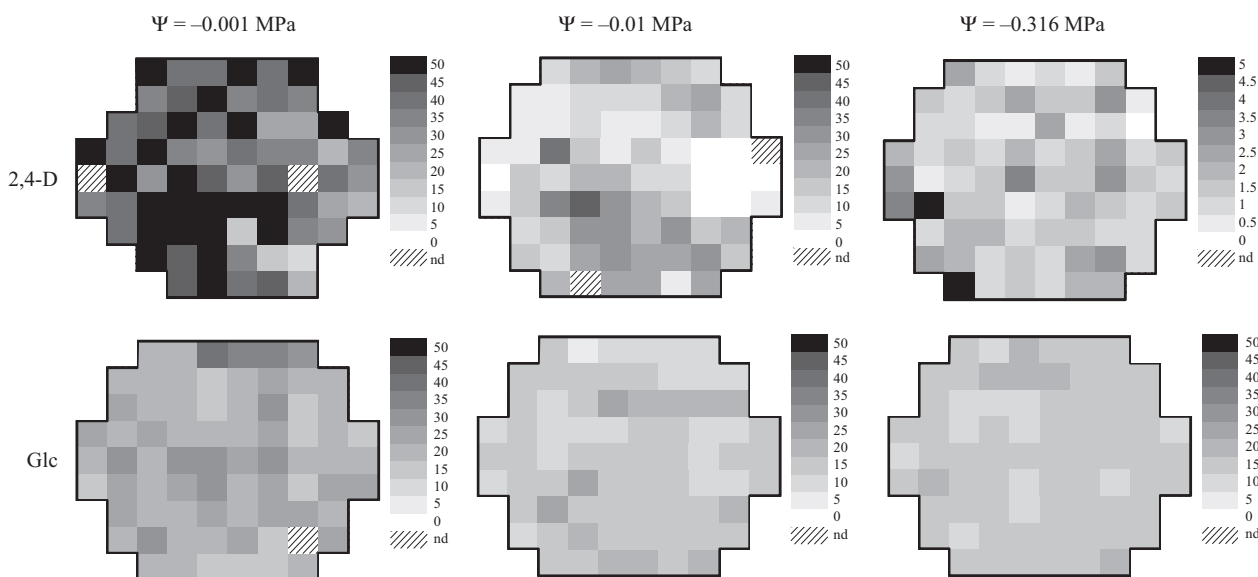
### Spatial structure of glucose and 2,4-D mineralization

The mineralization maps at the end of the incubations showed that the spatial structure of both glucose and 2,4-D mineralization at the millimetre scale was affected by the matric potential of the samples (Figs 2 and 3). 2,4-D mineralization displayed significant spatial autocorrelation at  $\Psi$  of −0.01 and −0.001 MPa and complete spatial randomness at −0.316 MPa (Fig. 3). The same was true for glucose mineralization but the spatial autocorrelation of 2,4-D mineralization was stronger and extended to greater distances (Fig. 3).

### Effect of matric potential on total and 2,4-D degrading bacterial communities

Between  $1.7 \times 10^6$  and  $3.8 \times 10^8$  bacteria (16S rRNA sequence) and between  $1.9 \times 10^4$  and  $3.8 \times 10^6$  degrading microorganisms (*tfdA* sequence) were found per g of dry soil at the end of the incubations (Table 1, Supporting Information, Fig. S1). There was no soil cube without 2,4-D degrading bacteria. The sequence copy numbers of both the 16S rRNA and the *tfdA* genes were significantly lower in samples incubated at  $\Psi = -0.316$  MPa than in the other samples ( $P < 0.01$ ,  $T = -3.41$  and  $P < 0.01$ ,  $T = -4.17$ , respectively), and no significant difference was observed between the other two treatments ( $P = 0.71$ ,  $T = 0.38$  and  $P = 0.63$ ,  $T = -0.49$ , respectively, Table 1, Fig. S1). The relative abundance of 2,4-D degraders in the samples (expressed as the *tfdA* sequence copy number per 16S rRNA sequence copy number) was also significantly lower in the drier samples ( $3.9 \times 10^{-3}$  copies of *tfdA*/16S at  $\Psi = -0.316$  MPa vs.  $1.5 \times 10^{-2}$  at  $\Psi = -0.01$  MPa and  $1.1 \times 10^{-2}$  at  $\Psi = -0.001$  MPa,  $P < 0.01$ ,  $T = -3.88$ ; Table 1, Fig. S1).

There were sufficient values for spatial analysis of the distribution of 2,4-D degrading genetic potential and the 16S rRNA gene in the samples incubated at  $\Psi = -0.001$  MPa only. At this matric potential, there was evidence for a weak spatial structure in the distribution of *tfdA* sequence copy number, but not for the 16S rRNA sequence copy number (data not shown).



**Fig. 2.** Spatial distribution of 2,4-D (top row) and glucose (bottom row) mineralization (represented as % of added substrates) at different matric potentials ( $\Psi = -0.001$ ,  $-0.01$  and  $-0.316$  MPa). Each square represents the mineralization in one spatially referenced soil cube. Note that the scale for the 2,4-D mineralization at  $\Psi = -0.316$  MPa is reduced to allow visualization of the spatial heterogeneity. (nd: not determined).

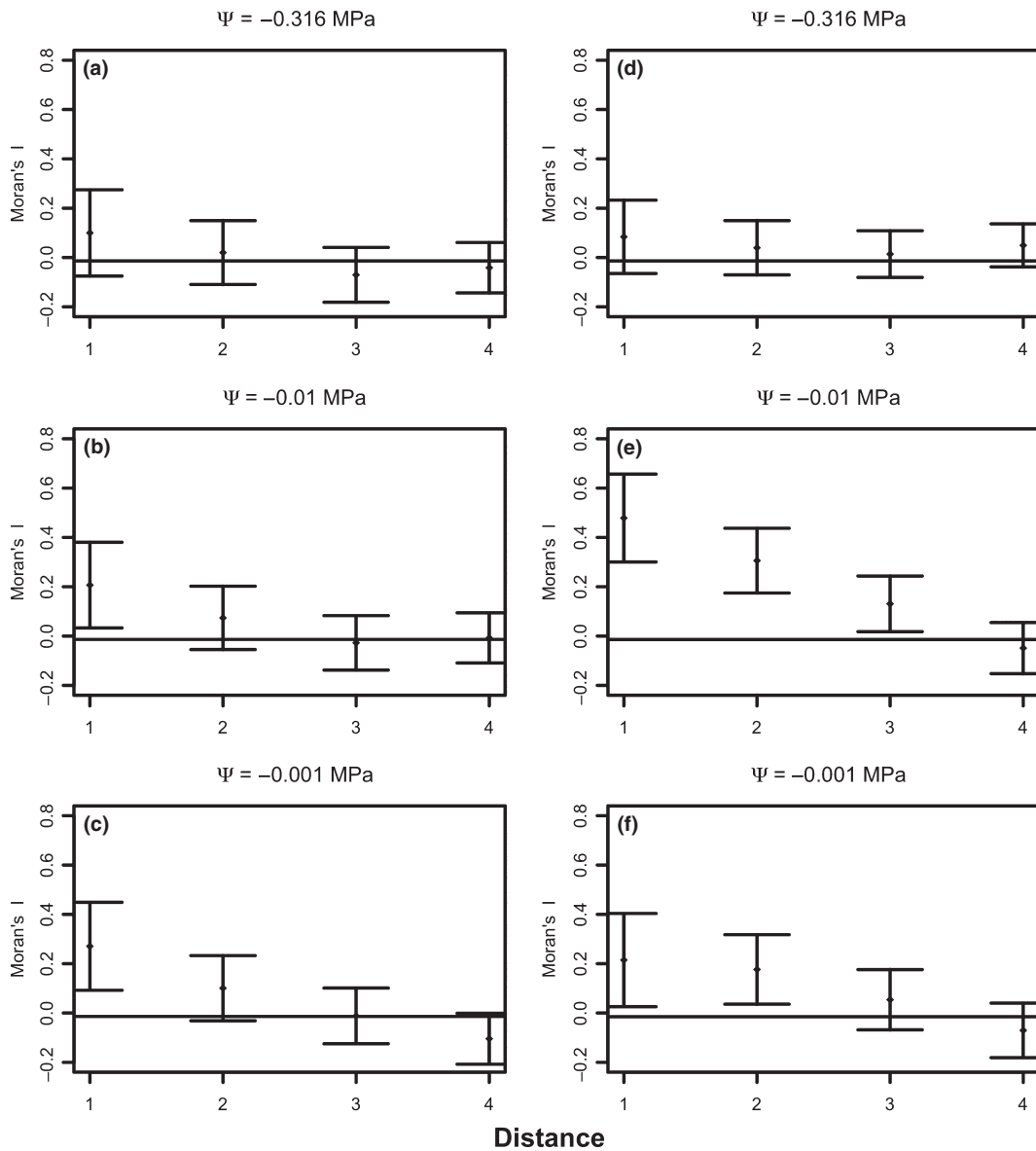


Fig. 3. Moran's I index of spatial autocorrelation for glucose (a–c) and 2,4-D (d–f) mineralizations at the three different matric potentials.

### Impact of matric potential on the relationship between the relative abundance of 2,4-D degraders and 2,4-D mineralization

Whatever the matric potential, no relationship between the abundance of total bacteria and 2,4-D mineralization was observed. The *tfdA* sequence copy number was significantly correlated with 2,4-D mineralization at  $\Psi = -0.316$  MPa ( $R^2 = 0.24$ ,  $P = 0.03$ , Figs S2 and S3) but higher significant relationships were observed between the relative abundance of 2,4-D degraders and 2,4-D mineralization at  $\Psi = -0.01$  and  $-0.001$  MPa ( $P < 0.01$ ); the strongest relationship being at  $\Psi = -0.01$  MPa

( $R^2 = 0.89$ , Fig. 4). The slope of the regression lines increased as water content decreased, meaning that when the soil was drier, the relative abundance of *tfdA* genes needed to be higher to obtain a given level of 2,4-D mineralization.

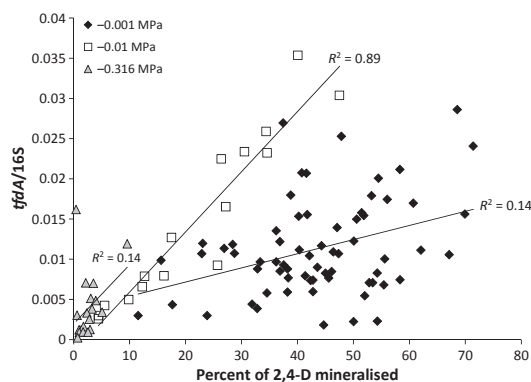
## Discussion

### Impact of matric potential on substrate mineralization

One of the objectives of this work was to study the impact of the soil matric potential on glucose and 2,4-D

**Table 1.** 16S rRNA and *tfdA* sequence copy numbers per g of dry soil and relative number of *tfdA* gene at the three matrix potentials

|                     | 16S     | <i>tfdA</i> | <i>tfdA</i> × 10 <sup>3</sup> /16S |
|---------------------|---------|-------------|------------------------------------|
| $\Psi = -0.001$ MPa |         |             |                                    |
| Mean                | 9.7E+07 | 9.7E+05     | 11.3                               |
| Min                 | 1.7E+06 | 2.6E+04     | 1.8                                |
| Max                 | 3.8E+08 | 3.8E+06     | 28.6                               |
| CV%                 | 64.6    | 71.9        | 53.3                               |
| $\Psi = -0.01$ MPa  |         |             |                                    |
| Mean                | 9.1E+07 | 1.1E+06     | 14.8                               |
| Min                 | 7.1E+06 | 1.6E+05     | 2.6                                |
| Max                 | 2.5E+08 | 3.3E+06     | 35.4                               |
| CV%                 | 66.4    | 89.5        | 71.8                               |
| $\Psi = -0.316$ MPa |         |             |                                    |
| Mean                | 3.6E+07 | 7.1E+04     | 3.9                                |
| Min                 | 2.4E+06 | 1.9E+04     | 0.2                                |
| Max                 | 9.3E+07 | 1.5E+05     | 16.2                               |
| CV%                 | 71.7    | 44.8        | 104.7                              |

**Fig. 4.** Relative number of *tfdA* gene (*tfdA* sequence copy number/16S rRNA sequence copy number) related to the percent of 2,4-D mineralized at  $\Psi = -0.001$  (diamond, black),  $-0.01$  (square, white) and  $-0.316$  MPa (triangle, grey).

mineralizations in millimetre-size structured soil units. Numerous studies have already shown that soil microbial activity increases with increasing moisture until an optimal moisture value is reached before decreasing (Linn & Doran, 1984; Prado & Airolidi, 1999). This has been shown to affect pesticide degradation (Helweg, 1987; Schroll *et al.*, 2006; Wang *et al.*, 2007) and more particularly 2,4-D degradation (Parker & Doxtader, 1983; Ou, 1984; Han & New, 1994), as well as simple substrates such as glucose (Barros *et al.*, 1995). The optimal moisture levels for soil microbial activity varied in the different studies but were generally close to field capacity (Barros *et al.*, 1995; Schroll *et al.*, 2006). The results obtained here in small-structured soil samples contrasted with previous studies carried out on larger samples in that the mineralization of both substrates increased with increasing water potential

from  $-0.316$  MPa to  $-0.001$  MPa (field capacity corresponding to a matrix potential of  $-0.032$  MPa).

### Impact of matrix potential on the variability and spatial structure of mineralization

Glucose and 2,4-D were chosen as substrates in this study because it was assumed that glucose mineralization would be less variable than that of 2,4-D. These two substrates would therefore allow the effects of the interaction between the fine-scale spatial distribution of degraders and matrix potential to be studied. As expected, less heterogeneity was observed in the mineralization of glucose than in that of 2,4-D. Changes in soil water content did not have the same impact on glucose and 2,4-D mineralization and their respective spatial heterogeneity. Increases in water content led to a reduction in the spatial heterogeneity of the pesticide mineralization, presumably by increasing the probability of degrader and substrate encounters. The variability of the glucose mineralization was smaller and more constant for the different matrix potentials probably because degrading microorganisms are more numerous and evenly distributed.

Under the wettest conditions, 2,4-D mineralization showed spatial structure at a similar scale to previous observations with hot spots of centimetre size (Gonod *et al.*, 2003, 2006). Here, however, the spatial structure was demonstrated to depend on the environmental conditions as there was a loss of spatial structure among samples incubated at  $\Psi = -0.316$  MPa (Fig. 3). Similar trends were observed for glucose mineralization, although the correlation length for glucose mineralization under the wettest conditions was shorter than that for 2,4-D mineralization. These data suggest that the mineralization of both substrates, but principally that of 2,4-D, depend on the connectivity provided by the water-filled pore network and that the spatial structure observed in the mineralization of the substrates is a reflection of the spatial organization of the pore network. The matrix potentials used in this study placed the substrates into pores with maximal pore neck diameters of 1, 30 and 300  $\mu\text{m}$ , resulting in differential substrate diffusion within the soil cubes. The limitation of substrate diffusion decreased probabilities of contact between substrate and degraders. The fact that the spatial structure was lost at  $\Psi = -0.316$  MPa (driest conditions) suggests that the delivery of substrate was a random process. The relatively high mineralization rates observed in the dry samples may have been performed by microorganisms that happened to be located in the small soil pores into which the substrate was placed. Grundmann *et al.* (2007) also concluded that diffusion-controlled isotropuron mass flow towards microbial hot spots was one of the main processes enabling increases in isotropuron

mineralization. Soil moisture is also essential for bacterial motility that can only occur if liquid films are of sufficient thickness (Gammack *et al.*, 1992). Dechesne *et al.* (2010) highlighted the potential value of flagellar motility in the mineralization of benzoate in a model system sufficiently moist to allow bacterial swimming (−8 and −50 kPa). Such bacterial motility has already been observed for 2,4-D degraders, and their spreading was associated with an increase in 2,4-D biodegradation (Pallud *et al.*, 2004). Finally, dry conditions may cause a decrease in microbial activity, a decrease in the number of microorganisms or a combination of these 2 factors, as shown by Han & New (1994). Therefore, higher soil water contents, by increasing both degrader growth, activity and spread as well as the mass transfer of the 2,4-D, may have increased the probability of encounter resulting in higher 2,4-D mineralization rates.

### Impact of matric potential on soil total bacteria and 2,4-D degraders

Total bacterial and 2,4-D degrader abundances were estimated at the end of incubation using the 16S rRNA and *tfdA* sequence copy numbers, respectively. Considering that the gene *tfdA* is mainly carried on a plasmid which is present at a single or low copy number per bacterium (Pemberton & Don, 1981), the *tfdA* quantity in soil is likely to reflect the size of the 2,4-D degrading bacterial communities possessing the *tfdA* gene.

Dryer conditions affected degrader population size as the *tfdA* sequence copy number was far lower at  $\Psi = -0.316$  MPa than at  $\Psi = -0.01$  MPa and  $\Psi = -0.001$  MPa: soil contained between 13.7 and 15.5 times more degrading bacteria at  $\Psi = -0.001$  and  $-0.01$  MPa, respectively, than at  $\Psi = -0.316$  MPa. Although the 16S rRNA sequence copy number was also affected by drier conditions, the difference was far smaller (2.5 times more copies at  $\Psi = -0.01$  MPa and  $\Psi = -0.001$  MPa than at  $\Psi = -0.316$  MPa). Therefore, the 2,4-D degrading communities carrying *tfdA* genes were much more sensitive to dry conditions than the total bacterial communities. 2,4-D degraders represented 1.0–1.2% of the total bacteria at  $\Psi = -0.001$  and  $-0.01$  MPa, respectively, but only 0.2% at  $\Psi = -0.316$  MPa. Han & New (1994) observed similar results, namely that 2,4-D degraders communities were more affected by dry conditions than the culturable aerobic heterotrophic microorganisms.

### Relationship between 2,4-D degraders and the mineralization function

The 2,4-D mineralization increases with the matrix potential appeared to be linked to the relative abundance of

*tfdA* even if the correlations were not all significant. Both Vieublé Gonod *et al.* (2006) and Baelum *et al.* (2008) observed a relationship between the *tfdA* sequence copy number and the rates of 2,4-D mineralization. It should be noted that the analysis of *tfdA* gene abundance was carried out after the samples (and therefore the microorganisms) had been exposed to 2,4-D. This would have resulted in an increase in the abundance of the *tfdA* gene, which may have affected the relationship between gene abundance and activity.

The activity per unit *tfdA* sequence copy number was significantly affected by matric potential. The data suggest that for a similar mineralization level to be reached, a higher proportion of degrading microorganisms among the total bacterial population is required in drier soils. The impact of matric potential on the relationship between the relative abundance of *tfdA* gene and 2,4-D mineralization can be explained in three ways. First of all, through short-term effects on microbial activity, water content may have a strong impact on gene expression and thus on the function encoded. This will result in a weakening of the relationship between degrader abundance, determined by the amount of functional genes, and the activity in question. Such differences between activity and abundance have already been observed for the total bacterial community and for specific 2,4-D degraders (Felske & Akkermans, 1998; Baelum *et al.*, 2008). Soil water content can also affect the relationship between the relative abundance of 2,4-D degraders and the 2,4-D mineralization by acting on the relative proportion and activity of bacteria and fungi in soil. It has been observed that whereas bacterial activity declines sharply as water potential falls from  $-0.05$  MPa the relative fungal activity increases (Griffin, 1981; Orchard & Cook, 1983). Cattaneo *et al.* (1997), in an experiment in which a 2,4-D degrading strain was inoculated into soil, found that the inoculated strain was the most abundant microorganism at field capacity ( $-0.03$  MPa) but that fungal species dominated in drier soil. Furthermore, Han & New (1994) observed that 2,4-D degrading fungi were more tolerant of dry conditions than were bacteria and that bacteria were efficient in degrading 2,4-D at matric potentials above  $-1.4$  MPa. Even if we did not test such dry conditions, fungal-bacterial competition may have been higher at  $\Psi = -0.316$  MPa than at the other matric potentials we tested. As only the bacterial *tfdA* gene was quantified here, the fungal contribution to 2,4-D mineralization was not accounted for, which may explain the absence of correlation between the relative number of *tfdA* gene and 2,4-D mineralization observed at the lowest water content tested. However, fungi that are more resistant to dry conditions are generally not able to mineralize 2,4-D. They are only capable of carrying out the

initial degradation steps and are responsible of the production of intermediary metabolites (Han & New, 1994; Vroumsia *et al.*, 2005). Finally, higher water contents might release and mobilize other organic substrates from soil (Young *et al.*, 2008) and thus enhance 2,4-D by co-metabolism, which does not involve the specific *tfDA* gene. Han & New (1994) and Vroumsia *et al.* (1999) also showed that 2,4-D degradation by fungi could be stimulated with a supply of an alternative source of carbon and energy.

## Conclusions

The use of <sup>13</sup>C-labelled substrates and the microplate system developed by Monard *et al.* (2010) allowed us to directly link a mineralization function to the size of the specific degrader community at a fine scale. The results highlighted the complex relationship that exists between degrader communities and their environment at small scales and how this relationship modulates degrader activity. As proposed by Crawford *et al.* (2005), soil should be viewed as a complex system in which the physical and biological components and their dynamics have to be accounted for. Small scale studies bring new insight into how microbial interactions within their microhabitat can impact soil processes at larger scale.

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**Fig. S1.** Box plot representation of the 16S sequence copy number per g of dry soil (a), *tfdA* sequence copy number per g of dry soil (b)  $tfdA \times 10^3/16S$  (c) at  $\Psi = -0.001$ ,  $-0.01$  and  $-0.316$  MPa.

**Fig. S2.** 16S sequence copy number per g of dry soil related to the percent of 2,4-D mineralised at  $\Psi = -0.001$  (diamond, black),  $-0.01$  (square, white) and  $-0.316$  MPa (triangle, grey).

**Fig. S3.** *tfdA* sequence copy number per g of dry soil related to the percent of 2,4-D mineralised at  $\Psi = -0.001$  (diamond, black),  $-0.01$  (square, white) and  $-0.316$  MPa (triangle, grey).

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