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Original article

Short-term effects of manure application on soil leachates in a mountain catchment

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Abstract – Twenty-six organic, mineral and microbiological parameters were determined on solutions leached from soils submitted to farmyard manure application (April 1st) on a mountain pasture in the Northern Alps, to assess potential short-term effects of manure spreading on soil and water quality, according to climatic conditions. For NH_4^+, total bacteria, total phenols and phenolic acids, the largest concentrations were observed one day after spreading; for Cl^-, Na^+, Mg^{2+}, active bacteria, and DOC one week after spreading and for hydrolytic activity and nitrate concentrations five weeks after. At the end of April, a wet period induced the disappearance of any differences between manured and control soil leachates, except for NH_4^+, DOC, total and active bacteria which had already returned to control soil levels before the rain. It is thus likely that the first significant rain after manure application in this mountain catchment will release concentrated solutions (nutrients and phenolic compounds) with potentially deleterious effects.

farmyard manure / soil leachate / nutrient / organic and phenolic compound / microbial activity

Résumé – **Effet à court terme d'application de fumier sur les lessivats du sol dans un bassin versant de montagne.** Vingt-six paramètres organiques, minéraux et microbiologiques ont été déterminés sur des solutions obtenues par lessivage de sols soumis à un amendement de fumier (réalisé le 1er avril) dans un paturage de montagne, afin de suivre les effets à court-terme de cet épandage sur la qualité des eaux et des sols, en fonction des conditions climatiques. Les concentrations les plus élevées ont été observées un jour après l'épandage pour NH_4^+, les bactéries totales, les phénols totaux et les acides phénoliques ; une semaine après pour Cl^-, Na^+, Mg^{2+}, les bactéries actives et le COD ; cinq semaines après pour l'activité hydrolytique et les nitrates. Fin avril, un épisode pluvieux intense a provoqué la disparition des différences entre les lessivats de sols amendés et de sols non-traités, sauf pour NH_4^+, COD, les bactéries totales et actives qui avaient déjà retrouvé des valeurs proches du contrôle avant le début des pluies. Les premières pluies significatives après un épandage sont donc susceptibles de provoquer le ruissellement de solutions concentrées (en nutriments, composés phénoliques) dont les effets potentiellement négatifs restent à étudier.

fumier / lessivat de sol / nutriment / composé organique et phénolique / activité microbienne

1. INTRODUCTION

Manure application in cropping systems is widely employed as an amendement for nitrogen and other nutrients, but also as a disposal method for large amounts of biodegradable organic waste. Its primary effects on the soil and the soil solution are well documented [13] and include an increase in soil organic matter, structural stability, water holding and cation exchange capacity.

In addition to these benefits as a soil conditioner, cases of surface and groundwater contamination by nutrients and bacteria have also been recorded [8]. Manure is also a source of numerous plant metabolites, including soluble (phenolic acids) and insoluble (lignin) phenolic compounds that are very stable compounds and only partially degraded in the bovine stomach [15]. Although some of them are deleterious to soil fertility [21] and animal health [12], their fate in manure has been very rarely studied [25, 29].

The objectives of this study were to describe the short-term influence (intensity and duration according to climatic conditions) of manure spreading on soil leachates, as a part of a general project on soil and water quality in mountain catchment

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areas. High altitude agroecosystems appear to be especially vulnerable to hazards related to farmyard manure management, since large quantities have to be spread between snow melting and the start of the growing season. Moreover, climatic conditions during the long winter storage period slow down the maturation processes, which is known to reduce the release of water-soluble toxic metabolites [24, 28]. As a consequence, heavy spring rains can release high concentrations of such metabolites, leading to an enrichment of soil solutions and associated groundwaters.

Variations of soil leachate composition were recorded through the analysis of 26 physical, chemical and biological parameters in the 16 weeks (from April to July) after farmyard manure application. The experiment was conducted in natura on a pasture, but to avoid the irregularity of in situ lysimeter sampling (subject to climatic conditions), we followed the fate of soluble compounds through weekly soil sampling followed by collection of artificial leachates in the laboratory. In addition to established indicators like dissolved organic carbon (DOC) and nutrients we also measured less frequently determined variables, including microbial activity and phenolic compounds.

2. MATERIALS AND METHODS

2.1. Site

The field site was located in the Vercors mountains which lie between Grenoble, Gap and Valence (South-Eastern France). This calcareous massif is essentially rural, but the population is greatly enlarged during the summer due to tourism. The demand for drinking water becomes very high during this period. Moreover, within the next few years the Vercors massif will become a drinking water resource for neighboring cities, including Valence and Grenoble.

The farm where the study was conducted was located in St Nizier du Moucherotte (20 km south of Grenoble), at a mean altitude of 1100 meters. Farming activities are essentially devoted to cattle and milk production (about thirty cows), on an area of 25 ha. The soil is an Udalf Alfisol or Luvisol [2] with 45% sand and 30% clay in the upper layer (0–15 cm). Precipitation and temperature were recorded daily by METEO-France (Lans-en-Vercors station).

A previous study using the same methodology had been done before manuring (21 March). On April 1st, 50 $t \cdot ha^{-1}$ of winter-stockpiled farmyard manure (mixed with hay) were scattered on a pasture dominated by *Dactylis glomerata* L. and *Trifolium* sp. The usual application rate for manure in this area is around 30 $t \cdot ha^{-1}$, but the rather high level of our study was selected to produce responses high enough to be followed for some months. Pure manure samples were collected and analyzed for water-soluble content and their water-soluble components using the same techniques as for soil (see below).

Soil samples were collected from manure-amended soil and from control (unamended) on 10 occasions over a 16-week period (1 April to 10 July), and also on 21 March from the control soil. At each sampling date, 20 × 30 cm undisturbed soil blocks 8 cm in depth (three from the manured area, one from control soil) were cut with a knife and immediately transported to the laboratory on polyethylene plates (zero-tension lysimeter). The covering vegetation (mainly grasses) was maintained. In the laboratory, one subsample of soil was used to determine moisture content (48 h at 105 °C). The soil samples stored on plates were immediately subjected to leaching (see below). After leaching, entire soil samples were dried and weighed (48 h at 105 °C).

2.2. Leachate sampling and chemical analysis

Undisturbed soil blocks stored on polyethylene plates were regularly and carefully watered with demineralized water untill 1 L was collected via a small hole opened in the plate. The average time for this collection was about 30 minutes. pH of the leachate was measured (Tacussel pH-meter), and subsamples were filtered for further mineral and carbon analysis through Whatman GF/A (1.2 µm) and GF/F (0.7 µm) glass-fiber filters to remove suspended matter.

2.2.1. Mineral analysis

A 0.2 µm cellulose acetate membrane (Millipore) was washed three times with 20 mL of organic-C-free distilled water and then used to filter about 150 mL of the leachate. Ten mL subsamples were frozen and later analyzed using a Capillary Ionic Analyzer (Waters Ltd) to determine Mg^{2+}, K^{+}, Na^{+}, Cl^{-}, $NO3^{-}$ and $SO4^{2-}$.

2.2.2. Carbon determination

A further 10 mL aliquot was kept for an initial DOC measurement using a Dohrman DC80 "Total Carbon Analyzer". Mineral carbon was first eliminated and organic carbon was measured after ultra-violet promoted potassium persulfate oxidation.

Biodegradable DOC is the part of total DOC concentration which is decomposed by autochtonous bacteria during a short-term incubation [33, 34]. The remaining 130 mL of filtered leachate were transferred to a 250 mL precombusted (550 °C, 4 h) glass bottle with an aluminium closure and inoculated with a suspension of bacteria (removed from the cellulose acetate filter), and incubated in the dark for 30 to 35 days, at 10 °C. The DOC concentration measured at the end of the incubation was considered as biologically refractory material (RDOC) [4, 33], and the difference between initial and final values of DOC was defined as the biodegradable fraction (BDOC).

2.2.3. Bacterial counts

Bacterial counts were performed using epifluorescence microscopy. The total number of bacteria was estimated after DAPI staining [10, 27]. One mL of water was filtered onto a GTBP-type membrane (Millipore), stained with a 40 $\mu g \cdot mL^{-1}$ DAPI solution (final concentration), for 10 min at room temperature, then washed and counted under immersion oil.

The number of active bacteria was measured using CTC staining [31]. One mL of water was stained with a 1.48 $mg \cdot L^{-1}$ CTC solution (final concentration) incubated for 3 h at 20 °C, and then filtered on a GTBP millipore membrane.

Table I. Climatic conditions during sampling period. †Date of manure spreading.

Date	03.21	04.01†	04.04	04.10	04.17	04.25	04.29	05.06	05.16	06.03	07.10
Week	1	2	3	4	5	6	7	8	9	11	16
Temperature of the sampling day (°C)	1.7	5.9	5.6	7.8	2.0	7.5	6.5	10.3	14.1	13.0	15.6
Mean temperature (over previous 7 days) (°C)	4.6	5.0	4.7	6.4	5.5	3.9	6.7	10.3	11.1	12.7	12.5
Cumulated precipitation (on previous 7 days) (mm)	19.6	3.0	3.2	0	0	6.8	61.4	69.1	15.8	7.4	49.4

Total biological activity was determined using fluorescein diacetate (FDA). The hydrolysis of FDA by enzymes of active microbial cells releases fluorescein that is then measured using a spectrophotometer [20].

2.2.4. Phenolic determination

Twenty mL of filtered leachates were used for the determination (in duplicate) of total phenols with Folin-Ciocalteu reagent using gallic acid as a standard.

After acidification by 2 N HCl, about 500 mL of leachate was extracted 3 times for phenolic monomers by ethyl-ether. After evaporation to dryness, residues were redissolved in ethanol and stored at –18 °C until HPLC analysis. Phenolic monomers were also extracted directly from manure samples by shaking 10 g of fresh manure three times with acidified ethyl-ether (150/100/100 mL). Chromatographic separation of phenolic monomers were performed on a Waters 600 Controller with a diode array detector Waters 996, using a 250 × 4.6 mm column filled with µBondapak C_{18}, 10 µm. Solvent A was 0.5% of acetic acid in distilled water and solvent B was 0.5% of acetic acid in acetonitrile. Phenolic acids were separated at a flow rate of 1.5 $mL \cdot min^{-1}$ by using a linear gradient from 0% to 20% of B in 45 min, with 15 min of re-equilibration between samples. They were further identified and quantified by comparison of UV spectra and retention time with standard compounds. The sum of all the identified phenolic acids was recorded, and each monomer expressed in % of this sum.

2.3. Manure analysis

Each manure soil sample was air-dried and passed through a 2-mm sieve, and pH was measured in 1:2.5 manure:water mixtures using a Tacussel pH-meter. Manure organic matter was oxidized at 1000 °C, and CO_2 measured by conductimetry (Carmhograph 8). Total nitrogen was measured by Kjeldhahl distillation using a Büchi 430 apparatus.

2.4. Statistical analysis

Differences in the content of components between the control leachates (one sample) and the amended leachates (mean value of three replicates) were analyzed using the Wilcoxon rank signed test. Differences between the dry and wet periods were tested using Kruskal-Wallis ANOVA of ranks because of poor homogeneity of variance for some parameters. All statistical analyses were performed using Statistica/W (version 5.0, StatSoft Inc.).

3. RESULTS

3.1. Climatic conditions during experiment

Two sampling periods were defined based on mean air temperature and, especially, rainfall intensity (Tab. I): the first five weeks after manure spreading were dry (total precipitation about 12.0 mm) and cold (mean temperature about 5.1 °C), resulting in a decrease in soil water contents from 46% on the day of spreading to less than 30% during the last three weeks of April. From April 29th to the end of the experiment (July 10th), the mean temperature increased (to 12.5 °C), and heavy rainfall (total precipitation of about 333 mm) induced an increase in soil moisture to a mean value of 55%.

Leachates from untreated soils exhibited slight differences in their concentrations between these two periods (Tab. II). Only ammonium and hydrolytic activity were significantly higher during the second period than during the first, while syringic acid was more concentrated in the leachate obtained during the first period.

3.2. Manure composition

The farmyard manure was a wet, alkaline substrate characterized by a high C content (22%) and high C/N ratio (31) (Tab. IIIa). Syringic acid was the main phenolic acid extracted by ethyl ether from fresh manure. Together with p-hydroxybenzoic acid and its associated aldehyde, p-hydroxybenzaldehyde, it accounted for more than 95% of the phenolic monomers.

Organic compounds were easily leached from fresh manure, which contained more than 600 $mg \cdot L^{-1}$ of DOC. DOC was mainly biodegradable (83%) and included 15% of phenolic compounds (184 $mg \cdot L^{-1}$ in gallic acid equivalent) (Tab. IIIb). Water extracts contained a large number of bacteria, of which 25% were active.

3.3. General effects of manure application

During the five first weeks after spreading, and whatever the components, manure application increased the content of soil leachates compared with that of the control. In addition, the water content was significantly greater for manured soils (Tab. II). Significant acidification of the leachate was also observed (Fig. 1). After five weeks, there was a change in the

Table II. Mean (and SD) of the 26 variables measured on leachates of untreated (control) and amended soils. n indicates the number of sampling dates. *P* gives the probability of a significant difference between control and amended soil (Wilcoxon test, ‡: $P < 0.03$; †: $P < 0.06$; NS: Non Significant). Different superscript letters between period I and II (a, b for control; c, d for amended) indicate significant differences (Kruskall-Wallis test, $P < 0.05$) between these two periods.

	Period I (1st–6th week) (dry and cold)			**Period II** (7th–16th week) (wet and warm)		
	Control (n = 5)	Amended (n = 5)	*P*	Control (n = 5)	Amended (n = 5)	*P*
Soil moisture (%)	31.6 (9.1)	36.4^{c} (7.9)	‡	42.8 (7.3)	51.5^{d} (6.6)	†
NH_4^+ ($mg \cdot L^{-1}$)	0.07^{a} (0.08)	4.62^{c} (7.03)	†	0.39^{b} (0.59)	0.12^{d} (0.03)	NS
K^+($mg \cdot L^{-1}$)	1.49 (1.17)	90.57^{c} (29.19)	‡	2.73 (2.90)	7.29^{d}(3.69)	‡
Na^+($mg \cdot L^{-1}$)	1.11 (0.42)	6.65^{c} (1.92)	‡	0.80 (0.39)	1.39^{d} (0.07)	‡
Mg^{2+}($mg \cdot L^{-1}$)	0.29 (0.11)	4.59^{c} (1.96)	‡	0.38 (0.23)	0.50^{d} (0.15)	NS
Cl^-($mg \cdot L^{-1}$)	2.43 (0.56)	95.62^{c} (23.82)	‡	5.22 (7.12)	6.34^{d} (2.21)	NS
SO_4^{2-}($mg \cdot L^{-1}$)	0.74 (0.30)	14.01^{c} (2.74)	†	0.77(0.63)	1.49^{d} (0.51)	‡
NO_3^-($mg \cdot L^{-1}$)	0.11 (0.07)	1.21 (0.74)	†	0.70 (0.59)	0.29 (0.16)	NS
DOC ($mg \cdot L^{-1}$)	16.5 (3.2)	241.9^{c} (55.3)	†	18.9(2.6)	29.9^{d} (4.4)	‡
RDOC ($mg \cdot L^{-1}$)	7.8 (2.3)	71.7^{c} (15.5)	‡	8.8 (1.4)	20.9^{d} (4.9)	‡
BDOC ($mg \cdot L^{-1}$)	8.7(4.0)	170.2^{c} (49.8)	‡	10.1 (1.9)	9.0^{d} (4.2)	NS
Total bacteria ($10^6 \cdot L^{-1}$)	16.6 (14.9)	77.3 (59.1)	‡	13.8 (9.1)	29.0 (11.5)	‡
ETS-active bacteria ($10^4 \cdot L^{-1}$)	8.4 (16.8)	546.1 (793.3)	‡	153.4(143.5)	253.1 (245.0)	NS
Hydrolytic activity ($\mu g \cdot L^{-1} \cdot h^{-1}$)	1.2^{a} (0.4)	12.6^{c} (3.8)	‡	4.0^{b} (1.9)	6.4^{d} (1.7)	‡
pH	6.8 (0.4)	6.4 (0.3)	†	6.6 (0.2)	6.3 (0.2)	‡
Total phenols ($mg \cdot L^{-1}$)	1.6 (0.3)	46.9^{c} (8.2)	†	2.9 (1.3)	6.3^{d} (2.4)	‡
Sum ($\mu g \cdot L^{-1}$)	3.9 (4.3)	2169.4^{c} (1181.7)	‡	2.9 (1.0)	3.6^{d} (1.5)	NS
Protocatechuic acid ($\mu g \cdot L^{-1}$)	0.1 (0.2)	122.6^{c} (49.3)	‡	0.1 (0.1)	0.3^{d} (0.2)	†
Gentisic acid ($\mu g \cdot L^{-1}$)	0.2 (0.3)	6.5^{c} (2.8)	‡	0	0.2^{d} (0.2)	‡
p-hydroxybenzoic acid ($\mu g \cdot L^{-1}$)	0.9 (0.8)	797.7^{c} (555.2)	‡	0.5 (0.2)	0.8^{d} (0.4)	NS
p-hydroxybenzaldehyde ($\mu g \cdot L^{-1}$)	0	16.9^{c} (9.9)	‡	0	0^{d}	‡
Vanillic acid ($\mu g \cdot L^{-1}$)	0.6 (0.7)	54.8^{c} (31.7)	‡	0.6 (0.4)	0.4^{d} (0.2)	NS
Caffeic acid ($\mu g \cdot L^{-1}$)	0.4(0.4)	3.0^{c} (2.2)	‡	0.7 (0.6)	0.8^{d} (0.4)	NS
Syringic acid ($\mu g \cdot L^{-1}$)	0.4^{a} (0.6)	1100.1^{c} (613.9)	‡	<0.1^{b} (<0.1)	0.3^{d} (0.2)	‡
p-coumaric acid ($\mu g \cdot L^{-1}$)	0.6 (0.7)	47.4^{c} (29.2)	‡	0.6 (0.2)	0.4^{d} (0.2)	NS
Ferulic acid ($\mu g \cdot L^{-1}$)	0.6 (0.6)	19.8^{c} (9.9)	‡	0.5 (0.3)	0.5^{d} (0.1)	NS

weather, which resulted in abundant rain and a differentiated response among the parameters investigated.

3.3.1. Mineral contents

Manure application induced an increase in the concentration of anions (thirty-one-fold and nine-fold for Cl^- and SO_4^{2-}, respectively), as well as in cation amounts (two hundred and eighty-seven-fold, twelve-fold and twenty-eight-fold, respectively, for K^+, Na^+ and Mg^{2+}) (Fig. 2). For all these elements, maxima were observed one week after spreading (April 4th). After the first rainy days, a strong decrease was recorded, though mineral contents remained significantly higher (by a factor of <10) in manured soil leachates than in control leachate, except for Cl^- and Mg^{2+} for which no more differences between control and manured soil leachates were found.

Distinct patterns were observed for NH_4^+ and NO_3^- contents. For NH_4^+, the maximum value of the first day (× 98) was immediately followed by a rapid decrease, and control values were reached by the 4th week despite the lack of rain. For nitrate, the highest values in manured leachates were

Table III. (a) Characteristics of manure. Syr: syringic acid; Aph: p-hydroxybenzoic acid; Hba: p-hydroxybenzaldehyde; Pro: protocatechuic acid; Ava: vanillic acid; Gen: gentisic acid; Caf: caffeic acid; Pco: p-coumaric acid; Fer: ferulic acid. (b) Characteristics of manure leachate. Total phenols are expressed in mg of gallic acid equivalent·L^{-1}.

(a)

Hum (%)	pH (H_2O)	pH (KCl)	C (% DW)	N (% DW)	C/N	Phenolic acids ($mg \cdot g^{-1}$ of DW)
369	8.5	8.1	22	0.71	31	0.42 including Syr = 56% Aph = 29% Hba = 10% Pro = 3% Ava, Gen, Caf, Pco, Fer < 1%

(b)

pH	Total bacteria ($10^9 \cdot L^{-1}$)	Active bacteria ($10^9 \cdot L^{-1}$)	DOC ($mg \cdot L^{-1}$)	Total phenols ($mg \cdot L^{-1}$)	Phenolic acids ($mg \cdot L^{-1}$)
8.1	3.86	0.97	617 RDOC = 102 BDOC = 515	184	14.6 including : Syr = 60% Aph = 32% Hba = 4% Pro = 3% Ava, Gen, Caf, Pco, Fer < 1%

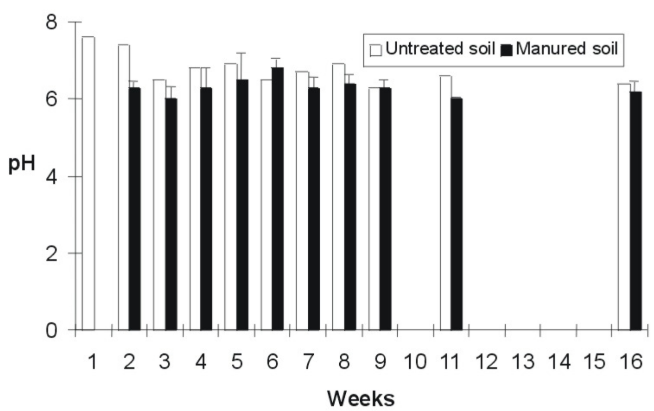

Figure 1. Variations of pH in soil leachates collected under untreated and manured soils (mean and SD of three replicates) during the 16 weeks of experimentation. Manure was spread at week 2.

observed at the end of the dry period (between the 4th and 6th week). As for other elements, the rain induced changes back to values similar to those in the control leachate.

No obvious temporal variations could be observed in control leachates for any of the elements. The unexpectedly high values of mineral contents (Fig. 2) recorded on the last sampling date (July 10th) were due to the spread of chemical fertilizers (such as nitrogen) by the farmer on the untreated site following hay harvest.

3.3.2. Microbiological parameters

Total bacterial counts in manured soil leachates (Fig. 3), estimated by DAPI coloration, strongly decreased from the first day of the experiment (23 times greater than for the control) to the 5th week, but then remained significantly higher than in the controls. In contrast to mineral elements, the weather pattern did not affect bacterial abundance.

ETS-active bacteria (estimated by CTC staining) showed a different pattern. The maximum value (20 times higher than the control) was observed at the second sampling day after manure spreading (Fig. 3). Following this date, very low activity was recorded, with no significant differences compared with controls. During May, a slight increase in bacterial activity both in control and manured samples could be linked to favorable pedoclimatic conditions (higher temperature and soil moisture).

Considering hydrolytic activity, significantly higher values were always measured in manured soil leachates compared with control leachates (Fig. 3), with maximum values observed at the end of the dry period (5th and 6th week). During this period, microbial activity was 25 times higher in manured than in control leachates. The differences between amended and untreated soil leachates became less as soon as the rain fell, but that was due to a decrease in the manured leachate and a simultaneous increase in the control leachates.

3.3.3. DOC content

During the month following manure application, dissolved organic content in leachates increased about 10 times compared with the control, with a maximum observed one week after manure amendment (Fig. 4). In control soil leachate, refractory and biodegradable fractions represented, respectively, half of the DOC. Manuring induced an increase in the biodegradable fraction in leachates by 80% during the first month. Total DOC rapidly decreased with the onset of rain, with different patterns between the fractions. In manured soil leachate, BDOC became similar to that from the control, while the refractory fraction was three times greater in manured than in untreated soil solution.

3.3.4. Phenolic content

Manure application increased the concentration of water-soluble total phenols in soil leachates thirty-fold, and the

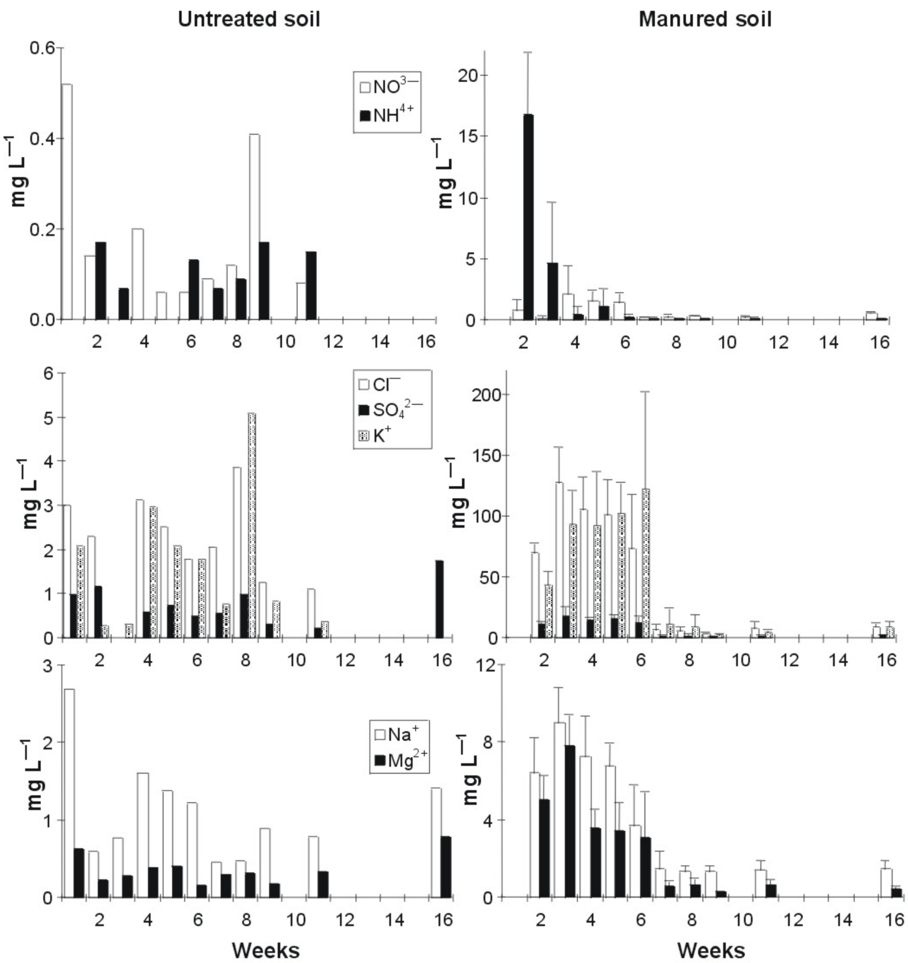

Figure 2. Variations of mineral concentrations ($mg \cdot L^{-1}$) in soil leachates collected under untreated and manured soils (mean and SD of three replicates) during the 16 weeks of experimentation. Manure was spread at week 2.

phenolic acid content increased more than 2000 times at the first sampling date (Fig. 5). Concentrations of total phenols decreased slightly during the first dry month. A significant decrease only occurred after the rain. Nevertheless, there were still significant differences between manure and control treatments, even after three months. In the control, total phenol concentrations showed higher values in early May, the beginning of the vegetation growth.

In contrast, there was a six-fold decrease in water-soluble phenolic acids during the first five weeks, and the change occurred much faster after the first rain, until concentrations returned to the same levels as those of the control. During the first five weeks, syringic and p-hydroxybenzoic acids accounted, respectively, for about half and one-third of the water-soluble phenolic acids (Tab. IV). At the onset of rainfall, the distribution of the phenolic monomers in the manured soil leachate was completely changed, and became similar to that of control soils, with caffeic, vanillic, p-hydroxybenzoic and p-coumaric acids as the main products.

4. DISCUSSION

Leaching under laboratory conditions was preferable to other methods (such as tension-free lysimeters) for collecting soil solutions as it allowed a regular characterization of soil leachates, irrespective of climatic conditions. However, the composition of these leachates may well differ from those occurring in the field, mainly because of the regular and high rate of the artificial flow. Nonetheless, some estimates of the quality and amounts of leachable compounds, as well as their temporal variabilities, were possible. Concentrations of DOC (between 13 and 22 $mg \cdot L^{-1}$), total phenols (between 1.2 and 4.3 $mg \cdot L^{-1}$) and nutrients (between 0.15 and 0.78 mg of $Mg^{2+} \cdot L^{-1}$) in the leachate from untreated soil leachates was about the same as or less concentrated than that obtained with tension-free lysimeters situated under organic layers [11]. Moreover, our regular watering, first on drying soils (during April) then on wet soils (May to July) clearly established the importance of soil water content for the transformation of manure constituents. It is therefore appropriate to discuss our results in terms of the two weather periods.

4.1. Manure characteristics

The manure used in this study exhibited a high C/N, in comparison with the value of 14.0 found by Dewes and Hünsche [7], and a high C content of 22% compared, for example, with the mean value of 14% obtained with four different manures used by Nyamangara et al. [22]. Leachable C was also abundant, with 617 $mg \cdot L^{-1}$, compared with 406 $mg \cdot L^{-1}$ found by

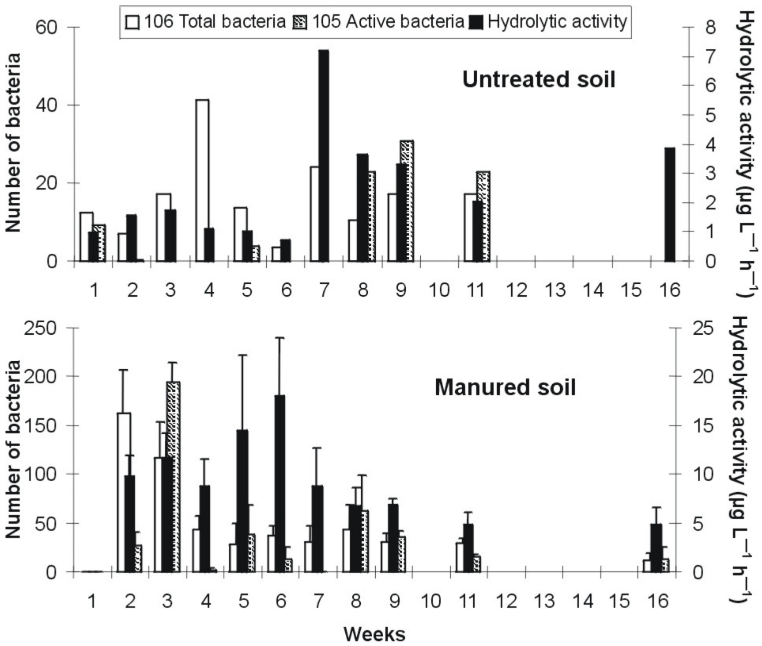

Figure 3. Variations of microbial activity in soil leachates collected under untreated and manured soil (mean and SD of three replicates) during the 16 weeks of experimentation. Manure was spread at week 2.

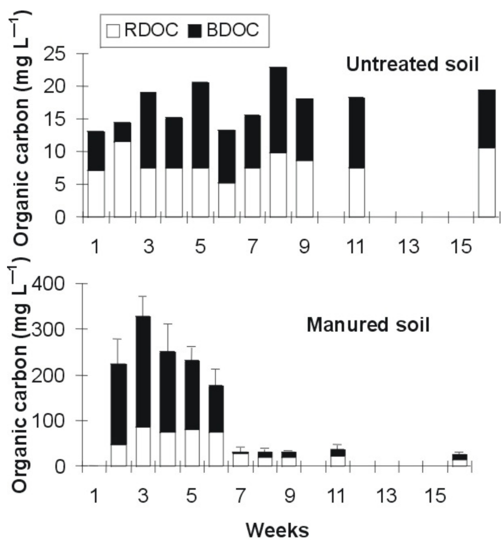

Figure 4. Variations of DOC, BDOC and RDOC concentrations ($mg\cdot L^{-1}$) in soil leachates collected under untreated and manured soils (mean and SD of three replicates) during the 16 weeks of experimentation. Manure was spread at week 2.

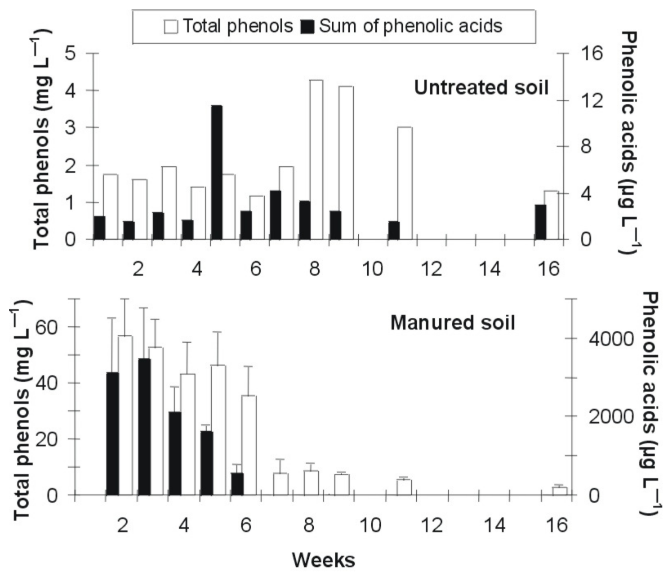

Figure 5. Variations of total phenol concentrations (mg of gallic acid equivalent$\cdot L^{-1}$) and phenolic acid concentrations ($\mu g\cdot L^{-1}$) in soil leachates collected under untreated and manured soils (mean and SD of three replicates) during the 16 weeks of experimentation. Manure was spread at week 2.

Riffaldi et al. [30] on farmyard manure leachates obtained after stirring for [22] h. In contrast, the latter found a higher phenol content (410 $mg\cdot L^{-1}$ of coumaric acid equivalent) compared with our results.

The DOC fraction of manure leachates was mainly composed of water-soluble biodegradable organic C, with only 17% of RDOC. The contribution of total phenols to DOC (calculated on a basis of 49% of C in gallic acid used as standard used in total phenols determination) was approximately in the same order as that of RDOC, which strengthens the hypothesis that total phenols contribute mainly to RDOC [5]. Phenolic acids contributed only about 1% to BDOC, and they seemed to be easily leached from manure, since the same composition was observed between solid manure and manure leachates.

Table IV. Phenolic composition of leachates collected under non-amended (control) and amended soils. Each phenolic acid is expressed as the % of the sum of all the phenolic acids. For amended soil, each value is the mean (and SD) of three replicates. †Date of manure spreading.

	Week	1	2†	3	4	5	6	7	8	9	11	16
Sum	Control	2.0	1.5	2.3	1.6	11.5	2.4	2.4	3.3	2.4	1.5	3.0
($\mu g \cdot L^{-1}$)	amended		3139.1	3470.1	2102.1	1608.6	539.2	2.6	5.7	2.7	4.7	2.2
including:			(1383.6)	(1314.5)	(663.7)	(173.2)	(247.3)	(1.0)	(3.5)	(<0.1)	(2.8)	(1.0)
Protocatechuic	Control	2.7	0.0	5.6	0.0	5.1	0.0	0.0	0.0	10.2	0.0	0.0
acid (%)	amended		3.9 (1.1)	5.3 (4.2)	5.3 (3.3)	10.4 (5.2)	8.5 (0.6)	7.4 (7.2)	2.1 (3.7)	6.4 (5.6)	10.1 (4.7)	9.0 (7.9)
Gentisic	Control	4.8	0.0	13.1	0.0	5.5	0.0	0.0	0.0	0.0	0.0	0.0
acid (%)	Amended		0.1 (<0.1)	0.1 (<0.1)	0.3 (0.2)	0.6 (0.2)	1.7 (0.5)	0.0 (0.0)	4.6 (7.9)	0.0 (0.0)	4.1 (7.1)	0.0 (0.0)
p-hydroxyben-	Control	17.8	23.5	28.0	28.2	20.9	31.0	12.1	16.1	11.9	22.2	22.1
zoic acid %	Amended		38.3 (4.6)	41.7 (4.3)	34.0(5.0)	28.2 (3.7)	19.1 (1.5)	18.6 (11.2)	25.4 (16.0)	22.0 (6.4)	13.3 (4.7)	31.6 (13.7)
p-hydroxyben-	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
zaldehyde (%)	Amended		0.6 (0.3)	0.8 (0.3)	0.7 (0.2)	1.1 (0.7)	0.5 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Vanillic	Control	21.7	29.6	17.8	0.0	15.7	18.3	16.7	20.7	16.9	0.0	32.7
acid (%)	Amended		0.6 (0.3)	1.3 (0.4)	2.9 (1.4)	6.4 (1.6)	10.5 (4.2)	3.0 (5.2)	13.3 (12.5)	6.0 (5.9)	12.7 (5.7)	21.0 (6.1)
Caffeic	Control	3.7	4.9	18.7	12.8	9.4	5.6	37.5	21.8	18.6	7.4	12.4
acid (%)	Amended		0.0 (0.0)	0.1 (0.0)	0.1 (0.0)	0.4 (0.2)	0.9 (0.3)	40.4 (26.0)	20.9 (12.3)	19.0 (5.7)	29.8 (2.3)	11.9 (10.7)
Syringic	Control	15.4	17.3	6.1	0.0	12.3	7.9	0.0	2.3	1.7	0.0	0.0
acid (%)	Amended		56.2 (5.4)	48.6 (0.9)	52.3 (3.8)	46.4 (3.9)	48.7 (9.9)	9.4 (10.3)	14.3 (4.0)	12.5 (4.1)	3.5 (3.0)	8.6 (10.2)
p-coumaric	Control	14.1	11.1	6.5	15.4	16.2	25.4	7.9	21.8	33.9	38.9	24.8
acid (%)	Amended		0.1 (0.0)	1.5 (0.9)	3.3 (0.7)	4.6 (1.1)	6.9 (3.8)	6.3 (11.0)	11.8 (14.8)	9.6 (6.7)	12.4 (9.4)	8.2 (8.1)
Ferulic	Control	19.7	13.6	3.7	43.6	14.9	11.9	24.2	17.2	6.8	31.5	8.0
acid (%)	amended		0.2 (0.0)	0.6 (0.4)	1.0 (0.4)	2.0 (0.5)	3.4 (2.0)	14.9 (16.4)	7.5 (7.2)	24.5 (1.2)	14.0 (13.4)	9.3 (9.8)

Manure phenolic acids can originate from forage cell solubles, but also from cell wall and lignin degradation occurring in the bovine stomach as a result of microbial activity [15] and during composting [17]. Nitrobenzene oxidation of lignin from faeces of sheep fed with lucerne (*Medicago sativa* L.) released in the order of abundance: ferulic acid > vanillin > syringaldehyde and then syringic acid [15]. Syringic acid, characteristic of dicotyledonous angiosperms lignin [19], was dominant in our free phenolic acid fraction, while ferulic acid and vanillin were only detected at trace concentrations. It can therefore be hypothesized that ferulic acid and vanillin were still bound to depolymerized fractions of lignin, while syringic acid occurred in free and thus leachable form.

The high C content and C/N ratio, and large amounts of leachable organic matter, were indicative of insufficiently humified manure [16].

4.2. Manure influence on soil leachates

The increase in soil moisture under farmyard manure-amended soil is a well-documented phenomenon [23], as is the increase in microbial biomass and activity [1, 9] and in organic and mineral compounds [13]. The intensity of these phenomena depended on the constituents: the most marked enrichment during the first month of experimentation was recorded for K^+ and NH_4^+ in the mineral fraction and for phenolic compounds (especially phenolic acids, as syringic acid). The lowest was noticed for Na^+, NO_3^-, SO_4^{2-}, RDOC and hydrolytic activity.

These trends directly reflected manure composition: K^+ is particularly abundant in farmyard manure [16] and its concentration slightly increased during the month of experimentation until the first significant rain. In contrast, it seems that after two weeks, even without rain, a great part of the available ammonium had already been volatilized [32] or nitrified. Nitrification could explained the few days of latency observed between manure spreading and NO_3^- enrichment, which only occurred after two weeks. Tenuta et al. [35] found the same trend of quick disappearance of NH_4^+ compared with a relative stability of NO_3^- concentrations over a 49-day field experiment that was conducted in weather conditions similar to ours (manure spread during a dry period, followed some weeks later by excess rain). But nitrate accumulation in the present study was relatively low and it could be suspected that a significant fraction of the ammonium was immobilized in microbial biomass, as suggested by the agreement between the quick disappearance of ammonium and the increase in active bacterial biomass.

The preponderance of manure composition over soil solution composition was also obvious when looking at the BDOC contribution to DOC on the first sampling date (80%), which was about the same as that observed in pure manure leachates (84%), while BDOC contributed less than 20% to total DOC in control soil leachates. This dressing of easily degradable organic matter was a potential pool for soil microbial activity, especially for both deeper layers of soils or aquatic ecosystems [18]. Hydrolytic activity that continually increased during the first five weeks after spreading reflected the utilization of this fraction (including some phenolic acids like p-hydroxybenzoic acid, that decreased during the first five weeks) by soil microorganisms such as bacteria [26]. Amongst the numerous bacteria from manure, a large part disappeared after two weeks from soil leachates, probably because they were Bacillus-like bacteria of fecal origin, unadapted to soil ecological conditions [6].

4.3. Rainy event influence

Twenty-five days after manure application, soil imbibition began due to a 7 mm rain, which was associated with an increase in hydrolytic activity in control leachates. It was followed by a rainy period of ten days (cumulated rainfall of 70 mm). In the same period, 1-day-mean temperatures increased from 6.0 to 12.0.

These climatic variations induced a general and rapid decrease in most of the water-soluble parameters (except for NH_4^+, total and active bacteria). This trend was particularly strong for BDOC and phenolic acids, when compared with RDOC and total phenols. Considering the five sampling dates from May to July, slight (less than three-fold) but significant differences between control and amended soil leachates were still recorded for soil moisture, pH, K^+, Na^+, Cl^-, SO_4^{2-}, DOC, RDOC, total bacteria, hydrolytic activity, total phenols and some phenolic acids including syringic acid. These 3-month effects were the result of particular organic matter biodegradation and mineralization by microorganisms, which slowly released low molecular weight compounds, including phenolic acids and mineral elements.

4.4. General discussion

As was expected, manure application at a high rate induced a global and immediate increase in all water-soluble components in soil leachates, even if for some parameters like hydrolytic activity and NO_3^-, increases were small and delayed for several weeks, waiting for biological processes to begin. The rate of disappearance of these elements from soil leachates will be more dependent on weather conditions (especially precipitation), and two classes of parameters have been roughly separated. Some components almost disappeared within a few weeks, irrespective of rainfall intensity: NH_4^+, total and active bacteria, and BDOC, including easily metabolizable phenolic monomers like p-hydroxybenzoic acid. On the other hand, minerals, RDOC, total phenols and syringic acid amounts changed only slightly during the first five weeks without rain events, to dramatically decrease with the first sufficient rain. It is thus likely that this first rain will be highly concentrated in these elements, some of them being potentially injurious to some organisms. For instance, syringic acid, identified here as a soluble and quantitatively important farmyard manure component, is also known (like many other phenolic acids) as a potential inhibitor of germination at 5×10^{-4} M [14] and of seedling growth at 10^{-5} M [3]. One week after manuring, soil solutions will contain around 2×10^{-5} M of phenolic acids (including 56% of syringic acid), that could induce some negative effects on the sensitive neighboring vegetation. More generally, the organic and mineral enrichment also represents a threat for water quality, since some of the maximum values registered during this study were far beyond the official levels accepted for drinking water. This was especially the case for NH_4^+ and K^+concentrations which reached more than 16 and 122 $mg \cdot L^{-1}$, respectively, while the authorized levels are 0.5 and 12 $mg \cdot L^{-1}$ [Décret n° 89-3 du 3 janvier 89]. These concentrations (although they resulted from a relatively high rate of spreading) seem high enough to cause restricted pollutions in the case of hard rain. In the mountain area where this study was conducted, the five weeks of dryness that followed manure spreading did not eliminate the deleterious potential of the manure and the first rain on this manure leached enough compounds to be a source of pollutants, minerals as well as organics.

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