Traçage des matières organiques dissoutes par fluorescence dans les bassins versants agricoles

Muhammad Bilal

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Traçage des matières organiques dissoutes par fluorescence dans les bassins versants agricoles

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Doctorate / AGROCAMPUS OUEST

Under the seal of European University of Brittany

To obtain the degree of:

DOCTEUR DE L'INSTITUT SUPERIEUR DES SCIENCES AGRONOMIQUES, AGRO-ALIMENTAIRES, HORTICOLES ET DU PAYSAGE

Specialization: Environmental science

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Fluorescence tracers of Dissolved Organic Matter in headwater agricultural catchments

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DEDICATED TO

MY PARENTS

Heartfelt appreciation for my parents who
taught me the first word to speak and brought me up with their
love and encouraged me for advance studies. I also wish to dedicate
my late sister, whose dreams come up. May her soul rest in peace. ameen!

My wife and my daughters Hadia & Zikra

Who make every moment of my life colorful
and have been great source of motivation and encouragement.

and

ISLAMIC REPUBLIC OF PAKISTAN and FRANCE

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I could never be able to Thank my parents and parents in law for their prayers, which enable me to achieve this goal, I am thankful to my sisters, uncles, cousins for their love and moral support. I am happy to be blessed from Allah for having good life partner who supported me well and my daughters who makes me joyful whenever, I feel their support.

Last but not least, a prayer for my late Sister and uncles whose dreams come up true, who gave me inspiration and dare to dream, May their soul rests in heaven (Ameen).

Table des matières

Table of contents

List of Figures

List of tables

Traçage des matières organiques dissoutes par fluorescence dans les bassins versants agricoles

Résumé :

La concentration en carbone organique dissout augmente depuis environ vingt ans dans les rivières de nombreux bassins versants en Bretagne et en Europe. Dans les petits bassins versants agricoles, les principales sources sont les sols et les effluents d'élevage. Afin de proposer des actions pour restaurer la qualité de l'eau, la fluorescence tridimensionnelle EEM (Excitation Emission Matrix) est utilisée pour tracer dans les sols et les cours d'eau la matière organique issue des effluents d'élevage.
Les traceurs de fluorescence sont mesurés sur les MOD issues de lisiers de porc, fumier de bovin et effluents compostés couramment recyclés sur les sols. Ces traceurs sont ensuite recherchés dans les eaux de ruissellement lors d'une simulation de pluie sur parcelle amendée par du fumier de bovin et du lisier de porc. La persistance des traceurs est évaluée dans une incubation de sol (deux mois) et sur deux dispositifs expérimentaux (Champ Noël, 0.9% de carbone total et Kerguehennec, 2.5% de carbone total) comparant des fertilisations minérale et organique (lisier, fumier) respectivement depuis 14 et 7 ans. Enfin, la relation entre les pratiques agricoles dans les zones humides de fond de vallée et la présence de ces traceurs dans les eaux de quinze bassins versants (BV) agricoles est explorée lors de trois crues. Ces zones sont reconnues comme étant les principales zones contributrices en MOD dans les BV bretons. Les pratiques agricoles (rotation, qualité et quantité de fertilisants, paturâge) dans les zones humides potentielles de fonds de vallée sont identifiées par enquête.
La fluorescence est intégrée dans deux régions du spectre (biochimique/géochimique, bio/géo), cinq régions détaillant les composés du type protéine, fulvique ou humique (I à V), et trois zones (Tryptophane (TRY), composés fulviques (FL) et humiques (HL)). La MOD issue des lisiers et fumiers possède une empreinte fluorescente biochimique qui les discrimine des effluents compostés présentant une empreinte plus géochimique similaire aux MOD issues des sols. Les traceurs bio :geo, TRY :FL, TRY :HL, et TRY:(HL:FL), TRY permettent de tracer les MOD issues d'effluents d'élevage dans les eaux de ruissellement quelques heures après l'épandage. Les MOD issues d'effluents bovins ne sont pas discriminées des effluents porcins. Un an après le dernier épandage, plusieurs traceurs des effluents sont retrouvés dans le sol à 0.9% de C, alors que sur le sol à 2.5% de C, seul le TRY persiste. Les résultats ne permettent de conclure sur l'effet cumulatif ou sur l'influence du dernier épandage. Les traceurs sont identifiés dans les BV les plus impactés par le recyclage d'effluents d'élevage. Certains BV ne sont impactés que par des MOD fortement humifiées issues des sols sans recyclage. La fluorescence tridimensionnelle permet donc de tracer des MOD issues des effluents d'élevage.

Mots-clés: matière organique dissoute, effluents d'élevage, pratiques agricoles, qualité des eaux, fluorescence, bassins versant.

Fluorescence tracers of Dissolved Organic Matter in headwater agricultural catchments

Abstract

Dissolved organic matter (DOM) concentrations are increasing in the streams at agricultural headwater catchments in French Brittany, an intensive agricultural region, and Europe during last twenty years. These increasing DOM concentrations are threat to water quality degradation. At small agricultural catchment scale, soil and farm wastes effluents are principle sources of DOM. To propose management actions and to restore stream water quality, three dimensional EEM (Excitation Emission Matrix) was applied to trace DOM issued from farm wastes in the soil and agricultural catchment stream.

Fluorescence tracers were measured on DOM produced from pig slurry, cow manure and composted manures which recycled commonly in cultivated soils. Afterwards, these tracers were analysed in rainfall simulation experiment in the cultivated plots amended with pig slurry and cow manures. The persistence of these fluorescence tracers was evaluated in soil incubation (two months) and in two different experimental dispositives (Champ Noël, 0.9% total carbon and Kerguehennec, 2.5% total carbon) as well as these tracers were compared in mineral vs organic (pig slurry and cow manure) fertilized plots with different recycling time of 14 and 7 years respectively. Finally, the relation between agricultural practices in Valley Bottom Wetlands (VBW) and the presence of these fluorescence tracers in 15 agricultural streams were explored during three storm events. VBW were identified as principle source of DOM in French Brittany catchments. The agricultural practices (crop rotation, quality and quantity of fertilizers, grazing meadows) in the VBW were identified by farm survey.

The fluorescence intensities were integrated in the two regions of EEM spectra (biochemical/geochemical, bio:geo), five regions composed of proteins like, fulvic and humic (I to V), and three zones (tryptophan (TRY), fulvic like (FL) and humic like (HL)). DOM produced from pig and cow demonstrated biochemical fluorescence signatures and discriminated from composted manures which showed geochemical signatures similar to soil DOM. The tracers bio:geo, TRY:FL, TRY:HL, TRY:(HL:FL), TRY trace the DOM issued from farming wastes in simulated runoff two hours after soil spreading. Cow manure DOM was not differentiated from pig wastes DOM with these fluorescence tracers. One year after last recycling, several tracers were found in soil 0.9% C while at the soil with 2.5% C, only TRY persisted. With these results, we are not clear whether the effect is cumulative or it's the influence of last farm wastes spreading. The fluorescence tracers were identified in the headwater catchments impacted by farm wastes recycling. Some catchments demonstrated highly humified DOM which resembled to soil DOM without recycling. Therefore, fluorescence spectroscopy permits to trace the DOM issued from farming wastes. Fluorescence spectroscopy is found a valuable tool for monitoring farming wastes DOM contamination and understanding the biogeochemistry of DOM in soil and water environment.

Key words: dissolved organic matter, farming wastes, agricultural practices, water quality, fluorescence spectroscopy, headwater catchments

GENERAL INTRODUCTION

General introduction

The increasing Dissolved Organic Carbon (DOC) concentrations in streams modify the physical, biological and chemical quality of natural waters, particularly via the transport of mineral or organic pollutants in agricultural catchment (Muller et al., 2007; Pedrot et al., 2008). High quality drinking water demands the control of source water pollution as source protection is often more reliable than treatment and reduces the cost of drinking water supplies. Incomplete removal of DOC in potable waters reduces the aesthetic quality and complete removal rises the cost during treatment. Besides this, during treatment process, formation of trihalomethane (THM) is enhanced which is a potential carcinogenic by-product (Galapate et al., 1999). In France, the legislation authorities required a DOC concentrations lower than 10mg L^{-1} in 95% of the water samples collected per year for potable drinking water supplies. In 2006, in French Brittany, an intensive agricultural region, 43% of the superficial resources for drinkable water supplies were not conforming to regulation.

Dissolved organic carbon (DOC) is operationally defined as organic carbon passing through filters of 0.45µm or 0.22µm. It includes chemically defined compounds such as carbohydrates and proteins, humic substances which include fulvic and humic acids which are operationally defined based on their solubility (Thurman, 1985). However, studies also reflect that humic substances are a complex mixture of both microbial and plant biopolymers, with their various breakdown products, and cannot be classed as a distinct chemical structure (Kelleher and Simpson, 2006). In general, dissolved organic matter (DOM) consists of a rapidly degradable fraction (labile DOM). The slowly degradable or relatively stable DOM fraction consists of structures not easily cleaved by enzymes, such as lignin or the compounds strongly altered in the preceding degradation steps (Joergensen, 1998).

Majority of the dissolved organic matter contents in streams are derived from allochthonous sources (Palmer et al., 2001; Gordon and Goñi, 2003): in general, litter leachates, root exudates and microbial degradation products (Zsolnay, 1996). It can comprise both young and old organic matter with varying biological recalcitrance (Raymond and Bauer, 2001). However, the age and DOM composition in stream can be changed depending upon discharge (Neff et al., 2006). The chemistry of DOM itself depicts the diverse classes of compounds with heterogeneity in molecular weight, reactivity and bioavailability (Seitzinger et al., 2005).

The diversity in DOM composition makes it a potentials tracer of source water and runoff generation pathways (Hood et al., 2006).

The knowledge of the factors controlling the variation of DOC in headwater streams is of particular interest for at least two reasons: i) the quantification of the overall carbon budgets draining into fresh water streams , ii) DOC as a vector of pollutant mobilization and transport in fresh water streams (Temminghoff et al., 1997).

Higher DOM concentrations have been observed during storm events (high water flows) in various aquatic ecosystems including hardwood forests (Inamdar et al., 2008), peatland (Worrall et al., 2002) and riparian wetland soils in headwater agricultural catchment (Morel et al., 2009). As the increasing DOM concentrations are associated with increasing discharge rate, so the storm events account considerable amount of DOM export from catchments (Hinton et al., 1997). Hydrological flowpaths can have a control on DOM export from surface water during high flows (Zhang et al., 2007).

Moreover, wetting and drying cycles (Lundquist et al., 1999) and climatic change (rising temperature and change in rainfall pattern) can also explain rising DOM concentrations (Freeman et al., 2001; Worrall et al., 2003).

In Brittany, a region of intensive agriculture, Valley Bottom Wetlands (VBW) are the main contributors of dissolved organic matter (DOM) in agricultural catchments (Morel et al., 2009). During storms, 64 to 86% of the DOC in the stream originated from the upper layers of the riparian wetland soils. Overall, VBW soils can be under intensive maize and wheat crop cultivation. During spring season, soils may be fertilized with farm wastes, and moreover, these VBW also serve as intensively grazing pastures. Thus, these agricultural intensive areas in VBW, with excess load of farm manure application, can take part in stream DOM contamination by two ways; firstly, by direct transfer of farming waste DOM during storm event from the intensively grazing areas or in the days after the farming waste supply on soil; secondly, these wastes can increase the water-extractable organic carbon of soil (Gregorich et al., 1998; Chantigny et al., 2002b) which can be flushed to the rivers when the groundwater level reached the surface horizon. The role of increased manure spreading on cultivated soils has been already highlighted in Brittany (Gruau and Jardé, 2005). These authors explained that a long-term DOM decrease was observed in an agricultural catchment marked by intensive spreading of pig manure and proposed the hypothesis that spreading of manure can acidify watershed soils, thereby promoting DOM adsorption on minerals which could ultimately limit the export to the river.

It is quite difficult to assess the role of farming waste recycling on the export of DOC in agricultural catchments, since some processes may increase the DOM production in soil, and other may favour its adsorption. Transfer of DOM from soils receiving pig slurry to the stream was verified (Jardé et al., 2005). This study provides evidence that manure spreading on catchment soils influences the water quality in rivers draining these catchments.

Changes in the DOM chemical characteristics were related to agricultural land use, nitrogen loading and wetland loss (Wilson and Xenopoulos, 2009). However, in agriculture headwater catchments, the links of agricultural practices with DOM concentration and composition as well as biogeochemical cycling remain poorly characterized. Hence, to restore water quality, it is essential to understand DOM sources in agricultural catchments and to investigate the impact of intensive farming practices in the Valley Bottom Wetlands.

DOC concentration alone is of limited interest as environmental tracer and more information on the nature of DOM is required. It is important to develop tools which enable to trace the fate of farming waste organic matter in the environment and to assess whether manure disposal on the catchment soils could affect the organic quality of rivers.

Fluorescence spectroscopy

In this aspect, 3-dimensional excitation emission matrix (3DEEM) fluorescence spectroscopy seems to be a good candidate due to its high sensitivity to physicochemical changes in DOM materials (Thacker et al., 2005). DOM concentration, composition, distribution and its dynamics has been studied by fluorescence in a range of aquatic environments. Principally, depending on the nature of the excited state, luminescence spectroscopy (the emission of light from any substance which occurs from electrons in excited states) is divided into fluorescence and phosphorescence. Phosphorescence is emission of light from triplet excited states, in which the electron in the excited orbital has the same spin orientation as the ground state electron (Lakowicz, 1983).

However, in fluorescence, measurements are performed in the UV-visible range (200 – 750 nm) in which first step involves molecular absorption of light (photons). The absorbing molecule is promoted from the ground state to an excited singlet state as shown in Figure 1. Part of the absorbed energy is then released during vibrational relaxation or internal conversion and the molecule reached the excited state of lowest energy (S_1). The rest of the absorbed energy is released in the form of light emission and occur generally 10^{-9} second after excitation, when electron decays back to ground state (S_0). The quantum yield is a measure of the efficiency with which absorbed light produces some effect. The emitted light always has lower energetic levels than the excitation and is thus detected at higher wavelengths. The shift

between excitation and emission wavelength is known as Stoke's shift. Fluorescence spectroscopy provides information about the presence of fluorescent molecules and their environment in analyzed samples (Lakowicz, 1983).

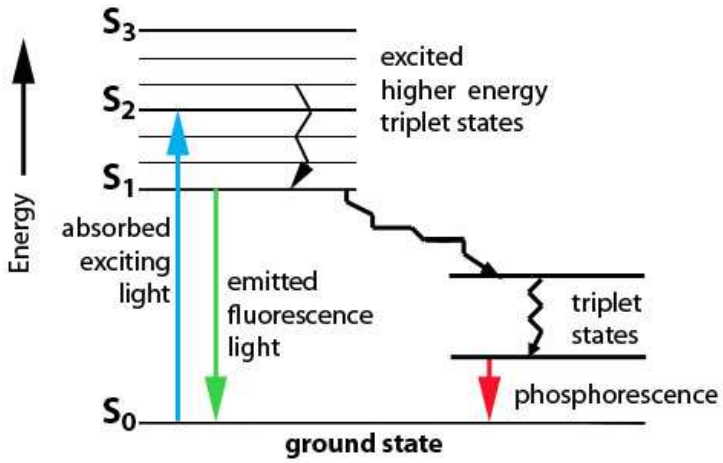

Figure 1 : Jablonski diagram of excitation and emission of a molecule in fluorescence or phosphorescence (Lakowicz, 1983)

The natural fluorescence can be used to elucidate the complex chemical composition and diverse sources of dissolved organic matter in natural waters (Coble et al., 1990).

In the study of organic matter, fluorescence typically occurs from aromatic molecules which provide good subject for study by fluorescence due to energy sharing, unpaired electron structure of the carbon ring. In the study of organic matter fluorescence, the compounds which absorb light are called chromophores and those which absorb and re-emit light are called fluorophores (Mopper et al., 1996). The commonly studied fluorophores in organic matter fluorescence are humic substance (breakdown products of plant material by biological and chemical process in terrestrial and aquatic environments) (Parlanti et al., 2002; Stedmon et al., 2003; Sierra et al., 2006) and amino acids in proteins and peptides.

Tryptophan and tyrosine are the fluorescent amino acids which indicate the presence of proteins. In these amino acids, fluorescence arises from indole group (a fused ring heterocycle containing both a benzene ring and heterocyclic nitrogen containing aromatic ring). These groups of fluorophores are commonly named as humic-like, fulvic-like and protein-like because standard materials of these substances demonstrate the fluorescence in the same area of optical space Tryptophan fluorescence has also been related to the activity of bacterial community (Elliott et al., 2006).

Excitation emission (EEM) fluorescence captures many spectral features by scanning over a wide range of excitation and emission wavelengths and generating a landscape surface

defined by the fluorescence intensity over excitation emission wavelength pairs (Wu et al., 2003; Sierra et al., 2005; Xie et al., 2008). In sea waters, since the work of Coble (1996) in tracing riverine DOC in sea water, application fluorescence spectroscopy increased in esturine and marine waters. Fluorescence properties of DOM serve as a tool for determining biological activity and associated protein fluorescence (Determann et al., 1998; Parlanti et al., 2000) and mixing of water bodies.

In fresh waters, the advances in fluorescence spectroscopy have been applied in tracking of dissolved organic matter (Thoss et al., 2000.; Newson et al., 2001). Different fluorescence peaks are reported from EEM and ascribed to protein like (tryptohan, tyrosine), fulvic like and humic like fluorophores in DOM in the aquatic and soil environment (Baker, 2002; Parlanti et al., 2002; Henderson et al., 2009).

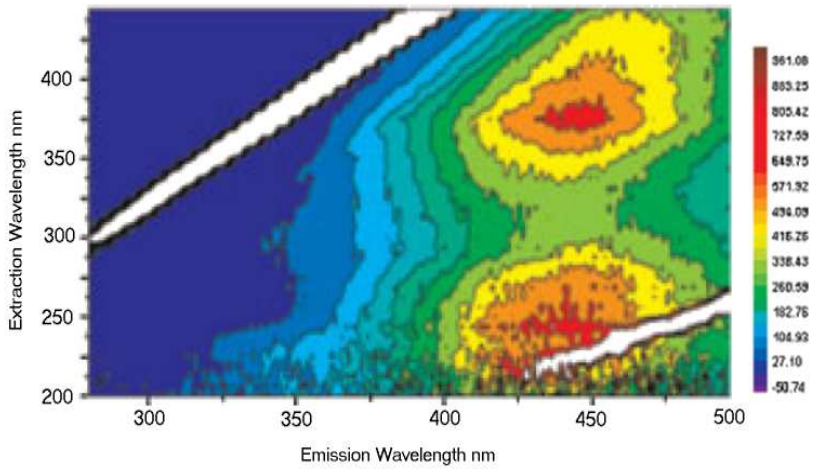

Figure 2 : Typical Excitation Emission Matrix (EEM) in a water sample (Hudson et al., 2007)

Relative strength of protein like and humic like fluorophores as well as their ratios has been used to differentiate various sources of DOM (Baker and Inverarity, 2004; Cumberland and Baker, 2007). Baker (2002) has indicated higher protein like fluorescence in animal wastes and demonstrated higher peak intensity ratio of tryptophan:fulvic like for animal wastes than stream water. However, unlike marine studies, application of fluorescence spectroscopy in fresh water environment is not yet widespread. A change of humic-like fluorescence has been identified from upland region to downstream with increasing anthropogenic input (Baker and Spencer, 2004). Lapworth et al., (2009) have used maximum peak fluorescence intensities of tryptophan like and fulvic like and observed more attenuation in tryptophan like compared to fulvic like fluorescence in hyporheic zone (0.5 meter below river bed) with changing surface

waters inputs from upstream processes in riparian areas. Fellman et al. (2009a) have shown changes in chemical quality of DOM in spring and fall wet season in bog, forested wetland and upland forests. They further showed the contribution of DOM from upland watersheds and the contribution of humic like fluorescence increased and protein like fluorescence decreased during stormflows (Fellman et al., 2009b).

Furthermore, in waste water (Baker, 2002) and treated sewage effluents or sewer discharge, tryptophan peak has been measured (Galapate et al., 1998; Baker et al., 2004). Sewage-derived material is rich in tryptophan-like fluorescence, and is observably different from rivers, where fulvic/humic like peaks predominate (Hudson et al., 2007). This is because DOM originating from clean river water is dominated by natural organic matter derived from plant material, whereas sewage-derived DOM is dominated by organic matter originating from microbial activity (Hur et al., 2008). Such differences in spectral signatures have facilitated the tracking of sewage contamination in river waters (Galapate et al., 1998; Baker, 2001; Baker et al., 2003; Chen et al., 2003a; Holbrook et al., 2005; Hudson et al., 2008). To trace DOM issued from recycling of farming waste on soils, we have to study purely agricultural headwater catchments.

Instead of using fluorophores peak fluorescence intensities, a chemometric approach of fluorescence regional integral integration proposed by Chen et al., (2003b) is getting popularity among the fluorescence users community. In drain flows, Naden et al. (2009) has adopted regional overlap of the anticipated fluorophores and proposed the TI:FI ratio (Tryptophan-like and fulvic/humic-like fluorescence) as tracer of cow slurry incidental losses in drain flow after slurry spreading.

However, in the literature, there is lack of some knowledge about the characterization of pure farm wastes, impact of farm waste supply on the production dissolved organic matter and its characterization by fluorescence spectroscopy. Besides this, literature also lacks the application of fluorescence spectroscopy to study the long term impacts of farm wastes supply on the soil DOM and there are no indications of fluorescence fingerprints in long term farm wastes soil supplies on gradient of dissolved organic matter concentrations.

Objectives of the thesis

The general aim of the thesis is to assess fluorescence as a tracer of Dissolved Organic Matter (DOM) issued from farming waste, in soils and stream water of agricultural headwater catchments.

The following questions will be considered keeping in view the gaps in the literature:

- What are the fluorescence tracers of different pure farm wastes?
- How are the fluorescence properties in natural streams impacted by known direct transfer of pig slurry and cow manure composts?
- Do fluorescence properties discriminate pig from cow manure contamination in runoff water?
- How evolve the fluorescence parameters of DOM in soils receiving farming waste in the days following the spreading? What is the persistence of the fluorescent tracers two months after the spreading?
- Does long term application (7-14 years) of pig slurry and cow manure wastes on cultivated soils significantly modify the fluorescence properties of DOM?
- Can we detect influence of farming waste recycling on soil or impact of grazing pasture in stream water in a network of 15 agricultural headwater catchments? Is there any relation between farming waste management (intensive recycling on soil or grazing pasture) in Valley Bottom Wetland and presence of fluorescence tracers in stream waters?

This thesis is organized in five chapters and has been written in publication format. In **chapter 1**, fluorescence characterization of various farm wastes was carried out and analysis of fluorescent tracers of pig slurry and cow manure composts was investigated in two different natural streams with known amounts of farming waste. Fluorescent parameters are proposed to trace DOM issued from farming wastes. In **chapter 2**, fluorescent tracers were analysed in runoff water collected in a simulation event (during spring) after pig slurry and cow manure supply on soil. In **chapter 3**, a biodegradation study is carried out to follow the persistence of these tracers during two months. A statistical approach is proposed to discriminate between types of farming wastes (cow manure, pig slurry and wheat straw). In **chapter 4**, impact of long term supply of pig slurry and cow manure was investigated in two soil types with different organic carbon contents. DOM production potential of pig slurry and cow manure supply was assessed and fluorescence discrimination of pig slurry and cow manure is discussed. In **chapter 5**, spatial and temporal variability of DOM fluorescence

properties were investigated in 15 agricultural headwater catchments during three storm events and possible farm wastes contamination was explored. Correlation between presence of fluorescence tracers and intensive agricultural practices (crop rotation, farming waste recycling on soils) in Valley Bottom Wetland was analysed. We thus adopted an original approach ranging from raw farming waste characterization of DOM fluorescent tracers to catchment scale detection of these fluorescence tracers.

CHAPTER 1

Chapter 1

EEM fluorescence characterization of farm manures and farm waste impacted natural water

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Abstract

In this work excitation emission matrix (EEM) spectroscopy coupled with fluorescence regional integration and chemometrics were used to observe the dissolved organic matter (DOM) quality differences among various farm manures extracts and the changes in cumulative regional intensity in EEM of natural water sample subsequent to farm manure contamination. Farm manures used in the study were pig slurries, cow faeces, fresh cow manures, cow manure composts and wheat crop residues. Fluorescence similarities in pig slurry and cow faeces depicted the similar DOM quality in two breeding animals although former is monogastric and the later is ruminant. Pig slurry and cow faeces were strong in biochemical fluorescence intensities (region II, IV), TRY zone and both were discriminated by ratios bio:geo, region IV:V, TRY:(HL:FL), TRY:FL, TRY:HL. Cow manure composts were discriminated with higher fluorescence intensities geochemical regions (region III, V) as well as fulvic like and humic like zones.

In the mixing up natural water sample and farm wastes (pig slurry and cow manure composts), pig slurry and cow manure composts have shown higher bio:geo, region IV:V, TRY:(HL:FL), TRY:FL, TRY:HL compared to natural water samples. All the ratios showed higher values for pig slurry than cow manure composts except TRY:(HL:FL) which had shown higher values for cow manure composts compared to pig slurry. Mixed water samples showed the impact of farm manures with increasing ratios in comparison with natural water. Pig slurry and cow manure composts were discriminated with lower region intensities (region III vs V in EEM) than natural stream waters. Results show that regional and zonal transformations in EEM could be an effective tool for the characterization of farm manures and for tracing the point source pollution of pig and cattle manures.

Key words: excitation emission matrix spectroscopy, characterization, farm waste, pig slurry, cow manure composts, point source pollution, composts

Introduction

Anthropogenic and natural sources of dissolved organic matter (DOM) have major impact on the stream chemistry in the catchments. Farm manure applications on agricultural soil allow the disposal of organic waste produced at cattle farms, which serves as valuable nutrient source for crops but its storage and application rate needs some management to prevent the DOM water pollution. Farming waste recycling on soil, intensively grazed pastures located near the river, piled stocks along the water course or incidental pollution can induce microbial and dissolved organic carbon contamination in stream water. Human and animal fecal contamination of coastal environment cause the economic losses from the closure of shellfish in addition to affecting the quality of shellfish and recreational water and finally bathing restrictions (Mieszkin et al., 2009).

The assessment of direct DOM pollution of natural water by farm manures is the main question which is addressed in this study. Although some tracing studies have been attempted at catchment scale to detect pig slurry DOM in natural river water via coprostanol signature (Jardé et al., 2007a), yet the question remains to trace the allochthonous sources of DOM in agricultural catchment.

Fluorescence spectroscopy has been proven to be an useful optical technique to observe the changes and transformations in fluorescent components of dissolved organic matter in the natural environments (Baker, 2001; Hudson et al., 2007). This optical technique is rapid, selective, sensitive and account low cost. DOM includes organic molecules with chromophoric (light absorbing) and fluorophoric (light emitting) molecules (Her et al., 2003). Generally two types of fluorophores are ascribed to the DOM spectra: the humic like and the protein like (Coble, 1996). This technique enables not only the qualitative differentiation of natural organic matter from various origins but also explains subcomponents of natural organic matter with varying composition and functional properties (Chen et al., 2003a). Structural similarities of humic solutes are closely associated with the fluorescence properties and differentiated untreated waste from fresh water samples according to the fluorescent materials (Peuravuori et al., 2002). Numerous analytical methods exist to depict the fluorescence signatures of DOM which include humification index (cumulative emission fluorescence intensities in the region $\sum$(300-345) divide by the sum of $\sum$(300-345) and $\sum$(435-480) (Ohno, 2002), the fluorescence intensities and their ratios (Baker, 2001; Yamashita and Tanoue, 2003). Animal wastes (silage liquor, pig, cow and sheep) have been

characterized with not only high protein-like (tryptophan and tyrosine intensity peaks) fluorescence but also very high ratios of tryptophan to fulvic like fluorescence peaks farm wastes compared to stream waters (Baker, 2002). Ratio of tryptophan to fulvic like is higher in silage liquor (>20) followed by pig and cow (2-5) and lowest in sheep barn wastes (0.5-4.0). However, this ratio has been more stable in sheep barn waste compared to silage liquor and more variables in pig as well cow slurries and the decrease in ratio has been associated with decrease in tryptophan fluorescence. But these studies include one (peak locations) or few data points. However, a new approach of fluorescence regional integration is capable for the quantification of whole EEM spectra of DOM (Chen et al., 2003b). Natural fluorescence was recently proposed to trace diffuse agricultural pollution from cow slurry spreading on intensively-farmed grasslands (Naden et al., 2009). The ratio of indices of tryptophan-like and fulvic/humic-like fluorescence was proposed as an indicator of cow slurry in drainage waters.

Fluorescence regional integration is an optical data learning technique in which a normalised region-specific volume reflects the abundance of molecular structures specific to that region. In this technique, five regions in the EEM are defined by aromatic proteins (regions I and II, tyrosine like), humic like substance (region III), tryptophan like (region IV) as well as fulvic like materials (region V). EEM regions have been accounted for humic, tryptophan, tyrosine peaks (Coble et al., 1990; Chen et al., 2003b). Peptides with aromatic residues such as tyrosine showed their fluorescence at shorter excitation and emission wavelengths (region I and II) (Determann et al., 1994; Ahmad and Reynolds, 1999). Regional transformations of fluorescence in EEM coupled with chlorine consumption have been used to predict trihalomethane and haloacetic acid formation (Johnstone and Miller, 2009). These regional fluorescence similarities and dissimilarities among the heterogeneous sources can be ascribed as tracers of the specific source.

There is scarcity of knowledge in the literature about fluorescence properties of farm manures and also how they impact fluorescence signals of dissolved organic matter in natural water streams. Besides this, to best of our knowledge, integrating EEM fluorescence by regions and zones have been hardly found in literature to point out pollution source in natural water by farm manures. Therefore, the objective of this study are threefold: (i) to characterize fluorescence properties of dissolved organic matter extracts from pig slurry, cow manure composts, fresh cow manures, wheat crop residues by using fluorescence spectroscopy coupled with fluorescence regional integration and chemometrics (ii) to identify the fluorescence tracers of cow manure and pig slurry (iii) finally to identify fluorescent

parameters suitable for tracing diffuse source pollution of natural waters streams by pig slurry and cow manure composts.

Material and Methods

Farm manures characteristics and sample preparation

Two pig slurries (PS1 and PS2), two cow manures (CM1 and CM2), three cow faeces (CF) and three cow manures composts (CMC) with various composting times were analysed. One compost sample (CMC1) with one month and another cow manure compost with 4 (CMC2) and six months (CMC3) of composting were sampled. Cow manures and compost manures included the incorporated wheat crop residues. The preparation of all the studied farm wastes is indicated in Table 1.1. One cow faeces (CFd), one wheat straw (WS), two pig slurries, two cow manures, three cow manure composts samples were air dried in the shade at room temperature and then grounded and passed through a sieve of 1mm mesh size. However, one fresh cow manure (CMf) and two cow faeces (CF1 and CF2) were analysed without drying. Most of the farm manures, used in this study, were obtained from livestock breeding unit at Kerguehennec station in Brittany, France.

Table 1.1 : Farm wastes preparation

Waste description	**Sample treatment**	**Name**
Pig slurry	Air dried, grounded, sieve 1mm	PS1, PS2
Cow manure	Air dried, grounded, sieve 1mm	CM1, CM2
	Fresh	CMf
Cow Faeces	Air dried and grounded, sieve 1mm	CFd
	Fresh, not grounded	CF1, CF2
Composted cow manure	CMC1 Air dried, grounded, sieve 1mm. (one month) CMC2 and CMC3, same compost sampled after 4 and 6 months of composting	CMC1, CMC2, CMC3
Wheat straw	Air dried, grounded, sieve 1mm	WS

Aqueous extracts of farm manures

DOC was extracted with 40:1 (V:W) ultra pure water to farm manure ratio. Each farm manure water suspensions (either dried or fresh phase) were kept in refrigerator at 4°C for 16 hours with periodic manual shaking. Then the farm manure water suspensions were centrifuged at 3000 rpm for 30 minutes and subsequently filtered through 0.7 and 0.22 µm nitrocellulose filters. To avoid any contamination, all the filters were rinsed with ultra pure water and dried overnight before vacuum filtration.

Aqueous extracts of soil DOM

One soil sample with three replicates was taken at 0-20cm soil depth from the cultivated experimental plot under chemical fertilization at Kerguehennec research station. Soil DOM extracts were obtained with 2:1 ultra pure water to soil ratio. Soil water suspensions were shaken mechanically on orbital shaker for 2h and then centrifuged at 3000 rpm for 30 minutes and filtered through 0.7 and 0.22 µm nitrocellulose filters.

Mixing experiment of farm manures and natural water

A pig slurry and natural water (NW) (Stream 1) mixing experiment was conducted in January 2008 to test the fluorescence parameters determined on raw farming wastes. Stream water was sampled on 18 January 2008 in an agricultural headwater watershed of Ducey, located in North-West France. Mixing experiment of cow manure compost (CMC3) and NW (Stream 2) was conducted in January 2009. For this experiment, water was sampled in a stream draining a natural wetland on 26 January 2009 at Haut Couesnon site, North-West France. Both stream water samples were taken during winter storm events. After sampling, stream water samples were kept at 4°C and filtered through 0.22 µm. The filtered samples were maintained in the refrigerator at 4°C prior to fluorescence analysis.

In each mixing experiment, NW samples and farm manures (PS and CMC3) samples were analysed for fluorescence properties at $5mgL^{-1}$DOC separately. A mixed sample of equal volume (50/50) of farm manure solution/natural water (each containing $5mgL^{-1}$DOC) was generated which results final DOC concentrations of $5mgL^{-1}$ in the mixture, was analysed. Two other mixed water samples containing 25/75 and 75/25 (DOC contribution from cow manure compost/natural water) were also tested.

Chemical Analysis

DOC concentrations were measured using Shimadzu TOC 5050 A total carbon analyzer. Accuracy on DOC measurements was ±5%, based on repeated measurements of standard solutions (K-phtalate). UV-Visible absorbance was measured on a Perkin Elmer Lambda 20 UV-Visible spectrophotometer with excitation wavelengths range of 200-600 nm 0.5nm data interval, slit width at 2 nm and scan speed was set at 120 nm/min. Specific Ultra Violet Absorbance (SUVA) was measured by multiplication of absorbance at 254nm with 100 and dividing by the DOC concentration in the solution ($5mgL^{-1}$DOC).

Fluorescence spectroscopic measurements of DOC were performed using a Perkin-Elmer LS-55B luminescence spectrometer. The spectrophotometer uses a xenon excitation source and slits were set to 5 nm for both excitation and emission. To obtain excitation-emission matrix spectra, excitation wavelengths were incremented from 200 to 425 nm at steps of 5 nm and emission was detected from 250 to 600 nm with a 0.5-nm step. Scan speed was set at 1500 nm/min, yielding an EEM in 22 minutes with 45 total scans. To minimise the temperature effect, samples were allowed to equilibrate with room temperature (20±2°C) prior to fluorescence analysis. The spectra were obtained by subtracting distilled water blank spectra to eliminate the water Raman scatter peak. Resonance peak (Fig. 1) on the lower side of three dimensional plots was also removed. Linearity was carried out between DOC concentration and fluorescence intensity with dilution of high DOC concentration samples. Inner filter effects were removed with the formula developed by (Ohno, 2002). The whole fluorescence dataset presented in this study was normalised at 5 mg L^{-1} DOC. To maintain the consistency of measurements and standardise the whole fluorescence data set, the corrected fluorescence intensities were normalised with daily determined Raman emission intensity units (26) of ultra pure water samples at 350 nm and 397 nm of excitation and emission wavelengths respectively.

Regional integration of excitation emission matrix (EEM)

An internal program was developed in the laboratory using the R® software (http://www.r-project.org) for the integration of fluorescence intensities across the whole EEM landscape (Annexes, at thesis page 206). Here peaks at shorter wavelengths (<250 nm) and shorter emission wavelengths (<380 nm) are related to simple aromatic proteins such as tyrosine and tryptophan (Regions I and II) Peaks at intermediate excitation wavelengths (250–340 nm) and shorter emission wavelengths (<380 nm) are related to soluble microbial by-product-like

material (Region IV) while peaks located at the excitation wavelengths (230–300 nm) and the emission wavelengths (380-575 nm) represent humic acid-like substances (Region III). Peaks at longer excitation wavelengths (>300 nm) and longer emission wavelengths (>380 nm) are related to fulvic acid-like organics (Region V). With this technique, EEM is divided into biochemical (bio) (I, II, IV) and geochemical (geo) (III, V) fluorescent regions (Figure 1.1a), (Table 1.2) and three peak intensity zones of tryptophan (TRY), fulvic like (FL) and humic like (HL) fluorescence (Figure 1.1b) (Table 1.2). Humification index (HIX) was determined according to Ohno (2002). 45 spectral loadings were used to reproduce three-dimensional plots of fluorescence intensity as a function of excitation and emission wavelengths.

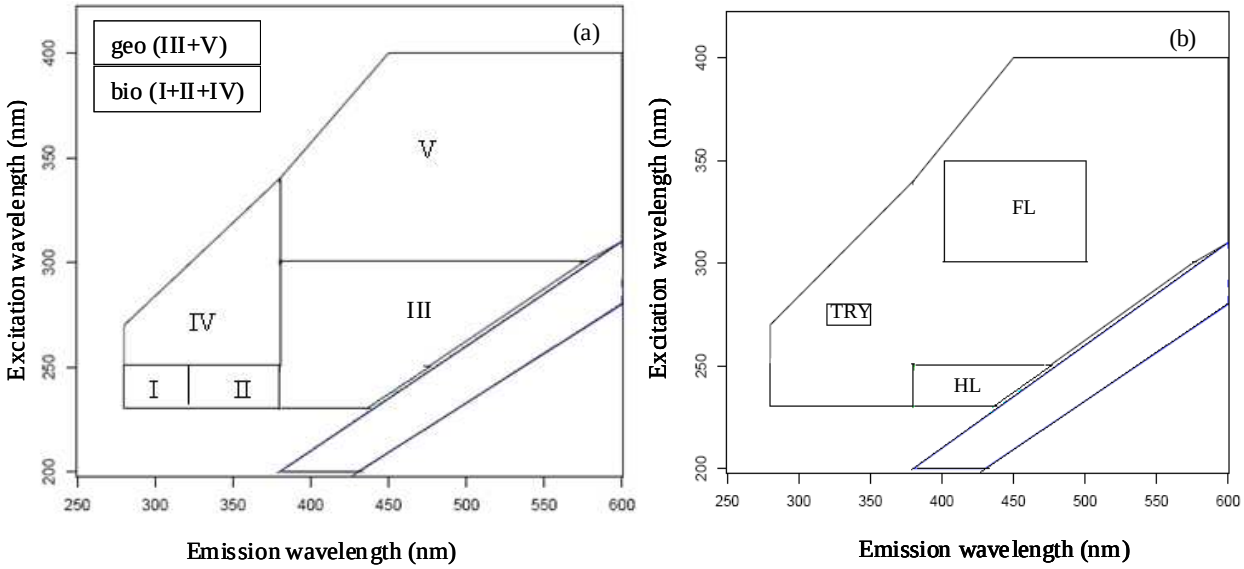

Figure 1.1: Integration of fluorescence intensities across regions (a) and maximum peak intensity zones (b).

Table 1.2 : Definition of excitation (Ex) - emission (Em) wavelengths (nm) ranges for each region and zones

	Ex (nm)	Em (nm)		Ex (nm)	Em (nm)
Regions			**Zones**		
region I	230-250	280-330	Tryptophan	270-280	320-350
region II	230-250	330-380	Fulvic like	300-350	400-500
region III	230-300	380-575	Humic like	230-250	360-420
region IV	250-340	280-380			
region V	300-400	380-600			
region bio	230-340	280-380			
region geo	230-400	380-600			

Statistical analysis

To observe the spectroscopic similarities and dissimilarities of farm manures studied, principal component analysis **(**PCA) was applied to the DOC normalised integrated fluorescence intensities of the studied farm manures with R® software (package ade4). Significant differences among the three groups of farm manures were tested by one way ANOVA ($p<0.05$) with STATISTICA (version 7.1).

Numerous replicates of fluorescent measurement on soil extraction in previous studies conducted with the same apparatus showed 5% coefficient of variation (unpublished data). Therefore we imposed this dispersion parameter to simulate three replicates per treatment in order to integrate potential analytical errors in the treatment comparison. Statistical analysis of the treatment means were run by one way ANOVA with STATISTICA 7.1 (Statsoft).

For the coefficient of variation (CV_R) of the ratio X/Y of two variables, we applied the approximation suggested by Holmes and Buher (2007): **Erreur ! Des objets ne peuvent pas être créés à partir des codes de champs de mise en forme.**, where CV_X and CV_Y are CVs of X and Y variables respectively.

Results

Spectroscopic characterization of farm effluents

Spectral differences and similarities among different types of farm manures were analysed by principal component analysis (PCA) on the fluorescence data set (Figure 1.2). PCA was performed on the integrated fluorescence intensities in five regions and three peak intensity zones along with their ratios of dissolved organic matter (DOM) in 12 farm effluents and one soil sample which was treated as illustrative individual (Figure 1.2). Both axes 1 and 2 of PCA explained the variability in the data set by 49% and 38% respectively (total variability of 87 %) as shown in Figure 1.2b. Data revealed clear differences and similarities among the studied farm effluents. Three groups were observed (Figure 1.2b): G1 containing all the pig slurry (PS) and cow faeces (CF) samples. In G1, Pig slurry along with one dry cow faeces (CFd) samples showed positive scores on axe1 and negative on axe2 and two fresh cow faeces reflected positive scores on axe1 and axe2. G2 consisted of two dry cow manures (CM1, CM32) and cow manure compost (CMC1; one month time duration) which showed positive scores on axe2 and negative scores on axe1. In G2, fresh cow manure (CFf) as well as wheat straw (WS) were positioned in the upper right quadrants with positive scores on both axes.

Third group (G3) was the cow manures composts (CMC2 and CMC3 with 4 and 6 months composting time) located in the lower left quadrants with negative scores on both axes.

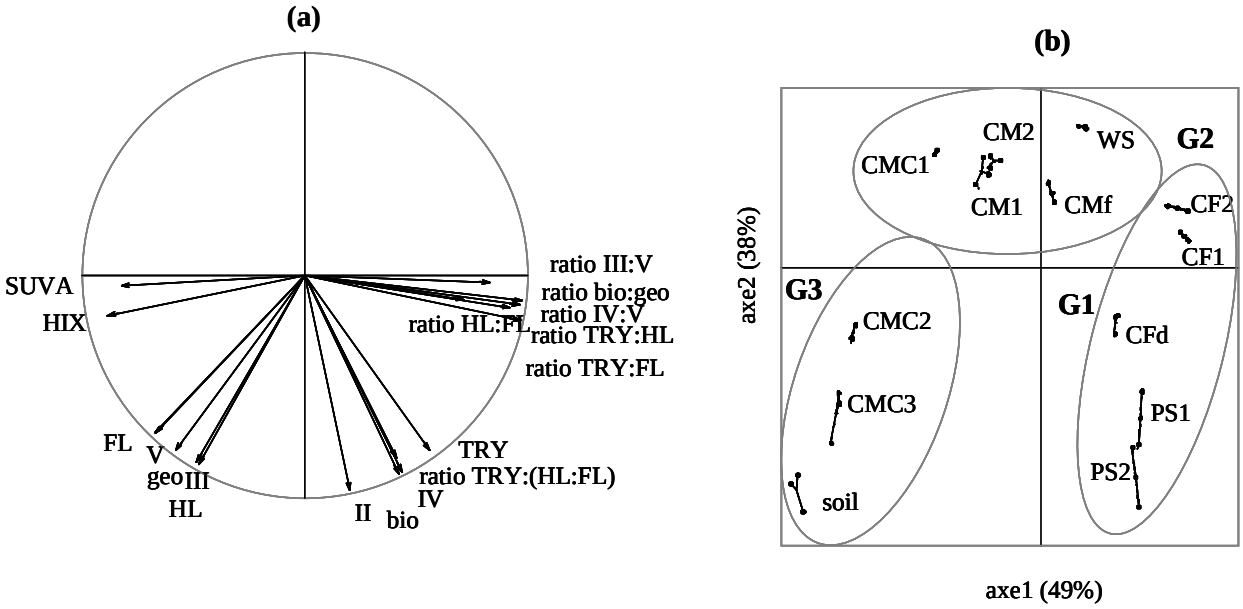

Figure 1.2 : PCA on the integrated fluorescence intensities of farm effluents: two pig slurries (PS), three cow faeces (CFd, CF1, CF2), one wheat straw (WS), three cow manures (CM1,2 & CMf) and three samples of cow manure compost (CMC) along axe1 and axe2 (b). (a) represents the factorial projection of variables along the both axes. G1, G2 G3 denote three groupings of the studied farm effluents.

Axe 1 of PCA showed significant differences ($p<0.05$) among the homogeneous groups G1, G2 and G3. It indicated positive average scores for G1 (2.08, $p<0.05$) and negative average scores of -0.80 and -5.00 for G2 and G2 respectively. Axe2 did not show significant difference between G1 (1.45, $p<0.05$) and G3 (2.38, $p<0.05$) but differentiated significantly G2 (-2.40, $p<0.05$) from G1 and G3. The integral fluorescence intensities across biochemical (bio), region II, IV and tryptophan (TRY) zone as well as ratio TRY:(HL:FL) showed highly negative correlation with axe2 and ratios bio:geo, IV:V, III:V, TRY:FL, TRY:HL demonstrated highly positive correlation with axe1 and separated the G1 effluents from G2 and G3. Integral intensities in geochemical (geo), region III, V and fulvic like (FL), humic like (HL) zones demonstrated highly negative correlation with axe2 and SUVA, HIX showed highly positive correlation with axe2 and discriminated the G3 samples of cow manure composts (CMC2 and CMC3). Moreover, group G2 manures (fresh and dried extracted cow manures, cow compost (CMC1) and wheat straw) were shown opposite fluorescence properties to pig manures and cow manure composts (CMC2 and CMC3) and separated by lower biochemical and geochemical fluorescence intensities compared (Figure 1.2b).

Likely we observed a significant shift of fluorescence properties in cow manure composts towards geochemical fluorescence properties and we hypothesize that cow manures after decomposition and biotransformation show their fluorescence properties close the humified soil DOM. Therefore, we put fluorescence properties of soil sample taken from cultivated soil under mineral fertilization as an illustrative individual in the PCA analysis and had observed the soil was projected with G3 effluents and showed almost similar DOM fluorescence properties to cow manure composts (CMC2 and CMC3).

Characterization of pig slurry contamination in natural river water

Regional fluorescence intensities

Pig slurry was discriminated by significant ($p<0.05$) higher fluorescence intensities in region IV (2742 RU) compared to higher natural water (2457 RU) (Figure 1.3). But in 50% mixed water sample, 50% DOC (5mgL^{-1} each from PS and NW sources), increase in region IV fluorescence was non significant.

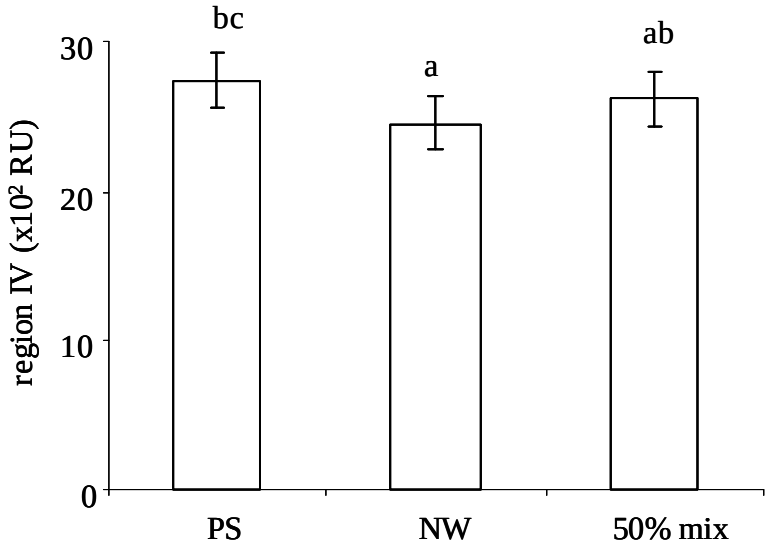

Figure 1.3 : Discrimination of pig slurry (PS) from natural water (NW) and a 50/50 mixture by fluorescence intensities in region IV. Confidence intervals were estimated assuming a coefficient of variation of 5%.

Biochemical to geochemical fluorescence intensity ratios

Ratios of regional integrated fluorescence discriminated well the PS from NW sample as shown in Figure 1.4a, b. PS sample showed significantly ($p<0.05$) higher bio:geo, IV:V ratios (0.32, 0.48 respectively) compared to NW sample with 0.12, 0.15 respectively. In mixed

water sample, ratio bio:geo, IV:V (0.18, 0.24) were significantly ($p<0.05$) higher compared to NW sample showing the impact of pig slurry.

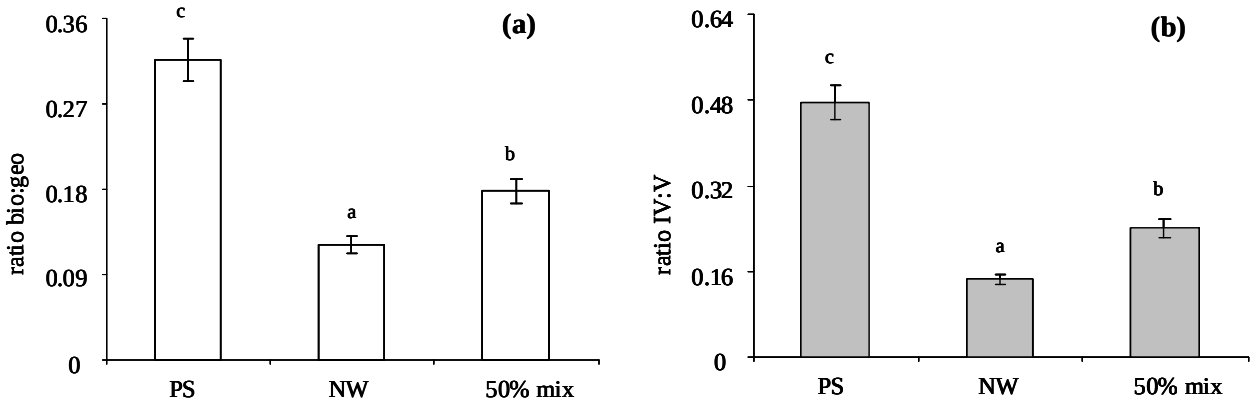

Figure 1.4 : Ratios of biochemical to geochemical regions (ratio bio:geo), region IV to V (ratio IV:V) in pig slurry (PS), natural water (NW) and a mixed water sample containing 50/50 DOC from each source. Confidence intervals were estimated assuming 5% coefficient of variation.

Peak zones fluorescence intensities

Pig slurry effluent showed significant higher TRY:FL, TRY:HL ratios (0.08 and 0.11) compared to natural river waters (0.01 and 0.02) (Figure 1.5). From the 50/50 PS and NW sample mixture, PS showed its impact by increasing TRY:HL as well as TRY:FL ratios by 0.03 and 0.05 respectively compared to NW source. Over all TRY:HL ratios were higher than TRY:FL ratios among all the sources.

Pig slurry showed significant higher ratio TRY:(HL:FL) compared to NW sample with values of 311 and 145 respectively. This ratio discriminated also the pig slurry contamination in 50% mixture of PS and NW with significant higher value of 258 compared to natural water.

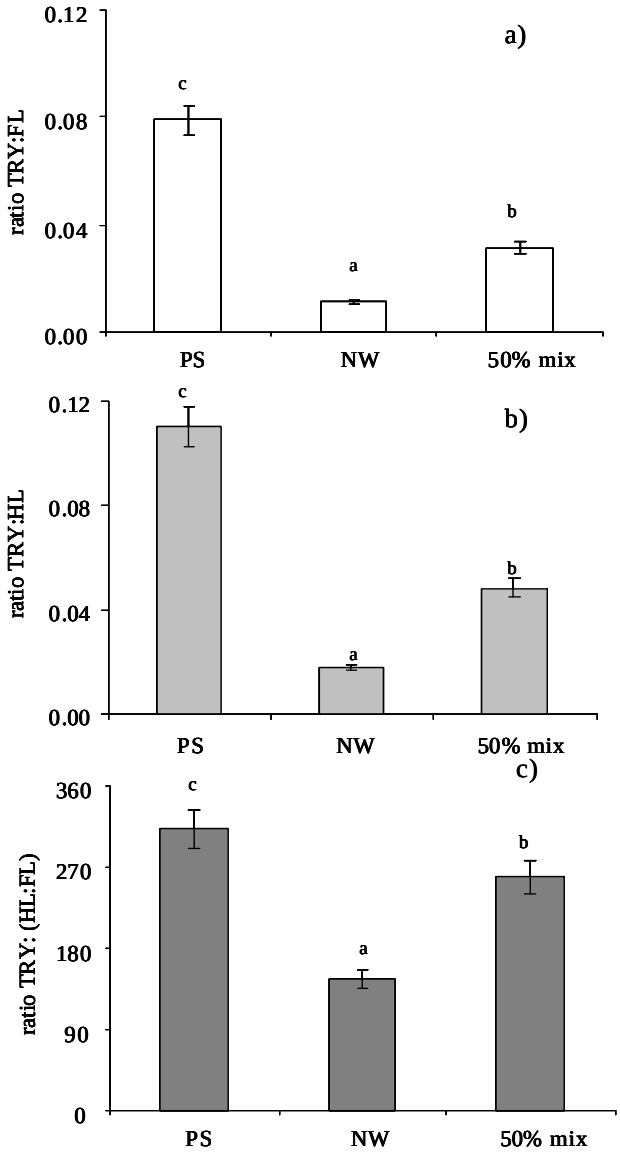

Figure 1.5 : Ratios of integrated fluorescence intensities between peak intensity zones of tryptophan (TRY), humic like (HL) and fulvic like (FL) in pig slurry (PS), natural water (NW) and a mixture of water sample containing 50% DOC from each source. Confidence intervals were estimated assuming a coefficient of variation of 5%.

Aromatic character of pig slurry and natural water samples

PS was characterized with significant lower SUVA (index of aromaticity) value (0.27) and HIX (0.73) compared to NW which showed higher humification (0.87) and aromaticity (1.65). 50% mixture of PS and NW showed almost 50% decrease in SUVA value (0.78) compared to NW. However, PS did not affect the HIX value in the 50% mix sample, as the HIX value was closer to NW source (Figure 1.6a).

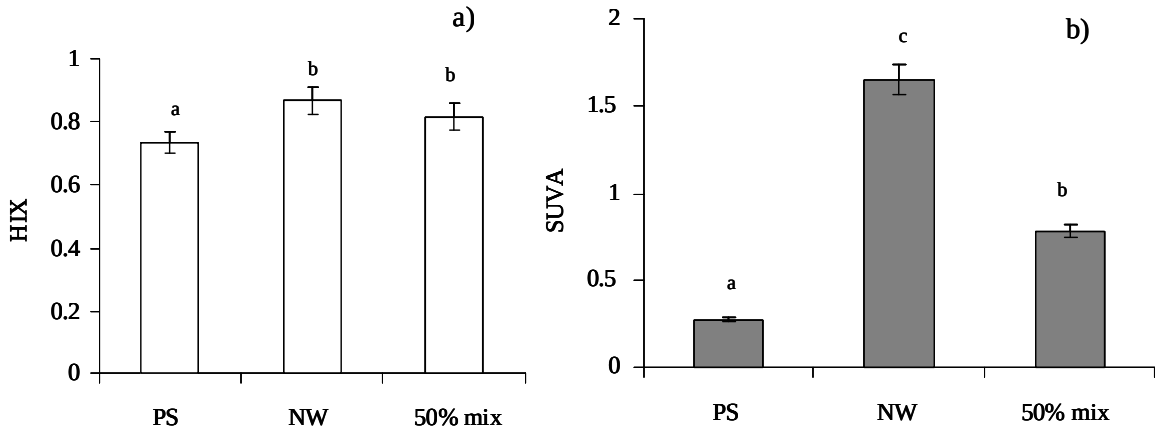

Figure 1.6 : Humification index (HIX) (a) and Specific Ultra Violet Absorbance (SUVA) (b) in pig slurry (PS), natural water (NW) and mixture of water containing 50% DOC from PS source. Confidence intervals were estimated assuming coefficient of variation of 5%.

Composted cow manure contamination in natural river water

Integrated fluorescence intensities across regions

CMC3 showed significant lower integral fluorescence in region III vs V (35055, 33921 RU) than NW sample (61038, 57624 RU) as shown in Figure 1.7. Cow manure compost showed its impact on the regional fluorescence in 50% mixture, by decreasing the values to (45939, 44339 RU) compared to NW source.

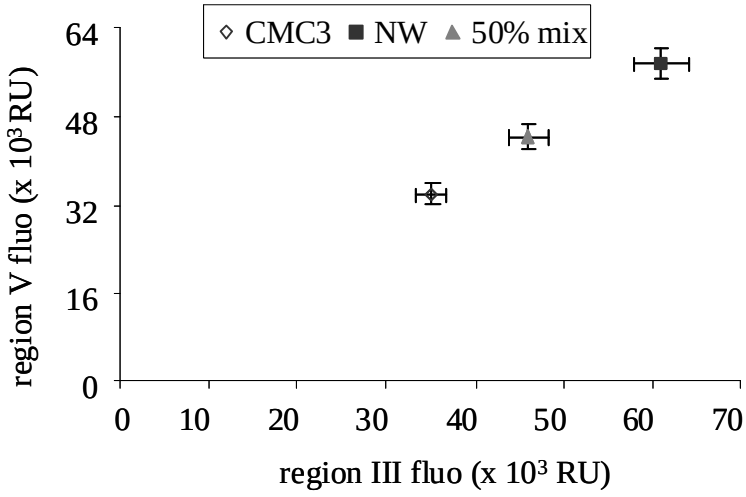

Figure 1.7 : Discrimination of cow manure compost (CMC3) from natural water (NW) and a 50/50 mixture by regional fluorescence across regions III and V. Confidence intervals were estimated assuming a coefficient of variation of 5%.

Biochemical to geochemical fluorescence intensity ratios

Cow manure compost (CMC3) was discriminated with significant higher bio:geo and IV:V (0.14 and 0.21 respectively) compared to NW sample with ratios 0.08 and 0.12 respectively

(Figure 1.8). Natural water used in this experiment was sampled from the stream draining a wetland and forest area, showing a lower bio:geo ratio (0.08) than the NW (0.12) draining the cultivated hillslope. It indicates the spatial variability of fluorescent DOM substances draining the heterogeneous soils. 50% CMC water mixture showed significant increase in bio:geo and IV:V ratios (0.10, 0.15 respectively) than NW sample.

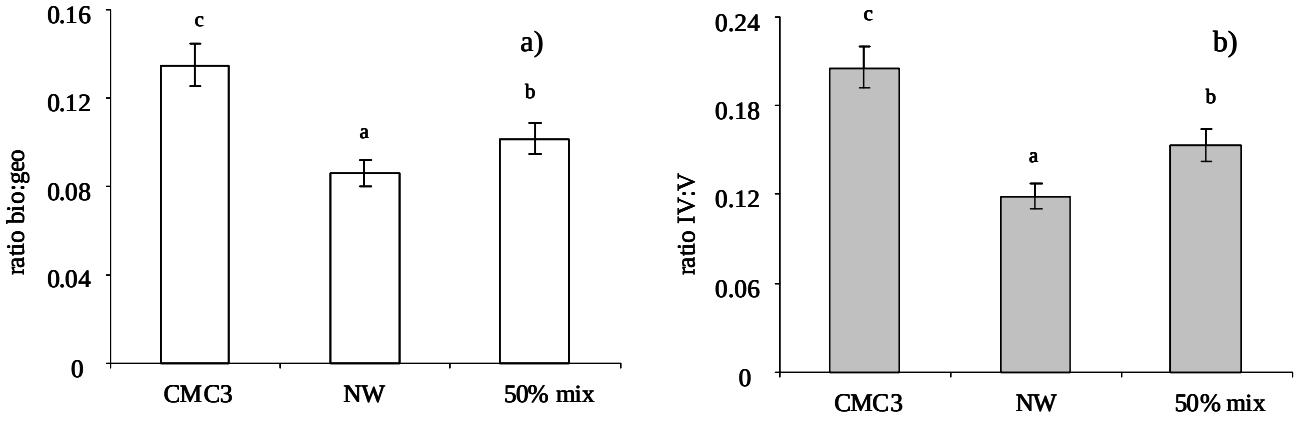

Figure 1.8 : Discrimination of cow manure compost (CMC3) from natural water (NW) by fluorescence intensities ratios of biochemical to geochemical regions (ratio bio:geo), region IV to V (ratio IV:V) and in a mixture of water sample containing 50% DOM from each source. Confidence intervals were estimated assuming a coefficient of variation of 5%.

Fluorescence ratios across zones

TRY:FL and TRY:HL ratios were significant higher in CMC3 (0.03 and 0.04 respectively) compared to NW sample (0.01 and 0.01) (Figure 1.9a,b). 50% water mixture was characterized with intermediate values of 0.02 and 0.03 respectively. Overall, CM, NW as well as 50% water mixture showed higher TRY:HL ratios compared to TRY:FL ratios.

Ratio TRY:(HL:FL) also showed significant higher values (730) in CMC3 than NW (414), while 50% mixture showed the impact of CMC3 source with significant higher TRY:(HL:FL) value (581) compared to natural water sample (Figure 1.9c).

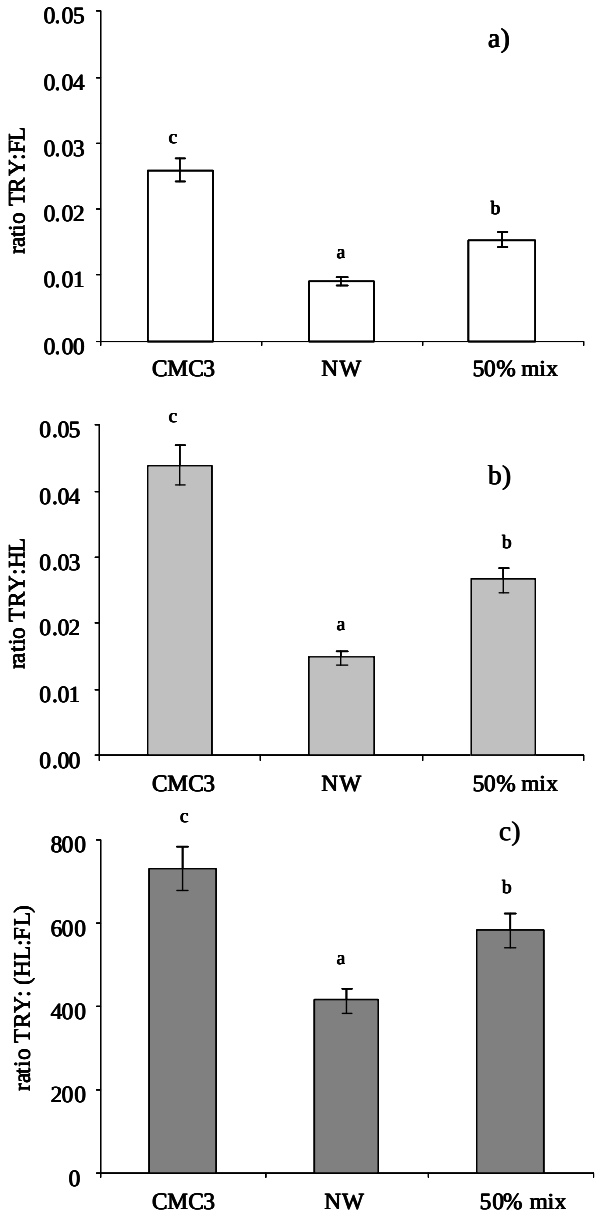

Figure 1.9 : Ratios of integrated fluorescence intensities across peak intensity zones of humic like (HL), fulvic like (FL) and tryptophan (TRY) in natural water (NW), pig slurry (PS) and a mixture of water sample containing 50/50 DOC from cow manure source. Confidence intervals were estimated assuming a coefficient of variation of 5%.

Aromatic character of cow manure and natural water samples

Specific ultra violet absorbance (SUVA) was significantly higher in NW (4.03) than CMC3 sample (3.40) (Figure 1.10b). 50% CMC3 water mixture demonstrated significant lower SUVA value (2.70) compared to both sources. It reflected the non preservative behaviour of absorbance parameter in mixing of cow manure and natural water. Humification index (HIX) was incapable to discriminate cow manure samples from natural water (Figure 1.10a), although it worked well in discrimination of pig slurry from natural waters (Figure 1.6a).

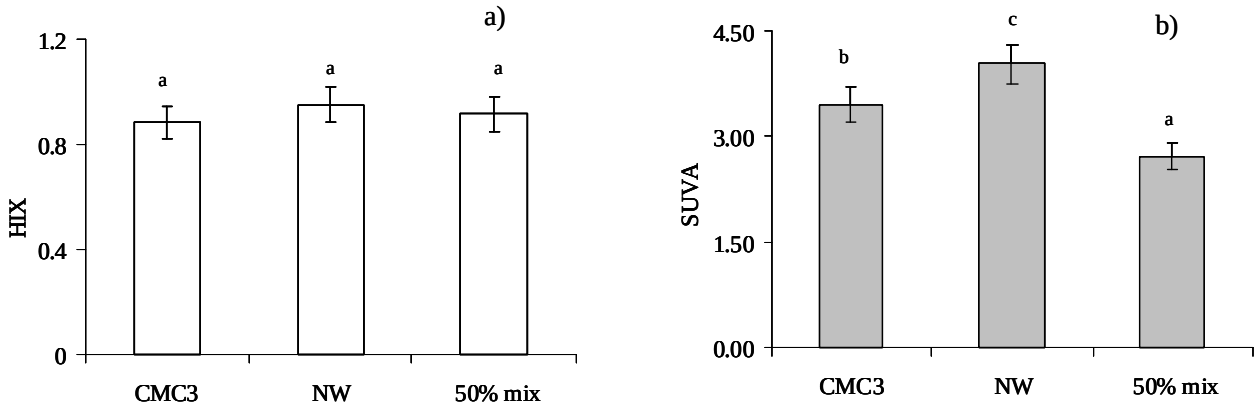

Figure 1.10 : Humification index (HIX) (a) and Specific Ultra Violet Absorbance (SUVA) (b) in cow manure compost (CMC3), natural water (NW) and mixture of water containing 50/50 DOM from each source. Confidence intervals were estimated assuming a coefficient of variation of 5%.

Discussion

Farm effluents characterization

After cancelling the initial DOC differences, fluorescence spectral dissimilarities in various farm effluents depict DOM quality differences. Principal component analysis (PCA) enabled the grouping of various farm effluents which have similar DOM spectroscopic properties. This classification reflects the origin of DOM by providing information on the similarity of fluorescence properties among various sources. The grouping of pig slurry (PS) samples in the same quadrant along with fresh cow faeces (CF) (G1, Figure 1.2b) indicate that DOM in the pig and cow excretions is of similar fluorescence properties and therefore, depict the similarity of digestive excretions in both breeding animals although the former is monogastric and the later is ruminant. Hunt and Ohno (2007) have shown the lack of consistent trends among the fluorescent components among the pig, cow and poultry manures as compared to plant extracted DOM fluorescence properties. The factorial projection (Figure 1.2a) suggests the possible discrimination of PS and CF samples from G2 and G3 effluents with strong biochemical integral fluorescence in the regions and TRY zone as well as ratios of bio:geo, IV:V, TRY:(HL:FL), TRY:FL and TRY:HL.

When the CF is mixed with wheat residues, it referred as cow manure (CM). Fresh and dried extracted cow manures and one month CMC and WS samples are grouped in G2 (Figure 1.2) which suggested the homogeneity of their DOM quality. It was also demonstrated that fluorescence properties of DOM extracted from cow faeces were changed after contact with

wheat crop residues. When CF interacts with crop residues, regional transformation results in higher HIX in CM.

Cow manure composts (CMC2 and CMC3 for 4 and 6 months time duration) are observed in the same group (G3, Figure 1.2) and exhibit similar spectral composition. The differentiation between cow manure and composted cow manure reflected different chemical composition with some condensed, aromatic and/or heterocyclic ring systems, a high degree of electronic conjugation and bearing suitable hydroxyl, alkoxyl and carbonyl groups which is consistant with the humification during the composting process (Senesi et al., 1991). Geochemical regions (region III and region V), peak intensity zones of FL and HL as well as HIX and SUVA are the most effective fluorescence discriminators of cow manure composts. As composting process proceeds, molecular complexity in the fresh organic materials (CMC1) is increased (CMC2, CMC3). This increasing molecular complexity could also be related to increase in volumic intensities in humic acid like and fulvic acid like regions (region III and region V) (Marhuenda-Egea et al., 2007). Geochemical fluorescence intensities as well as SUVA and HIX in CMC2 and CMC3 can be related to increased carboxylic, phenolic carbon groups and polymerization and cross-linking that lead to the formation of larger molecules (Liang et al., 1996).

The decreased fluorescence intensities in region I, II and IV in cow manure composts could be due to the degradation of the fresh materials as a result of microbial activity. Fluorescence properties of composted cow manure DOM are not different from soil DOM.

Pig slurry was not discriminated from fresh cow faeces. So a diffuse pollution by direct transfer of DOM from pig or cow slurry after spreading on soil or in intensively grazing pasture area not be discriminated with fluorescence characterization. On the contrary, a direct transfer of DOM from cow manure or composted cow manures should be discriminated from cow faeces or pig slurry pollution with lower fluorescence intensities in region II, IV, bio, TRY zone, ratio TRY:(HL:FL) in fresh cow manures or intense fluorescence in geochemical regions, FL and HL zones.

Discrimination of point source pollution of pig and cow manure compost

A mixing experiment was carried out in the laboratory to trace the general impact of pig slurry contamination on the natural river water DOM fluorescence and to find out some pertinent fluorescence indicators of pig slurry contamination. Simulated level of contamination was low because the DOC concentration of the mixing between slurry or compost and natural water was $5 mgL^{-1}$. After slurry spreading, DOC concentration in runoff

ranged between 100 to 250 mg L^{-1} (Royer et al., 2007b). The mixing sample was composed of a solution with 50% from stream and 50% of DOC from pig slurry wastes (50/50 DOC; 5 mgL^{-1}DOC from each source), but it does not represent a mixing of 50% of pure slurry in stream water.

PS effluent is characterized by significant higher ratios of bio:geo, IV:V, TRY:FL, TRY:HL, TRY:(HL:FL) and lower fluorescence intensities in regions III and V as well as significant lower values of SUVA and HIX and an opposite trend for these indices is observed in natural river waters.

In the pig slurry/natural water sample, all the ratios are tended towards PS source. This is because region II and IV are connected to tyrosine, tryptophan and protein like components (Chen et al., 2003b). Total hydrolysable amino acids correlate with peak fluorescence intensities of tyrosine and tryptophan (Yamashita and Tanoue, 2003) which are located in biochemical regions (I and IV). Therefore, upon the addition of 50% DOM from PS source, these nitrogen containing components increase in the mixture and result in stronger ratios. Lower ratios bio:geo, IV:V, TRY:FL, TRY:HL, TRY:(HL:FL) in NW sample suggest the microbial transformation of biochemical fluorescent components (region I, II, IV, as well as TRY zone). Direct pollution source can be of two types: (i) either from the stockpiled manures (at various stages of humification and aromaticity) along the water courses or from animal faeces in the intensively grazed pastures near the stream channels. In case of stockpiled manures, composts are suitable for studying their point source contamination. Stream fluorescence shows a great variability depending not only of allochtonous source of pollution but also of soil type, vegetation, and landuse (Cumberland and Baker, 2007; Hudson et al., 2007). Therefore, to explore the mixing of two highly aromatic and humified DOM substances, the natural water was sampled in a stream draining a peat wetland and the composts (CMC3) at six month of time duration was taken.

Initial higher ratios of bio:geo, IV:V, TRY:FL, TRY:HL, TRY:(HL:FL) were observed in CMC3 samples compared to NW samples. Although DOM from CMC3 is highly aromatic yet it influences the DOM fluorescence properties in river water by increasing ratios and decreasing the aromaticity of NW.

In the peat wetland catchment, DOM is enriched with polyphenolic rich fractions and fluorescence in the geochemical regions (region III and V, in our case) is more important compared to the stream 1 (NW sample from stream draining Ducey catchment). Intense fluorescence in the geo region was measured compared to carbohydrate and protein rich

(Chen et al., 2003a) biochemical regions of EEM and consequently lower ratios in the peat wetland catchment.

SUVA is a good indicator of aromatic carbon contents of the organic matter (Weishaar et al., 2003).The SUVA decrease in the water mixture (PS and NW) as well as (CMC3 and NW) which confirms the lower aromaticity and humification (Huber et al., 1994) after mixing with farming wastes. Humification index reflect that CMC3 and wetland catchment NW are at the similar stage of humification.

Implication for water quality monitoring in agricultural headwater catchment

Non significant difference in ratio bio:geo between stream 1 and pure composted cow manure reflected the higher background ratio in stream 1 and it also indicated that the ratio will not differentiate the impact of composted cow manures on stream 1 (Figure 1.11). A contamination of water with pig slurry would be clearly detected in all streams, however spatial variability of the fluorescence properties of stream should be explored.

These results also show the necessity of monitoring the stream fluorescence properties over a long time to initiate stream water quality database for each catchment and to analyse temporal variation of the fluorescence properties.

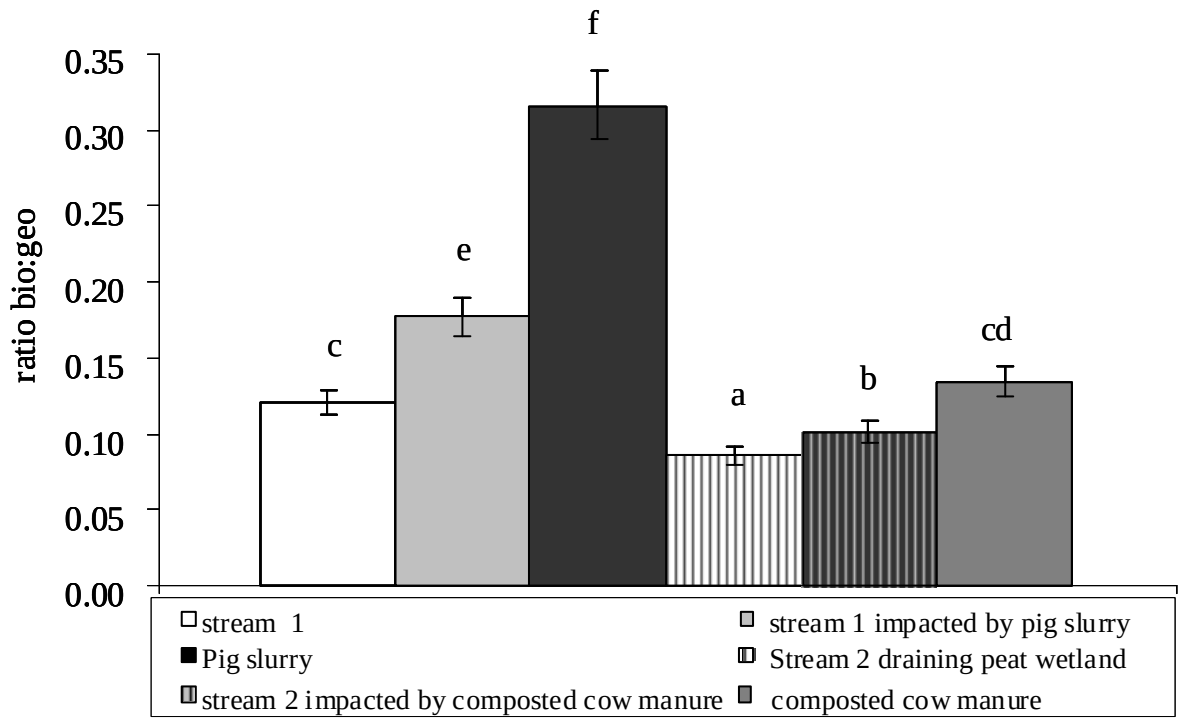

Figure 1.11 : Ratio bio:geo in the stream 1 impacted by pig slurry and the stream 2 (peat wetland) impacted by composted cow manure. Confidence intervals were estimated assuming a coefficient of variation of 5%.

Quantifying the increasing impact of composted cow manure on stream2 fluorescence

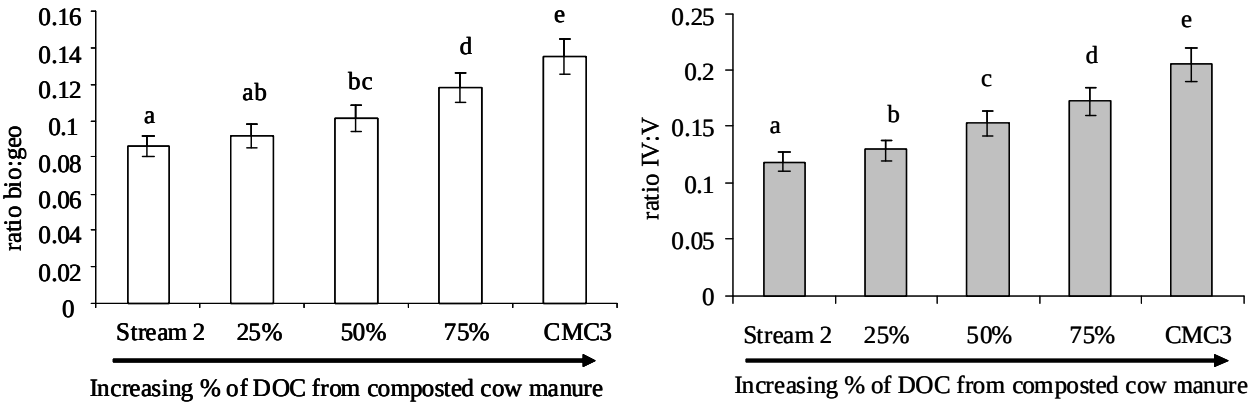

Figure 1.12 : Impact of increasing DOM from cow manure compost (CMC3) on the stream 2 fluorescence. Confidence intervals were estimated assuming a coefficient of variation of 5%.

Increasing impact of composted cow manures showed significant increase in bio:geo ratio (50% to 75% compared to stream2) and the ratio IV:V (25% to 75% compared to stream2) (Figure 1.12). Ratio IV:V is found more sensitive in detecting a pollution of natural water even the cow manure compost impact is 25 % (5 mgL^{-1}) on the stream water.

Discrimination of the type of farming waste contamination

The fluorescence indicators proposed in this study allow to identify a farming waste contamination of DOM in agricultural headwater streams (ratios bio:geo, IV:V, TRY:FL, TRY:HL) but the type of farming waste suspected in the pollution can't be discriminated. The PCA analysis conducted on the farming waste extract enable to discriminate pig slurry and cow faeces from cow manure and composted cow manure. A mixing of one stream sample with different farming waste extracts and a PCA analysis should be conducted to conclude the nature of the farming waste contamination since one value of ratio is not sufficient to conclude. An increase in the bio:geo ratio for example is observed after addition of pig slurry or cow compost in the stream water. The variation of the ratio is dependant upon the nature but also upon the quantity of farming waste DOM supply to stream.

Conclusion

Fluorescence spectroscopy coupled with PCA showed the DOM quality differences among the various farm manures applied to cultivated soils. Important outcomes from this study are following:

- Fluorescence similarities in pig slurry (PS) and cow faeces (CF) depicted the similar fluorescence DOM quality although pig is monogastric and cow is ruminant with different digestive process.
- PS and CF were strong in biochemical fluorescence intensities (bio), (region II, IV) and TRY zone and differentiated from fresh and composted cow manures with higher ratios of bio:geo, regional ratio IV:V, TRY:(HL:FL), TRY:FL, TRY:HL.
- Cow manure composts were discriminated by higher fluorescence intensities geochemical regions (region III, V), fulvic like and humic like zones as well as humification index.
- Composted cow manures depict DOM fluorescence properties similar to soil DOM.

Discrimination of farming wastes in natural stream water

- Ratios bio:geo, IV:V, TRY:FL, TRY:HL and TRY:(HL:FL) are found as pertinent tracers of farming wastes (pig slurry and cow manure composts) in the mixed water samples.
- Direct transfer of pig slurry waste in stream can be differentiated with higher bio:geo ratios.
- TRY:(HL:FL) which had shown higher values for cow manure composts compared to pig slurry
- Increasing impact of farming wastes can be quantified with increasing bio:geo and region IV:V ratios

Implication for water quality monitoring

These results show the necessity of monitoring the stream fluorescence properties over a long time to initiate stream water quality database for each catchment and to analyse temporal variation of the fluorescence properties rather than interpreting absolute measure of fluorescence ratio.

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CHAPTER 2

Chapter 2

Tracing of farming waste fluorescent DOM during a runoff simulation

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Abstract

Fluorescence properties of dissolved organic matter (DOM) were used to characterize the diffuse DOM pollution by pig slurry and cow manure. A simulating runoff experiment was conducted on a microplot of 1m² a few hours after farming waste spreading. Three repetitions for each treatment (control, pig slurry and cow manure at agronomic rates) were tested in April 2008.

A rainfall simulation was conducted with intensity of 67 $mm.h^{-1}$. Cumulated runoff was about 16 L for each plot. Regional integration was applied on fluorescence measurements. Ratio bio:geo (biochemical fluorescence in the region I, II and IV / geochemical fluorescence (humic/fulvic like- fluorescence, region III and V) and ratio III/V (humic-like fluorescence) discriminated the farm wastes DOM from soil source. The fluorescence properties on first runoff samples from farming waste amended soils were identical to those measured on raw farming waste. This indicated that a spring storm event which occurred a few hours after the spreading lead to transfer of DOM from farming waste. The ratios bio:geo and III:V were significantly higher than those measured in control in the first 6L runoff in pig slurry treatment and in all the runoff samples collected in two repetition on cow manure. However in the last cow manure simulation replicate, DOM transfer was from soil source. Region V also discriminated the soil DOM with significantly higher fluorescence from the farming wastes. It was impossible to discriminate pig slurry from cow manure contamination since fluorescence properties measured on cow faeces were identical to pig slurry. Air drying treatment modifies the fluorescence properties of the farming waste. To detect farm waste contamination in stream, fresh effluent analysis dataset have to be investigated to explore the variability of the farming wastes fluorescence properties.

Key words: farm wastes, Pig slurry, cow manure, rainfall simulation, runoff, fluorescence tracers,

Introduction

In modern agricultural systems, the widespread use of farm wastes fertilization serve as a valuable source of crops nutrients (Moral et al., 2005) and a mean of alternative source of chemical fertilizers which cause excess nitrates and phosphorous loads in the catchment streams (Granger et al., 2010) during intense rainfall events. Water contamination can be aggravated if rainfall event occur shortly after the supply of farm manures and transport the DOM towards the stream by modifying the water pathways via surface runoff or preferential flow via tile draining (Royer et al., 2007a; Hernes et al., 2008; Naden et al., 2009).

The excess DOM production pollutes the natural resource water quality. In the French legislation, [DOC] concentration in superficial water should be under 10 mgL^{-1} during 95% of sampling time for drinking water supplies. Moreover, DOM act as a vector in the transport of pesticides, metals and viruses etc towards streams (Williams et al., 2005; Song et al., 2008). In the literature, there is scarcity of knowledge about the net impact of different farm manures supply on the DOM production in soil if rainfall coincides with the fertilization time. The new directive on Bathing water quality 2006/7/EC (Anonymous, 2006) strengthens the concept of bathing water management by introducing bathing water profiles designed to identify pollution sources in bathing waters and of other surface waters in the catchment area of bathing water concerned. One of the major sources of fecal pollution which may contaminate bathing waters is associated with the practice of land spreading of animal wastes, especially in intensive agricultural areas such as Brittany (France). It is well know that cattle and pig manures contain pathogenic microorganisms (Guan and Holley, 2003; Omisakin et al., 2003) and that land spreading of manure constitutes a human health risk (Thaddeus et al., 2008). It is thus important to use methods to identify livestock contamination in surface water and to discriminate cow manure from ping slurry contamination.

Identifying sources of contamination required the development of tracers of DOM. EEM Fluorescence spectroscopy appears as a interesting tool and has been applied to trace diffuse agricultural pollution from dairy slurry spreading on intensively-farmed grasslands (Naden et al., 2009). Excitation-emission matrix spectra (EEM fluorescence) are obtained by incrementation of excitation and registration of emission spectra. Four major peaks are generally observed in DOM samples. However, in this current study, instead of taking few data points in the form of peak picking, the whole 3D-EEM spectra is analysed quantitatively with fluorescence regional integration (Chen et al., 2003b). Peaks at shorter

wavelengths (<250 nm) and shorter emission wavelengths (<380 nm) are related to simple aromatic proteins such as tyrosine and tryptophan (Regions I and II). Peaks at intermediate excitation wavelengths (250–340 nm) and shorter emission wavelengths (<380 nm) are related to soluble microbial by-product-like material (Region IV) while peaks located at the excitation wavelengths (230–300 nm) and the emission wavelengths (380-575 nm) represent humic acid-like substances (Region III). Peaks at longer excitation wavelengths (>300 nm) and longer emission wavelengths (>380 nm) are related to fulvic acid-like organics (Region V). Relative importance of these peaks or region had been used to trace DOM from diverse sources. Baker (2002) has demonstrated that intense ratio tryptophane-like to fulvic/humic like fluorescence are higher in farming wastes compared to stream water. Naden et al., (2009) also used the ratio of indices of tryptophane-like and fulvic/humic like fluorescence (TI:FI) to distinguish incindental losses of dairy slurry in drainage waters.

In the presented study, we (i) investigated the impact of pig slurry and cow manure wastes on the production of dissolved organic mater during spring storm events by analysing runoff water quality by simulation (ii) test the potential of fluorescence spectroscopy as tracer of farm manure DOM during three rainfall simulation events, and finally try to discriminate between pig and cow manure contamination in runoff water.

Materials and methods

Experimental dispositive

A rainfall simulation field experiment was conducted in April 2008 at the agricultural experimental research center of Kerguehennec Brittany, France. Three treatments were evaluated: control (C), pig slurry (PS), cow manure (CM) with three replicates (R1, R2, R3) which produced a 9 plots experimental design.

A fellow plot of 10m*30m was selected with average slope of 3% and tilled to a depth of 15 cm. Nine tilled plots (0.75m*1.5m) were arranged into rectangular shape. The soil was a cambisol (41% sand, 42% silt, 16% clay) with 2.5% organic matter contents in the Ap horizon**.** Before each rainfall simulation, each plot was hydrologically isolated with galvanized iron (15 cm above and below the soil surface). The runoff collector was composed of a 10 cm diameter polyvinyl chloride gutter (PVC) with 1 cm slit. This gutter was connected to a 2.5L distilled water washed plastic beaker via PVC pipe as indicated in Figure 2.1.

Rainfall simulation

The rainfall simulator was placed under a tent to prevent wind and natural rain perturbation. Rainfall intensity was adjusted to 67 $mm.h^{-1}$ to generate an extreme spring storm flow. Rainfall intensity was determined by measuring the volume collected on a $2m^2$ recipient after one minute. The duration of the simulation period varied from 40 min to 75 min to obtain the same cumulated runoff quantity required. The whole runoff was collected. Eight runoff samples (750 mL, 4L, 750 mL, 4L, 750 mL, 750mL, 750 mL) were collected for each plot and was analysed for current analysis (microbiological, pH, suspended matter, dissolved organic carbon). The two large volume samples of 4L were required by all the partners working with various molecular tracers techniques of DOM because specific analytical methods required large volumes. The time required to collect the samples was measured to compute runoff rate and volume. Runoff water was agitated well before sampling in sterile plastic bottles for microbial analysis. Samples were stored at 4°C until analysis.

Cow manure and pig slurry characteristics and application rates

Cow manure and pig slurry were collected from two farms located near the experimental research center. Cow manure was collected in plastic bags and pig slurry was collected in 15L plastic containers. Pig slurry was stirred before manual application. Pig slurry was applied with manually watering sprayer at 2.4 kg m^{-2} (24 Mg ha^{-1}) and the quantity of applied slurry corresponds to agronomic dose of Nitrogen requirement for maize crop. Cow manure was also manually spreaded at 3.2 kg m-² (32 Mg ha^{-1}). Pig slurry and cow manure consisted of carbon contents 41.7 and 47.2 $g.kg^{-1}$DM (dry matter) respectively and nitrogen contents 2.2 and 1.6 $g.kg^{-1}$DM respectively. Both wastes were spreaded on the experimental plot two hours before the rainfall simulation.

Water used for rain simulation

The rain water used during the simulation experiment was taken from the drilled well at 5 meter depth. This water was free of microbial contamination of Enterococci and had low dissolved organic carbon (DOC) 1.39 ±0.12 mg L^{-1} with pH 6.59.

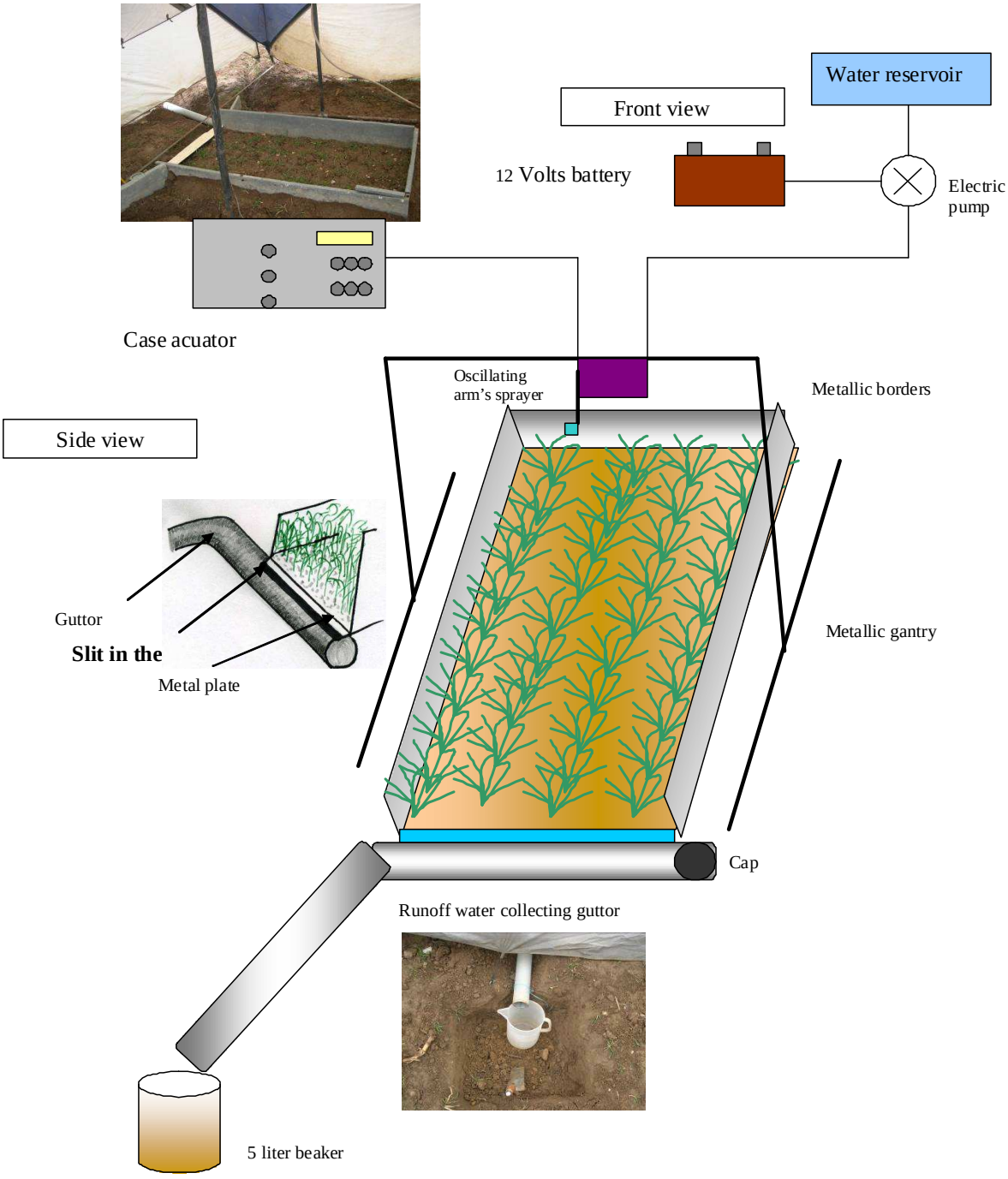

Figure 2.1 : Experimental dispositive of rainfall simulation

Aqueous DOM extracts of farm manures and soil

DOM was extracted with 40:1 (V:W) ultra pure water to farm manure ratio. Pig slurry samples were separated into its liquid and solid parts through centrifugation at 3000 rpm for 30 minutes. Liquid pig slurry sample was filtered and referred as PS fresh slurry sample in the current study. Solid part of pig slurry and cow manures samples were air dried and grounded at 700 turns per minutes and sieved through 1 mm mesh size and referred as PS dry and CM

dry DOM extracts in the current study. Moreover, DOM extractions were also undertaken for the fresh farm manures (cow manures (CM fresh) and cow faeces (CF fresh) and utra pure water suspensions. Extraction procedure for aqueous DOM extracts was similar for each of farm manures either fresh or dried. Each farm manure water suspensions were kept in refrigerator at 4°C for 16 hours with periodic manual shaking. Then the farm manure water suspensions were centrifuged at 3000 rpm for 30 minutes and subsequently filtered through 0.7 and 0.22 µm nitrocellulose filters.

For soil, DOM was extracted with 2:1 ultra pure water to soil ratio. Soil water suspensions were shaken mechanically on orbital shaker for 2h and then centrifuged at 3000 rpm for 30 minutes and filtered through 0.7 and 0.22 µm nitrocellulose filters. To avoid any contamination, all the filters were rinsed with ultra pure water and dried overnight before vacuum filtration. The values of fluorescence indices studied in the farm manures were enlisted in Table 2.2.

Physical and chemical water analysis

Suspended sediments were determined by weighing sediment after drying overnight at 105°C. Samples were filtered through 0.22 µm membranes (Millipore Millex-GV). pH was determined on 20 mL filtered water samples using a digital pH-meter (WTW) calibrated with buffers (WTW) of pH 4 and 7. [DOC] was measured on a Shimadzu TOC 5050 A total carbon analyzer. Accuracy on DOC measurements was ±5%, based on repeated measurements of standard solutions (K-phtalate). DOC concentrations were highly elevated in cow faeces (3050 mgL^{-1}) followed by the pig slurry (2167 mgL^{-1}), cow manures (1580 mgL^{-1}). But we presented the data of farm wastes normalised at 5 mgL^{-1} and presented the DOC level of 100 mg L^{-1} in by diluting with a factor of 30, 20 and 15 times CF, PS and CM respectively (Figure 2.6 and 2.7, in the discussion section of the current study). UV-Visible absorbance was measured on a Perkin Elmer Lambda 20 UV-Visible spectrophotometer across 200-600 nm excitation wavelengths range with data interval 0.5nm, slit width 2 nm and scan speed 120 nm/min.

Fluorescence measurements of DOM were performed using a Perkin-Elmer LS-55B luminescence spectrometer. The spectrophotometer uses a xenon excitation source and slits were set to 5 nm for both excitation and emission. To obtain excitation-emission matrix spectra, excitation wavelengths were incremented from 200 to 425 nm at steps of 5 nm and emission was detected from 250 to 600 nm with a 0.5 nm step. Scan speed was set at 1500 nm/min, yielding an EEM in 22 minutes with 45 total scans. To minimise the temperature

effect, samples were allowed to equilibrate with room temperature (20±2°C) prior to fluorescence analysis. The whole fluorescence dataset presented in this study was normalised at 5 mg L^{-1} DOC. Linearity was carried out between DOM concentration and fluorescence intensity by dilution of high DOM concentration samples. To eliminate the second order Raleigh light scattering, excitation and emission cutoff filters were applied at 230-310 nm and 380-600 nm respectively on the lower side of three dimensional plots (Figure 2.2). Inner filter effects were removed with the formula (Ohno, 2002). To maintain the consistency of measurements and standardise the whole fluorescence dataset, all the integrated fluorescence intensities were normalized to average Raman emission intensity units of 31 for daily determined ultra pure water samples at excitation and emission wavelengths of 350 nm and 397 nm respectively. A Raman normalised integrated EEM spectrum of ultra pure water was subtracted from the data sample to eliminate the water Raman scatter peak. To minimise the effect of temperature, all samples were allowed to reach laboratory temperature prior to measurement and the analysis was performed at a laboratory temperature of 20 ± 2°C.

Regional integration of excitation emission matrix (EEM)

An internal program was developed in the laboratory using the R® software (http://www.r-project.org) for the integration of fluorescence intensities across the whole EEM landscape (Annexes, at thesis page 206). Here peaks at shorter wavelengths (<250 nm) and shorter emission wavelengths (<380 nm) are related to simple aromatic proteins such as tyrosine and tryptophan (Regions I and II) Peaks at intermediate excitation wavelengths (250–340 nm) and shorter emission wavelengths (<380 nm) are related to soluble microbial by-product-like material (Region IV) while peaks located at the excitation wavelengths (230–300 nm) and the emission wavelengths (380-575 nm) represent humic acid-like substances (Region III). Peaks at longer excitation wavelengths (>300 nm) and longer emission wavelengths (>380 nm) are related to fulvic acid-like organics (Region V). With this technique, EEM is divided into biochemical (bio) (I, II, IV) and geochemical (geo) (III, V) fluorescent regions (Figure 2.2a) (Table 2.1) and three peak intensity zones of tryptophan (TRY), fulvic like (FL) and humic like (HL) fluorescence (Figure 2.2b) (Table 2.1).

The quantitative analysis included the integration of fluorescent volume beneath each region and zone. Moreover, ratios TRY:HL, TRY:FL, HL:FL, bio:geo, IV:V and III:V were also calculated. 45 spectral loadings were used to reproduce three-dimensional plots of fluorescence intensity as a function of excitation and emission wavelengths. Humification index (HIX) was determined according to Ohno, (2002).

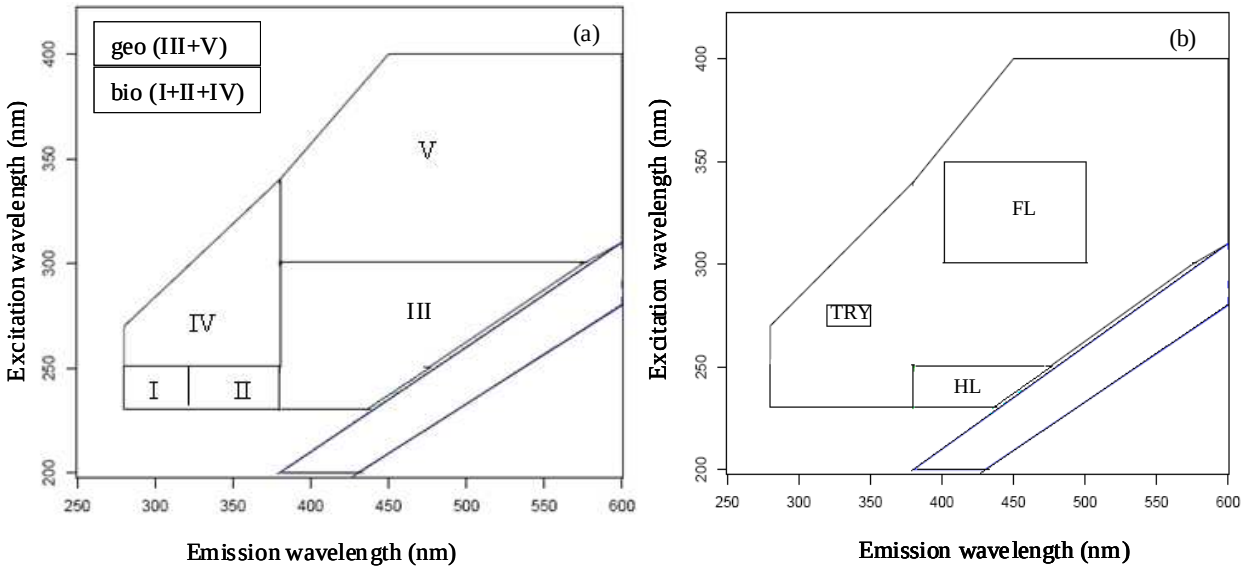

Figure 2.2 : Integration of fluorescence intensities across regions (a) and maximum peak intensity zones (b).

Table 2.1 : Definition of excitation (Ex) - emission (Em) wavelengths (nm) ranges for each region and zones

	Ex (nm)	Em (nm)		Ex (nm)	Em (nm)
Regions			**Zones**		
region I	230-250	280-330	Tryptophan	270-280	320-350
region II	230-250	330-380	Fulvic like	300-350	400-500
region III	230-300	380-575	Humic like	230-250	360-420
region IV	250-340	280-380			
region V	300-400	380-600			
region bio	230-340	280-380			
region geo	230-400	380-600			

Table 2.2 : Fluorescence indices in farm manures and soil

DOM extracts	region V	ratio III:V	ratio bio:geo
CF fresh	11692	1.66	0.39
CF fresh	12374	1.68	0.38
CF fresh	11555	1.67	0.39
CM dry	7924	1.04	0.24
CM dry	7246	1.03	0.25
CM dry	6516	1.02	0.24
CM fresh	8612	1.29	0.30
CM fresh	9291	1.34	0.31
PS dry	6238	1.32	0.47
PS dry	7525	1.34	0.47
PS fresh	10874	1.27	0.29
soil extracts	12994	1.11	0.11
soil extracts	18104	1.12	0.09
soil extracts	12934	1.12	0.11

Results

Heterogeneous response of runoff water under the control of effluent type

During three simulation events, we considered average rainfall intensity of 67 mm h^{-1} which corresponded to a spring rainfall event in the context of Brittany France. Weaker rainfall intensities were measured in control (soil alone) R1 and R2 as well as pig slurry (PS) R1 and R2. Rainfall intensities were highly elevated in cow manure modality R3 as shown in Table 2.3. However, required volume in cow manure R3 was obtained after 120 min of simulation time and this modality was particularly different with respect to simulation time and higher rainfall intensity to attain required volume as compared to other modalities. In this modality, soil surface (either due to soil cultivation or cow manure land spread) favoured the infiltration of rainwater.

Overall, the runoff time and simulated runoff in all the simulation events of cow manure were comparable to control and pig slurry modalities. Besides this, cow manure surface application delayed the release of water streaming that required intense rainfall to generate the needed runoff volume.

Table 2.3 : Hydrological charateristics of rainfall simulation events RI (rainfall intensity), CR (cumulated rainfall), RT (runoff time)

Treatment	RI (mm/h)	CR (mm)	ST (min)	(L / plot)
CR1	60	43	43	13.02
CR2	62	78	75	14.86
CR3	68	69	61	16.85
PSR1	61	46	45	16.82
PSR2	60	62	62	16.37
PSR3	66	46	42	17.23
CMR1	62	76	74	15.81
CMR2	66	99	90	15.56
CMR3	79	157	119	15.2

Dynamics of microbial parameters and dissolved organic carbon concentrations

In control (Soil alone), E. Coli and Enterococci were lower than the detection limits. Escherichia Coli and Enterococci showed stable dynamics in both modalities of PS and CM soil amendments in the runoff water collected during three rainfall events (Figure 2.3).

Particulate matter (PM) contents were stable during three repetitions R1, R2, R3 and the PM values were ranged 3 to 5 g L^{-1}. Dissolved organic carbon (DOC) concentrations in control (soil alone) treatment were low; between 2 to 5 mgL^{-1} in the first three samples (6L runoff)) and 2 to 4 mgL^{-1} in the last four samples (14L runoff).during all the rainfall events R1, R2 and R3.

In pig slurry (PS) soil amendment, in rainfall events R1 and R2, DOC concentrations ranged between 33 to 71 mgL^{-1} and 20 to 65 mgL^{-1} respectively during the first three water samples (6L runoff). However, during simulation event R3, DOC concentrations in PS amendment were remained constantly higher from 65 to 87 mgL^{-1} in the first six liters runoff . In the next 14L runoff water, DOC concentrations ranged between 21 to 28 and 9 to 17 mgL^{-1} during R1 and R2 in PS soil amendment respectively. Moreover, during R3, PS showed higher DOC concentration 38 to 65 mgL^{-1} compared to R1 and R2.

In cow manure amendment, DOC ranged from 23 to 94 mgL^{-1}, 11 to 34 mgL^{-1} and 15 to 29 mgL^{-1} during R1, R2 and R3 respectively during first three water samples (6L runoff). In last four samples, DOC ranged from 51 to 71 mgL^{-1}, 24 to 38 mgL^{-1} and 13 to 15 mgL^{-1} during R1, R2 and R3 respectively.

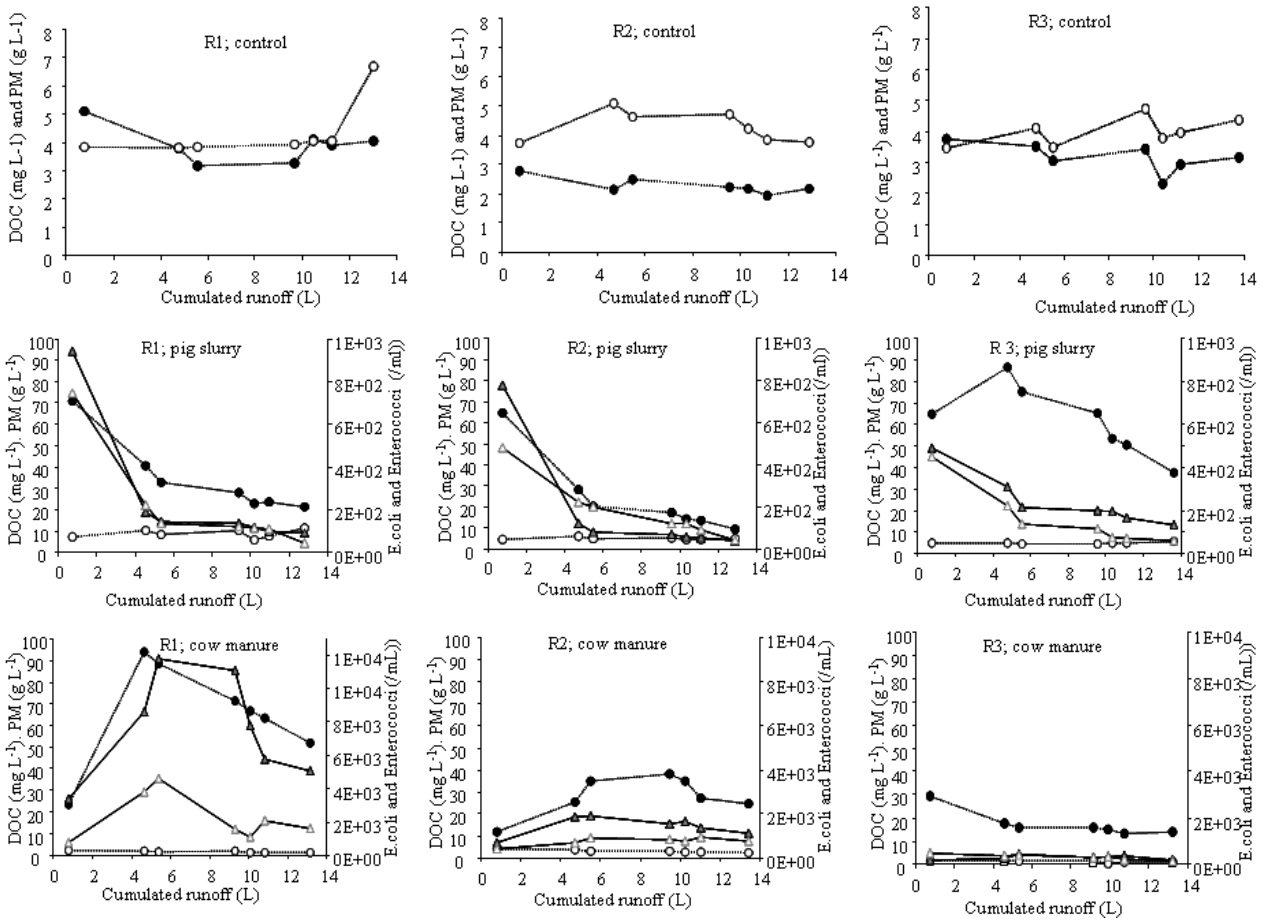

Figure 2.3 : Dynamics of dissolved organic carbon (DOC) (—●—), particulate matter (PM)(—○—), Escherichia coli (—▲—), Enterococci (—△—) in runoff water collected during three rainfall events control (mineral soil), pig slurry (PS) and cow manure (CM).

Discrimination of farm wastes DOM in runoff water

Ratio of biochemical to geochemical fluorescence

Biochemical to geochemical fluorescence intensities ratios discriminated significantly ($p<0.05$) the soil alone (0.09-0.13) from PS (0.25-0.33) and CM (0.29-0.31) treatments during rainfall event R1, R2 and R3 except CM treatment in R3 showing no significant difference to control (Figure 2.4). In R1 and R2, ratio bio:geo differentiated significantly CM treatment with higher values compared to PS. However, CM modality demonstrated significant lower values compared to PS treatment in R3. In last four samples of simulated runoff, ratio bio:geo discriminated significantly PS from control in R1 and R3 as well as CM from control in R1 and R2. PS was not differentiated from control in R2.

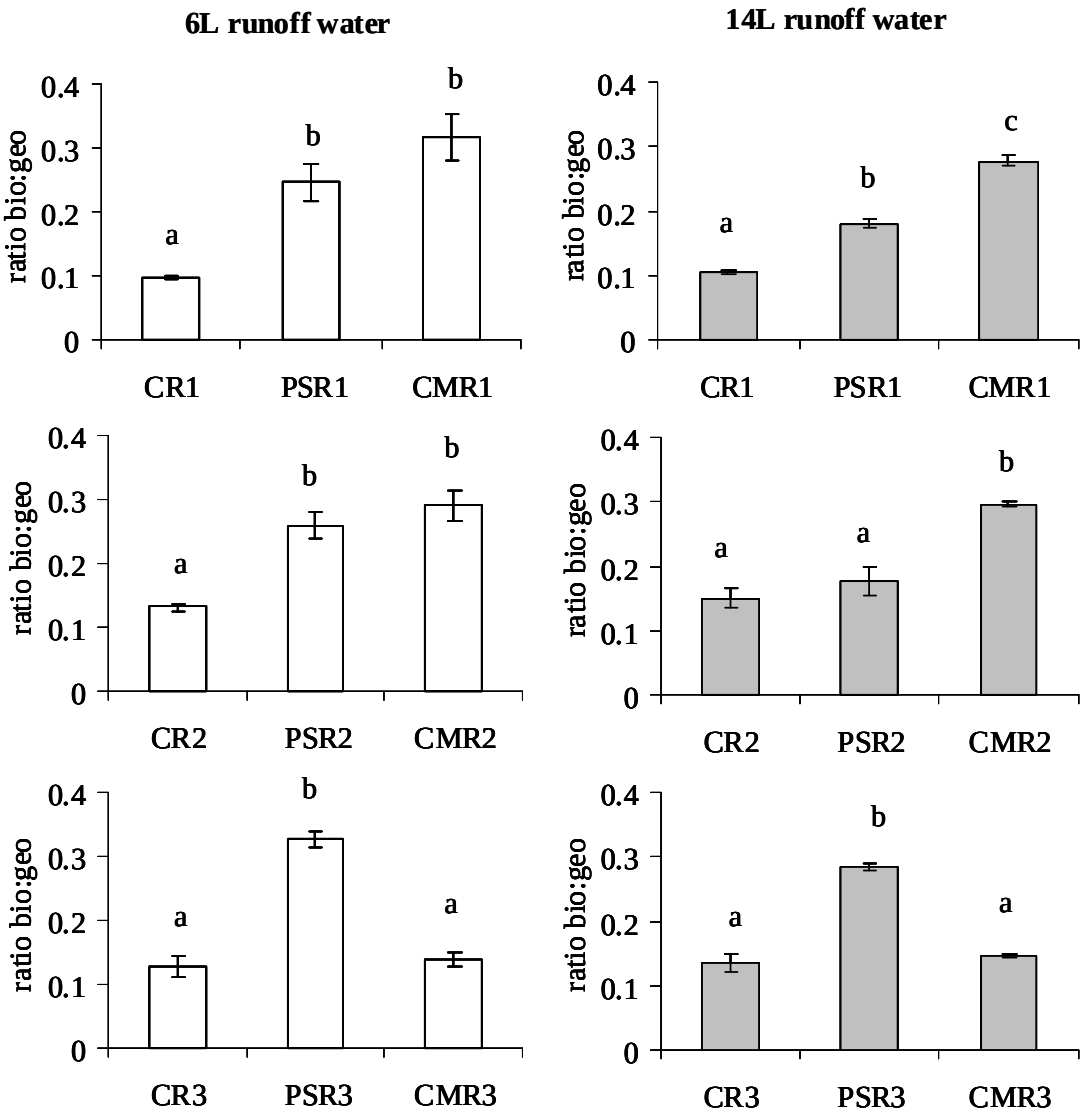

Figure 2.4 : Discrimination of control (mineral soil) from farm wastes (pig slurry (PS), cow manure (CM)) by biochemical to geochemical ratio (ratio bio:geo) in the 6 Litters and the 14 Litter next runoff water collected during three rainfall events (R1, R2, R3). Bars represent standard error and different letters indicate significant mean differences ($p<0.05$) ANOVA (one way).

Integral fluorescent volume in region V

Fluorescence intensities in region V discriminated the control (mineral soil) from PS and CM soil treatments with significant higher values ($p<0.05$) during all the studied rainfall events R1, R2 and R3 in 6L simulated runoff and 14L runoff water as shown in Figure 2.5. In 6L simulated runoff, fluorescent volume in region V differentiated control treatment (mineral fertilized soil) with significant higher values (31399 RU and 22357 RU) from PS (14571 RU and 11974 RU) and CM (7624 RU and 9347RU) in R1 and R2 respectively. Moreover, PS showed significant higher values than CM amendment in R1 and R2. In R3, in 6L simulated

runoff, control treatment (22177 RU) was differentiated by region V from PS (9112) and CM (14349 RU). However, region V fluorescence lowered significantly in PSR3 compared to CMR3 treatment.

In 14L simulated runoff, region V discriminated control treatment (33986 RU and 20161 RU) from PS (19787 RU and 16144 RU) and CM (10766 RU and 8600 RU) amendment in R1 and R2 respectively. Similarly to 6L simulated runoff, PS showed significant higher fluorescence in region V compared to CM simulated runoff. In R3, 14L simulated runoff, control treatment was differentiated significantly with higher values (21953 RU) from PS (9623 RU) and CM (14304 RU).

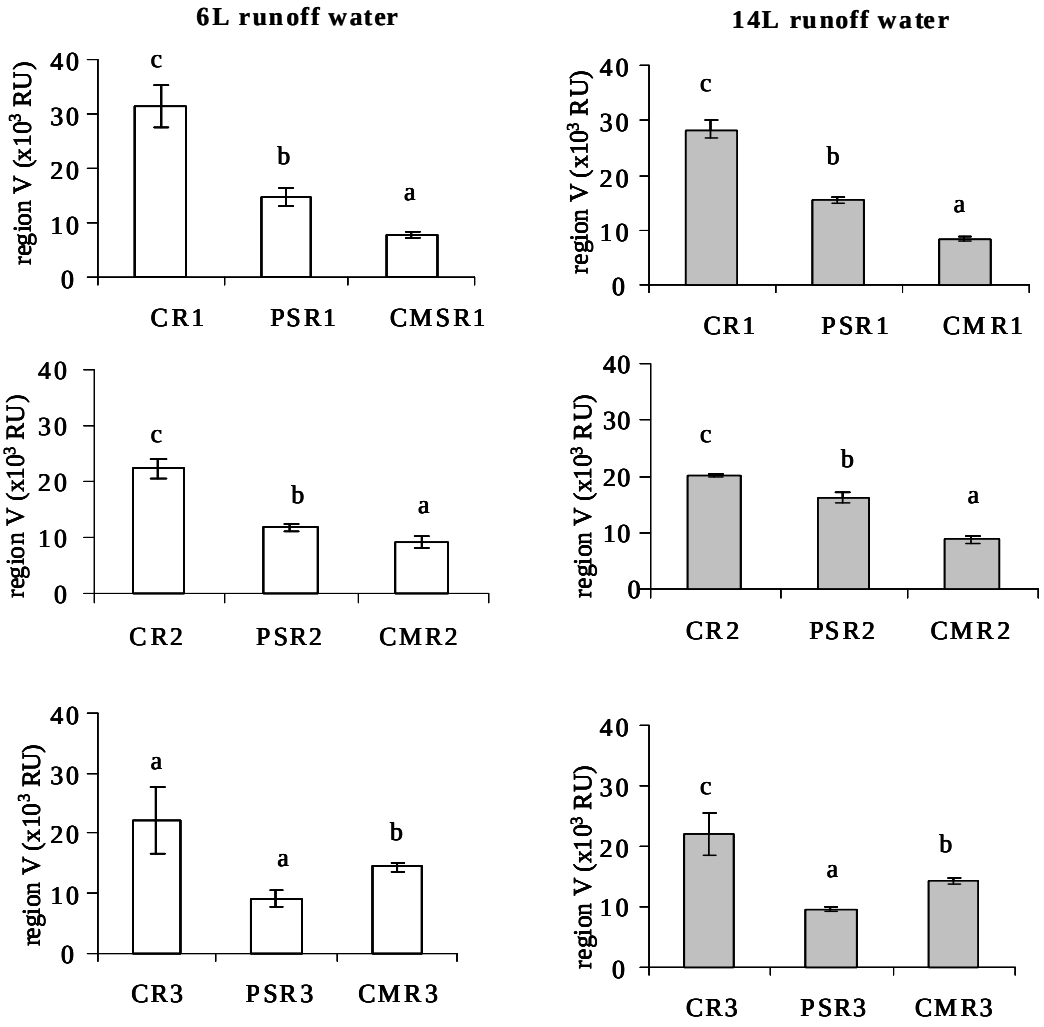

Figure 2.5 : Discrimination of control (mineral soil) from farm wastes (pig slurry (PS)), cow manure (CM)) by integral fluorescence intensities in region V (RU) in 6L and 14L runoff water collected during three rainfall events (R1, R2, R3). Bars represent standard error and different letters indicate significant differences ($p<0.05$).

Correlation between humification index (HIX) and geo fluorescence

Pearson product moment correlation (r) between HIX and geo fluorescence intensities was shown in Table 2.4. We observed significant positive correlation ($r=0.98$ $p<0.0001$) between HIX and geo fluorescence in mineral soil in the first samples of runoff and a lower correlation (0.76, $p<0.001$) in the last runoff samples. In cow manure amended treatment, the correlation is important in the beginning and in the end of the runoff event ($r=0.94$ and 0.95 respectively). In PS treatment, there was no significant correlation between HIX and geo fluorescence in 6L simulated runoff but it was significant in 14L simulated runoff. The correlation was not significant for the first samples on pig slurry treatment ($r=0.53$, $p<0.15$), but better in the end of the runoff event ($r=0.79$).

In case of pig slurry, we hypothesize that just after pig slurry spreading, rainfall export the larger proportion of DOM from pig slurry source which is poor humified. After flushing of significant portion of pig slurry DOM in runoff, correlation between HIX and geo developed as more humified DOM is exported. Soil DOM dominates in the last runoff sample as DOC decreased in pig slurry treatment. Humified DOM seems a constant source release during CM simulation and keeps correlation strong in the runoff.

Table 2.4 : Correlation between humification index and geo fluorescence

	6L simualted runoff	14L simualted runoff
	r value	r value
Control (soil alone)	0.98 ($p<0.0001$)	0.76 ($p<0.001$)
Pig slurry	0.53 ($p<0.15$)	0.79 ($p<0.002$)
Cow manure	0.94 ($p<0.001$)	0.95 ($p<0.001$)

Discussion

Direct impact of farm waste on DOM production in runoff water

Farm waste amendments had shown the net impact of farm wastes modalities on DOC concentrations and it was almost 18 times higher than control modality during first runoff samples in three rainfall simulation events R1, R2 and R3. In the PS modality, DOC highly elevated in first sample (R1, R2) or first three samples of R3 event and the values ranged between 60 and 87 mgL^{-1} DOC. Then the concentrations decreased rapidly as simulation proceeded. It reflected that larger part of DOC mobilised rapidly in first flush of runoff water

of rainfall simulation. At the end of simulation experiment of pig slurry, DOC pool depleted, however, DOC concentration still remained higher than mineral soil with values 8 to 34 mgL^{-1}. These values were four time higher than measured in control at the end of experiment.

However, in cow manure simulation, DOC concentrations were higher (about 50 mgL^{-1}) in R1 compared to R2 and R3 that marked strong variability in first event of cow manure simulation However, in R2 and R3, DOC did not exceed to 40 to 30 mgL^{-1}DOC respectively.

Moreover, at the end of simulation experiment, DOC concentrations in cow manure were globally higher compared to pig slurry treatment. This variability could be related to the presence of cow dung in cow manure wastes, wheat straw in cow manure get washed and only cow dung generated DOC. Globally microbiologic indicators were stable in farm wastes modalities and the concentration of particulate matter were constant in control (mineral soil), pig slurry and cow manure.

DOM quality after farm manures supply

Fluorescence spectroscopy has enabled us to quantify DOM export and to study chemical characteristics after pig slurry and cow manure supply on soil in a runoff simulation experiment conducted in small surface area (1.12 m^2).

Fluorescent DOM characteristics of pig slurry changed with sample preparation. Air dried pig slurry showed higher bio:geo and III:V ratio than fresh pig slurry (Figure 2.6a). Runoff generated on mineral soil (control) during all the rainfall simulation events R1, R2 and R3 are characterized by lower bio:geo and III:V ratio and are identical to values obtained on soil extracts.

Thus, two sources of DOM are evident in these runoff simulations. Fresh PS waste extract showed bio:geo and III:V ratios close to majority of the values observed in PS simulated runoff which reflect similar DOM quality in simulated runoff and pure PS waste. A general trend of decreasing bio:geo and region III:V ratios was observed as simulation proceeded (shown with arrow in Figure 2.6a). These decreasing ratios approached to the simulated runoff in control soil, especially in PSR2, where last sampling point was positioned in the controlled soil DOM. It reflects the export of more indigenous soil DOM than the exogenous applied through PS waste as rainfall simulation event proceeds.

Figure 2.6b demonstrated the cow manure rainfall simulation events (CMR1, CMR2 and CMR3) along with controlled treatments (CR1, CR2, CR3) as well as pure cow manure wastes either extracted after drying or fresh phase and fresh cow faeces. DOM extracted from Cow faeces (CF) is characterized by higher bio:geo and region III:V ratios. Fluorescence

properties of cow manure (cow faeces mixed with wheat straw) extracted DOM are characterized by lower bio:geo and III:V ratio than cow faeces. There is effect drying on the fluorescence signature and also decreased these ratios.

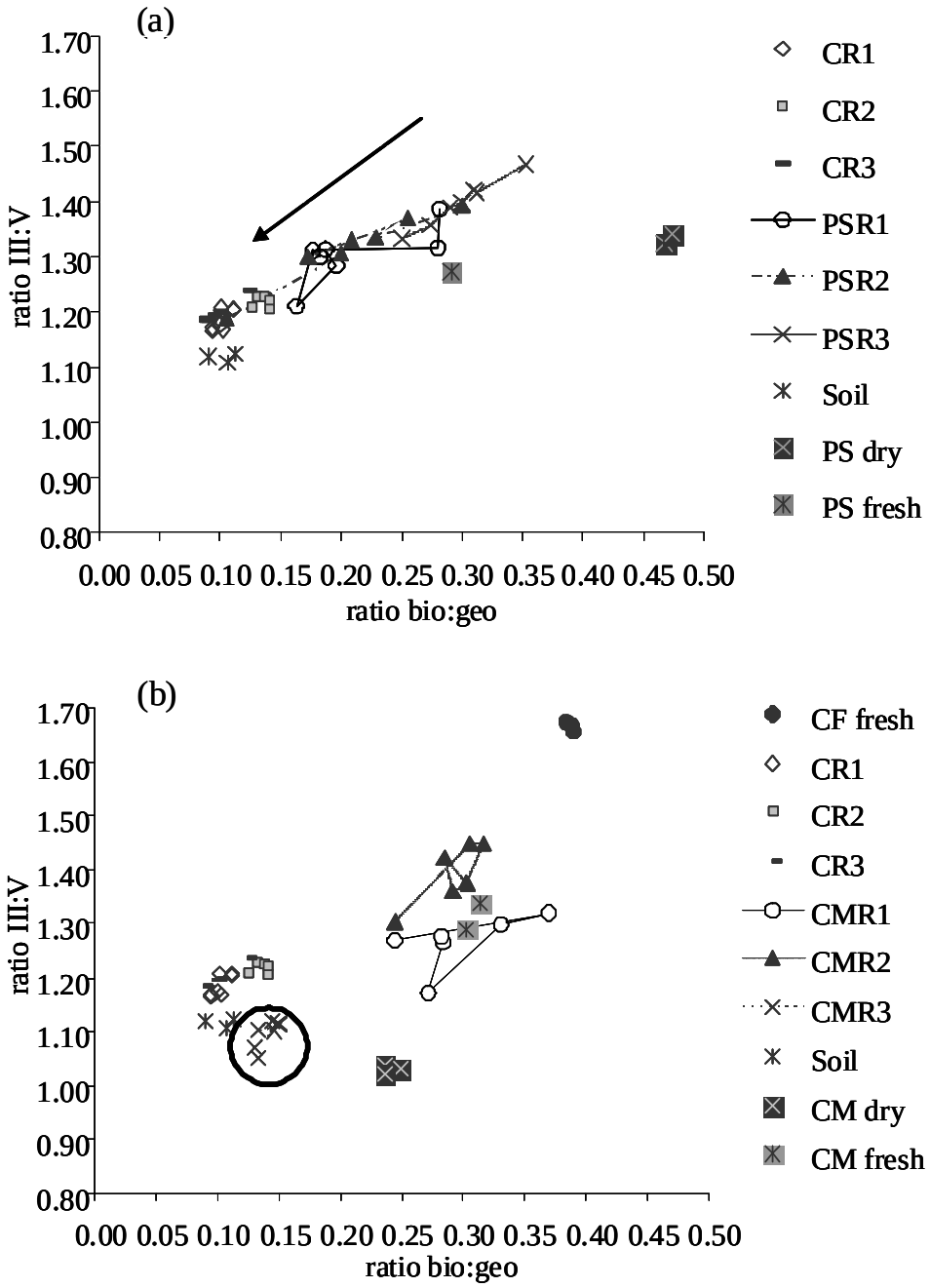

Figure 2.6 : Dynamics of ratio bio:geo and ratio III:V in simulated runoff in control (C) (a) and cow manure (CM) in three rainfall simulation events R1, R2 and R3 (b).Cow Faeces (CF) and Cow manure (CM) extracts are also reported.

However, in cow manure simulated runoff, DOM in R1 and R2 showed almost similar ratios of bio:geo and region III:V and grouped with aqueous extracts of fresh cow manure wastes. It reflects that majority of DOM released during CM simulated runoff in rainfall events R1 and

R2 originate from exogenous applied CM waste. But CMR3, black line circle in Figure 2.6b, reflected that most of the DOM substance was of indigenous soil origin.

However, ratio bio:geo and III:V can not discriminate a pig slurry contamination from a cow manure. DOM issued from soil is clearly discriminated from DOM issued from farming wastes with rapid fluorescence measurement and characterization of bio:geo and III:V ratio by regional integration.

Ratio bio:geo as a potential tracer of farming waste DOM

At the start of rainfall simulation events (R1, R2 and R3) conducted on pig slurry treatment, runoff water showed higher DOC and bio:geo ratios and approached values obtained on pig slurry effluents diluted 400 times (Figure 2.7a). As the rainfall simulation proceeded, the ratios gradually decreased and approached to bio:geo values in runoff collected from control plots. DOC export from PS amended soil plots also decreased as simulation proceeded.

In CM modalities (Figure 2.7b) during R1 and R2, DOC increased at the start of rainfall simulation and then decrease. In CMR1, highly DOC concentrated runoff samples showed bio:geo ratio close to cow faeces and cow manure DOM extracts. In CMR2, although, DOC was lowered than CMR1 yet it demonstrated strong bio:geo ratios which were in between the cow manure and cow faeces.

While CMR3 reflected strong control of indigenous (soil) DOM fluorescence with lower bio:geo ratio and approached to bio :geo ratios in simulated runoff in control plots. In modality R3, we hypothesize the major contribution from soil DOM in the labile organic carbon.

The correlation between HIX and geo fluorescence was proposed here to investigate the DOM sources in runoff water. The correlation is important in soil and cow manure runoff water and less important on runoff water generated on pig slurry treatment. The strength of this relation coupled with bio:geo and III:V ratio could be use to discriminate cow manure from pig slurry DOM contamination. However, it was not analysed in this study and should be explored in further studies.

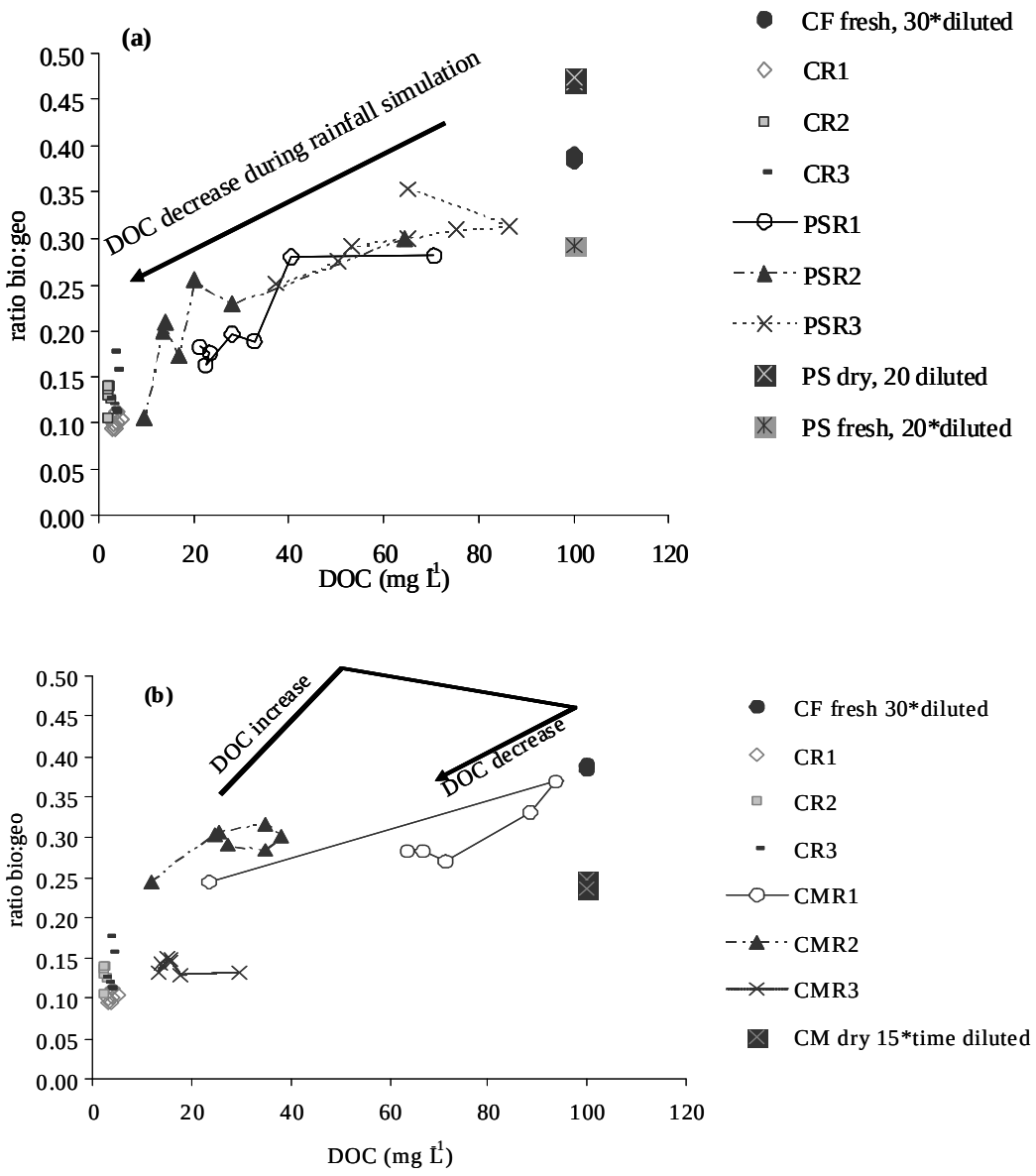

Figure 2.7 : Dynamics of ratio bio:geo with increasing DOC concentration in simulated runoff in three rainfall simulation events R1, R2 and R3 of pig slurry (PS) (a) and cow manure (CM) (b)

Conclusion

The main results obtained in this runoff simulation experiment in soil after receiving pig slurry or cow manure wastes demonstrated that:

- Under natural field soil conditions, net impact of farm wastes modalities on DOC concentrations was almost 18 times higher than mineral soil (control) in simulation during first runoff samples. At the end of experiment, DOM concentrations in runoff samples during pig slurry and cow manure treatment remained significant higher than mineral soil.

- The regional integration of the fluorescence signal and the characterization of bio:geo and III:V ratio are useful to distinguish slurry and cow manure DOM from soil DOM.
- The first runoff samples fluorescence properties on farming waste amended soils are identical to those measured on raw farming waste. This indicated that a spring storm event which occurred a few hours after the spreading lead to transfer of DOM from farming waste. Thus transfer of associated contaminants such as viruses or antibiotics is also possible to occur.
- The ratios bio:geo and III:V are significantly higher than those measured in control in the first 6L runoff in pig slurry treatment and in all the runoff samples collected in two repetition on cow manure. However in the last cow manure simulation, DOM transfer was from soil source.
- Region V fluorescence discriminated the soil DOM from farm wastes.
- It is impossible to discriminate pig slurry from cow manure contamination since fluorescence properties measured on cow faeces are identical to pig slurry.
- Air drying treatment modifies the fluorescence properties of the farming waste. To detect farm waste contamination in stream, fresh effluent analysis dataset have to be investigated to explore the variability in the farming waste.

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CHAPTER 3

Chapter 3

Discrimination of farm waste contamination by fluorescence spectroscopy coupled with multivariate analysis during a biodegradation study

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Abstract

Persistence of potential tracers of dissolved organic matter (DOM) generated from farm waste amended soil were investigated by fluorescence spectroscopy coupled with classification and regression tree (CART) and principal component analysis (PCA) during short term (8 days) to mid term (60 days) biodegradation study. Pig manure (PM), cow manure (CM), wheat straw (WS) and soil alone (SA) treatments inputs were used. Waste amendments were potential sources of higher DOM concentrations. PCA revealed the DOM quality differences between farm wastes and soil alone as well as a significant shift observed from biochemical to geochemical fluorescent fraction in SA and PM treatments. Ratio tryptophan:humic like and tryptophan zone were the potential discriminators of recent and mid term pollution by farm wastes. Integral intensities of fulvic like zone and region III discriminated the PM from CM and WS during the 60 days. CART analysis showed 90% and 100% potential for farm wastes discrimination from soil during P1 and P2 respectively. Prediction success was 72% and 57% for PM from other wastes and 60% and 100% for WS during both periods. Fluorescence spectroscopy in combination with CART analysis can be a non-destructive innovative method for monitoring susceptible farm waste contamination.

Key words: Farm waste, soil, fluorescence spectroscopy, CART analysis, biodegradation

Introduction

Elevated dissolved organic matter (DOM) concentrations have been reported in fresh water environments across Europe and North America (Worrall et al., 2003). This increase has significant impact on functioning of aquatic ecosystems (Kalbitz and Wennrich, 1998) and lead to formation of carcinogenic disinfection by-products such as trihalomethan (THM) (Sirivedhin and Gray, 2005) during the chlorination process of water treatment.

Agricultural land spreading of farm wastes for plant nutrient recycling and crop production improves soil quality (organic matter contents, physical properties such as aggregate structural stability, texture, porosity, infiltration, water holding capacity and biological activity (Barzegar et al., 2002)).However, it also increases the potential for negatively impacting the environmental quality through significant higher dissolved organic matter level in soils(Kalbitz et al., 2000) which ultimately reaches rivers draining these cultivated amended soils (Jardé et al., 2007a). Plant biomass, litter leachates, root exudates, soil humus and microbial degradation products are also considered as the main sources of DOM in soil (Kalbitz et al., 2000). Agricultural intensification has a major impact on the increasing DOM concentration through land use change and soil disturbance, farm waste soil amendments (Chantigny, 2003; Royer et al., 2007b; Molinero and Burke, 2009a) as well as higher mobilization of native soil carbon due to animal waste (Bol et al., 1999; Shand et al., 2000). It is thus essential to gain insight into how DOM issued from these farm wastes changes upon decomposition when it comes in contact with soil after amendments.

Biodegradation kinetics of soluble organic matter highlight two fractions: a rapidly decomposable fraction with a turnover time of less than one day (containing 29-36% of the total carbon) and a slowly decomposable fraction with a turnover time of about 80 days (Gregorich et al., 2003). However much less research has been done to acknowledge the biodegradation potential of farm wastes dissolved organic matter (DOM) after soil amendment. Animal faecal contamination in rivers has been investigated with biomarkers of sterol and bile acids (Tyagi et al., 2009), sterol/stenol in pig slurry (Jardé et al., 2007b). The characterization of these tracers requires solvent extraction and chromatographic detection. There is a need to develop cheap and non-destructive tools for tracing these heterogeneous sources of DOM as a prerequisite to management actions for river water quality restoration at catchment scale.

In various environmental applications, 3-dimensional fluorescence excitation-emission matrix (3D-EEM) spectroscopy has been used for monitoring and discrimination of organic matter in

soil and lakes considering fluorescence intensity peaks and their ratios with peak picking method (McKnight et al., 2001). Humic like peak C and tryptophan and tyrosine like peaks T and B have been used for monitoring of DOM in treated effluents, farm wastes, treated sewage wastes and sewer discharge (Baker, 2002; Baker and Inverarity, 2004; Lee and Ahn, 2004; Saadi et al., 2006) and in coastal environments subjected to anthropogenic inputs (Parlanti et al., 2000). However, in the current study, instead of taking few data points in the form of peak picking, the whole 3D-EEM spectra is analysed quantitatively with fluorescence regional integration (Chen et al., 2003b).

Besides this, machine learning multivariate analysis is an ideal tool for the exercise when large datasets are involved. Recent literature highlights the performance of multivariate techniques (principal component analysis, PCA) in fluorescence fingerprinting of DOM for water treatment EEM (Tartakovsky et al., 1996; Peiris et al., 2010) and hierarchical clustering method for DOM sourcing of marine water samples (Jiang et al., 2008). Parallel factor analysis (PARAFAC) also helped to characterise fluorescent landscape of DOM from aqueous extracts of soils and soil amendments by decomposing the fluorescent EEM into different independent fluorescent components (Ohno and Bro, 2006).These methods have advantage of time saving and more accurate analysis over the traditional peak picking technique. In the current study, we introduced classification and regression tree (CART) analysis, a nonparametric data mining approach, for the class membership of categorical dependent variable without getting any assumption about the distributions of the variables (Breiman et al., 1984).

The aims of this study are twofold: (i) to investigate the potential of 3-dimensional fluorescence spectroscopy coupled with CART analysis to identify the optical tracers of DOM released from soil alone and from farm wastes amended soil (pig manure, cow manure and wheat straw); (ii) to analyse the short-term to mid-term persistence of fluorescence indices of farm waste contamination during a biodegradation experiment.

Material and Methods

The topsoil horizon from an agricultural field was sampled after wheat crop harvest from the experimental station of Kerguéhennec in Morbihan, East Brittany, France. The soil, derived from mica schist, is a Humic Cambisol (FAO) with a loamy texture (17% clay, 42% silt, 41% sand), an organic matter content of 37 g kg^{-1} and a pH (H20) of 6.0.

Organic products characterization and experimental design

A crop residue (wheat straw (WS)) and two farm manures i.e. pig slurry (PS), cow manure (CM) were used as organic amendments. Pig slurry samples were separated into its liquid and solid parts through centrifugation at 3000 rpm for 30 minutes. Solid pig slurry was used and referred as pig manure (PM) in this study. Total organic C and N contents of these materials were determined by elemental analyser (Flash EA 1112, Thermofinningan, Milan, Italy). The C:N ratio of PM, CM and WS were 10, 33 and 110 respectively. C:N ratios of PM and CM were comparable to farm wastes studied by Morvan (Morvan et al., 2006) in which C:N ratios for pig manure and cow manures were <15 and >25 respectively.

Experimental conditions of biodegradation

In the laboratory, soil samples were air dried and crumbled manually by removing the unrefined residues of organic matter. Soil aggregates were chosen after sieving through 3.15 to 5 mm mesh size and then stored in the darkness at 4°C. The aggregates were moistened by capillary action then subjected to 2.5 pF to attain a water holding capacity of 21.2 %. Soil samples were pre-incubated at 25°C during 6 days before the experiment to minimize microbial activity variation due to temperature change. The organic materials were air dried and crushed to 1-mm particle size and then incorporated homogenously into the moist, sieved and pre-incubated soil at a rate of 4 g $C.kg^{-1}$ dry soil. The soil mineral-N content was adjusted to 75mg N / kg dry soil by adding potash fertilizer (KNO_3) solution to ensure mineral nitrogen availability for the microorganisms during biodegradation and a follow-up for mineral nitrogen content was done during the whole study time. Samples were incubated at 25°C in hermetically closed jars in the darkness. A tube containing 40 ml deionised water was introduced in each jar to minimise sample desiccation. The atmosphere in jars was regularly renewed to maintain aerobic environment for microbial degradation. All the treatments were sampled after 0, 3, 7, 15, 30 and 56 days after incubation along with three replicates. We divided the whole data for fluorescent DOM characterisation into period P1 (0 -7 days after incubation) and period P2 (08-56 days after incubation). We marked periods P1 as short-term and P2 as mid-term farm wastes pollution.

Extraction of dissolved organic matter (DOM)

DOM was extracted with 2:1 ultra pure water to soil ratio. Soil water suspensions were shaken mechanically on orbital shaker for 2h and then centrifuged at 3000 rpm for 30 minutes

and filtered through 0.7 and 0.22 µm nitrocellulose filters. To avoid any contamination, all the filters were rinsed with ultra pure water and dried overnight before vacuum filtration.

Chemical Analysis

Dissolved organic carbon (DOC) in each solution was measured on a Shimadzu TOC 5050 A total carbon analyzer. Accuracy on DOC measurements was ±5%, based on repeated measurements of standard solutions (K-phtalate). UV-Visible absorbance was measured on a Perkin Elmer Lambda 20 UV-Visible spectrophotometer across 200-600 nm excitation wavelengths range with data interval 0.5nm, slit width 2 nm and scan speed 120 nm/min.

Fluorescence measurements of DOM were performed using a Perkin-Elmer LS-55B luminescence spectrometer. The spectrophotometer uses a xenon excitation source and slits were set to 5 nm for both excitation and emission. To obtain excitation-emission matrix spectra, excitation wavelengths were incremented from 200 to 425 nm at steps of 5 nm and emission was detected from 250 to 600 nm with a 0.5-nm step. Scan speed was set at 1500 nm/min, yielding an EEM in 22 minutes with 45 total scans. To minimise the temperature effect, samples were allowed to equilibrate with room temperature (20±2°C) prior to fluorescence analysis. The whole fluorescence dataset presented in this study was normalised at 5 mg L^{-1} DOC. Linearity was carried out between DOM concentration and fluorescence intensity with dilution of high DOM concentration samples. To eliminate the second order Raleigh light scattering, excitation and emission cutoff filters were applied at 230-310 nm and 380-600 nm respectively on the lower side of three dimensional plots (Figure 3.1).

Inner filter effects were removed with the formula (Ohno, 2002). To maintain the consistency of measurements and standardise the whole fluorescence dataset, all the integrated fluorescence intensities were normalized to average Raman emission intensity units of 19 for ultra pure water samples (n=25) at excitation and emission wavelengths of 350 nm and 397 nm respectively. A Raman normalised integrated EEM spectrum of ultra pure water was subtracted from the data sample to eliminate the water Raman scatter peak**.**

Regional integration of excitation emission matrix (EEM)

An internal program was developed in the laboratory using the R® software (http://www.r-project.org) for the integration of fluorescence intensities across the whole EEM landscape. Here peaks at shorter wavelengths (<250 nm) and shorter emission wavelengths (<380 nm) are related to simple aromatic proteins such as tyrosine and tryptophan (Regions I and II) Peaks at intermediate excitation wavelengths (250–340 nm) and shorter emission wavelengths (<380 nm) are related to soluble microbial by-product-like material (Region IV) while peaks

located at the excitation wavelengths (230–300 nm) and the emission wavelengths (380-575 nm) represent humic acid-like substances (Region III). Peaks at longer excitation wavelengths (>300 nm) and longer emission wavelengths (>380 nm) are related to fulvic acid-like organics (Region V). With this technique, EEM is divided into biochemical (bio) (I, II, IV) and geochemical (geo) (III, V) fluorescent regions (Figure 3.1a) and three peak intensity zones of tryptophan (TRY), fulvic like (FL) and humic like (HL) fluorescence (Figure 3.1b).

The quantitative analysis included the integration of fluorescent volume beneath each region and zone. Moreover, ratios TRY:HL, TRY:FL, HL:FL, bio:geo, IV:V and III:V were also calculated. 45 spectral loadings were used to reproduce three-dimensional plots of fluorescence intensity as a function of excitation and emission wavelengths. Humification index (HIX) was determined according to Ohno, (2002).

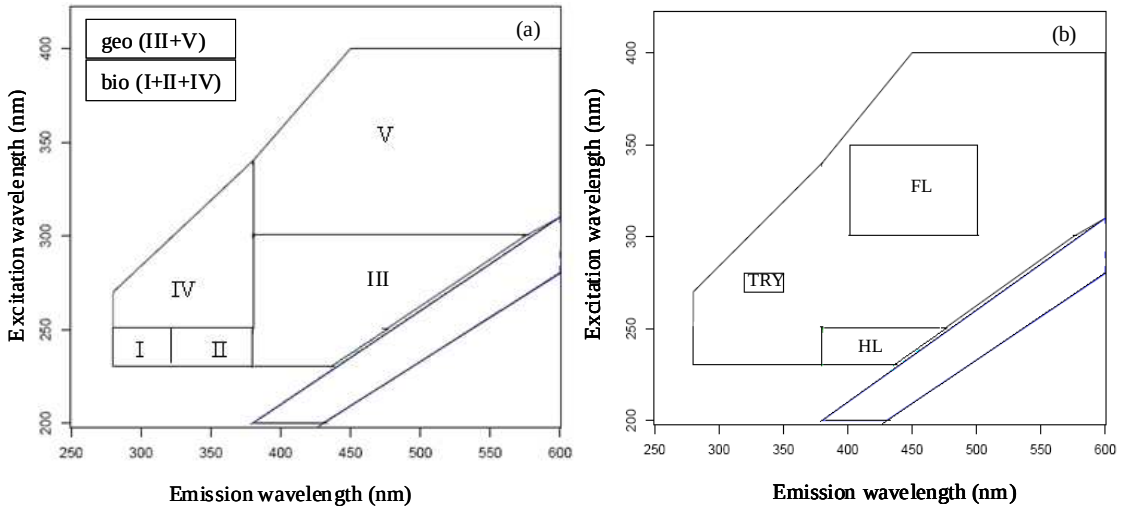

Figure 3.1 : Integration of fluorescence intensities across regions (a) and maximum peak intensity zones (b).

Statistical analysis

Principal component analysis (PCA) was applied to the spectroscopic data of DOM issued from farm wastes and SA during biodegradation study periods P1 and P2 with R® software (package ade4). Significant differences among the temporal shift of treatments were tested by one way ANOVA ($p<0.05$).

Unlike traditional statistical techniques, we applied CART tree approach (Breiman et al., 1984) as they were adopted to predict a qualitative property by selecting the most discriminant quantitative predictors. It can also handle numerical data that are highly skewed or multi-model with categorical predictors having either ordinal or non ordinal structures. CART used an optimal univariate splits by carrying out an exhaustive search for all possible

splits for each predictor variable and find the best split having higher improvement in the prediction accuracy. The tree structure started with the root node which contains all the observations of SA, WS, PM and CM treatments in the form of histogram plots. The splitting of root node results the child nodes which again becomes parent node if division continues and the nodes where division finishes or homogeneity occurs called terminal nodes. CART analysis was performed with STATISTICA (version 7.1).

Results

Temporal dynamics of dissolved organic carbon (DOC)

Period P1was marked by strong variations of DOC concentration in the farm wastes amended soil. After three hours, DOC in PM, CM and WS treatments were 73.8±5.7 mg L^{-1}, 42.5±3.6 mg L^{-1} and 28.6±2.9 mg L^{-1} respectively compared to soil alone 11.6±0.2 mg L^{-1} (Figure 3.2). Within 24 hours, DOC decrease was more important in PM and CM treatment (31.1 and 10.9 mg L^{-1}) than in WS treatment (2.9 mg L^{-1}).

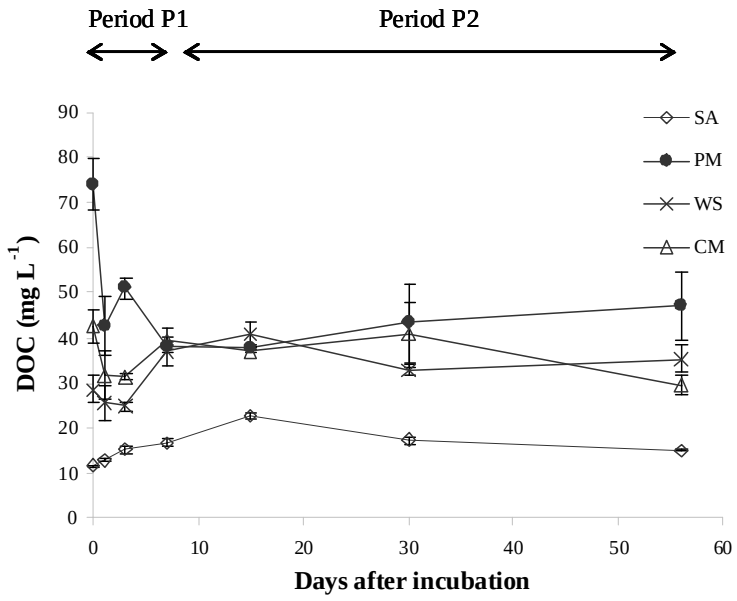

Figure 3.2 : Time series of DOC concentrations of four treatments. Abbreviations are SA- soil alone, WS- wheat straw, PM-pig manure and CM-cow manure. bars indicate standard error (SE) and N =3.

DOC concentrations were almost same in all the farm waste treatments (38 mg L^{-1}) on 7th day after incubation. During period P2, DOC concentrations were almost stable in farm wastes treatments. At the end of study period, PM treatment showed higher DOC values 47.2±7.5 mg L^{-1} compared to WS and CM treatments with 35.3±2.9 mg L^{-1} and 29.2±2.1 mg L^{-1}

respectively. During the whole study period, farm wastes showed higher DOC compared to soil alone. DOC concentration in SA treatment varied between 11.6 to 16.9 mg L^{-1} during period P1 but during P2, DOC dynamics was stable except a peak of 22.8 mg L^{-1} on 15^{th} day after incubation.

Spectral differences among the farm wastes treatments and soil alone

Principal component analysis (PCA) was applied to the integrated fluorescence properties of farm wastes and soil alone treatments, to investigate the spectral differences as well as to retrieve the additional information on temporal shift of the observed indices during period P2. A preliminary comparison of average was conducted to select the pertinent spectroscopic indices which discriminate the modalities

The axe 1 and axe 2 of the PCA explained 47.5% and 28.6%, respectively, of variability in 14 spectroscopic indices of SA, PM, CM and WS treatments distribution during both degradation periods P1 and P2 (Figure 3.3). SA treatment was clearly separated from the farm wastes treatments in opposite quadrants with negative scores on axe 1 during period P1 and positive scores on axe 2 in period P2 as shown in Figure 3.3. The average axe 2 score for SA treatments (2.59) was significantly higher in period P2 than during period P1 (0.63) ($p<0.05$). Geochemical integrated fluorescence intensities across the regions geo (III+V) and the zones HL and FL, ratio HL:FL and HIX had strong negative weightings on axe1 (Table 3.1) which separated SA treatment from the farm wastes during period P1. However, during biodegradation period P2, only HIX separated the SA treatment with its positive weightings on axe2. SA treatment during P2 showed negative Pearson correlation (r) to TRY (- 0.68) and to ratios TRY:HL (- 0.92), TRY:FL (- 0.88), bio:geo (- 0.93) and IV:V (- 0.96).

Among the farm wastes, ratios bio:geo, IV:V, TRY:HL, TRY:FL had strong positive weightings on axe1 (Table 3.1) where CM and WS treatments grouped together and separated from PM treatment in both periods (Figure 3.3). Axe 2 of PCA discriminated the PM treatment from the WS and CM treatments during both periods. There were significant higher ($p<0.05$) average score for PM during P2 (-0.64) compared to P1 (-2.66). Biochemical integrated fluorescence intensities across the region IV and TRY zone had strong negative weightings on axe 2 (Table 3.1) and separated the PM during P1 from rest of the farm wastes and SA treatments. But during P2 in PM treatment, fluorescence indices shifted from biochemical (TRY zone, region IV) to geochemical (geo, FL, HL) fluorescent fractions.

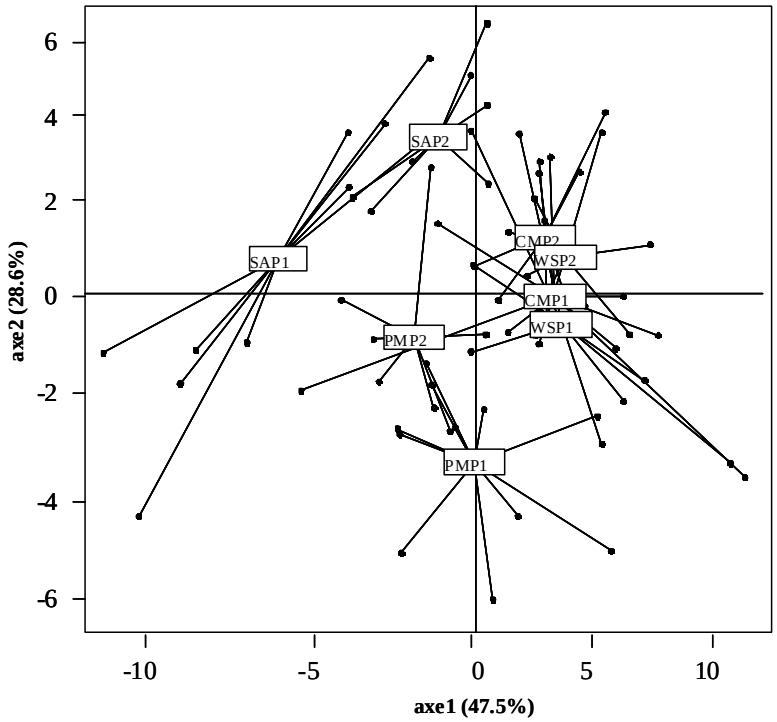

Figure 3.3 : PCA of farm wastes biodegradation study for two periods P1 (0-7 days after incubation) and P2 (8-56 days after incubation). Abbreviations of farm wastes treatments: wheat straw (WSP1, WSP2), pig manure (PMP1, PMP2), cow manure (CMP1, CMP2) and a control treatment i.e. soil alone (SAP1, SAP2). PCA run include the distribution of 16 variables (Table3.1) of the integrated fluorescence properties of DOC and absorbance $A_{(365)}$ on axe 1 and axe 2.

Table 3.1 : PCA weightings for the spectroscopic parameters (variables) during biodegradation study periods P1 and P2.

Variables	axe 1	axe 2
region IV	-0.34	-0.92
region III	-0.89	-0.44
geo	-0.88	-0.44
ratio bio:geo	0.68	-0.60
ratio III:V	-0.45	-0.16
ratio IV:V	0.75	-0.62
FL	-0.88	-0.43
HL	-0.91	-0.27
TRY	0.25	-0.93
ratio TRY:HL	0.78	-0.59
ratio TRY:FL	0.70	-0.52
ratio HL:FL	-0.73	0.11
$A_{(365)}$	-0.13	0.05
HIX	-0.79	0.54

Classification and regression tree (CART) analysis

Farm wastes tracer during period P1

Different tree structures for P1 dataset are shown in Table 3.2 and tree number 2 was chosen as an optimal tree (marked "*") with the minimal cost-complexity measures (Cross validation (CV) cost-misclassification costs of test samples, resubstitution cost-misclassification cost of learning sample dataset) and node complexity (a penalty for additional terminal nodes).

Table 3.2 : Cost complexity measures of all possible trees for period P1 dataset.

All possible trees	Terminal nodes numbers	CV cost	CV std. Error	Resubsititution cost	Node complexity
1	8	0.325	0.067	0.000	0.000
2*	**6**	**0.302**	**0.067**	**0.045**	**0.023**
3	5	0.373	0.069	0.095	0.050
4	3	0.395	0.068	0.295	0.100
5	2	0.500	0.031	0.500	0.205
6	1	0.750	0.000	0.750	0.250

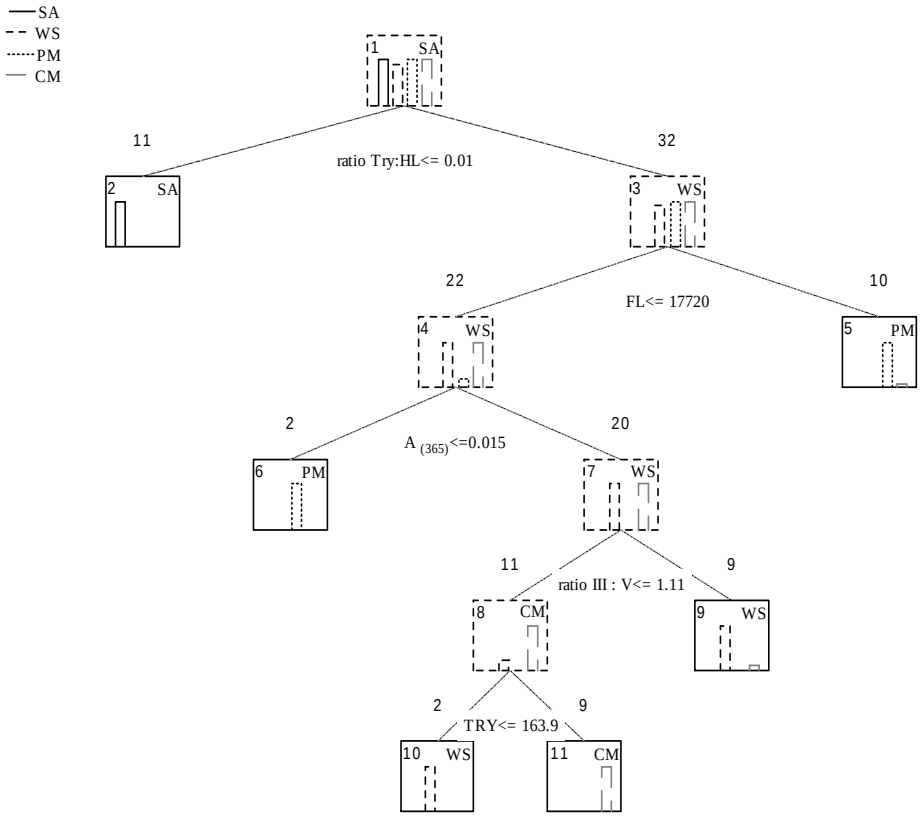

Figure 3.4 : Optimum tree for the fluorescence properties of DOC issued from the farm wastes during biodegradation for period P1 (0-7 days after incubation). Treatment abbreviations are Soil Alone (SA), Wheat Straw (WS), Pig Manure (PM), Cow Manure (CM). Predictor variables abbreviation are integrated fluorescence intensities of across zones of fulvic like (FL), humic like (HL) and tryptophan (TRY), ratio TRY:HL and regional ratio III:V of integral intensities across regions III and V, spectral absorbance $A_{(365)}$.

Terminal nodes numbers described the complexity measurement. Tree structure complexity decreased from tree 1 to 8. Tree structure with one terminal node showed equal misclassification costs (CV cost and resubstitution cost). The optimum tree structure obtained at the end of pruning is drawn in Figure 3.4.

In this optimal tree constructed, there were 5 child nodes (dotted line squares) and 6 terminal nodes (solid black line squares). Integrated fluorescence intensities ratio Try:HL was the first splitter which divided the root node into a terminal node containing all the observation of SA treatment and a child node separating the farm wastes treatments.

Among the farm waste treatments, integrated fluorescence intensities across FL zone classified PM treatment from CM and WS at node#3. Second discriminator of farm waste treatments was ratio III:V which separated the WS from the CM treatment. Finally TRY zone differentiated the CM from WS treatment and allocated it to terminal node#11. However confusion remained in the discrimination of CM treatment as often it misclassified with WS treatment.

Table 3.3 : Confusion matrix of predicted versus observed treatment resulting from cross-validation procedure applied on optimum tree for period P1.

Predicted	Observed			
	SA	WS	PM	CM
	n=11	n=10	n=11	n=11
SA n=10	**90.9%**	0%	0%	0%
WS n=16	0%	**60%**	30%	63.63%
PM n=11	0%	20%	**72.7%**	9.09%
CM n=6	10%	20%	0%	**27.27%**
Total accuracy rate (n=43)				62.79%

Prediction accuracy was assessed by cross validation approach as shown in Table 3.3. Overall prediction accuracy of farm wastes treatments as well as soil alone was 62.7 % for the period P1 dataset. Optimum tree (Figure 3.4) demonstrated a high accuracy (90.9%) in predicting SA treatment, relatively high (72.7%) for PM treatment and fair prediction accuracy (60%) for WS treatment but CM treatment was poorly predicted (27.3%). Among the farm wastes, there was almost complete discrimination between PM and CM treatments with only 9 % CM misclassification rate with PM treatment. However, misclassification rate of CM treatment was high (63.6%) with WS treatment.

Farm wastes tracer during period P2

All possible trees for period P2 are shown in Table 3.4 with tree# 3 marked ("*") as an optimal tree after pruning. Optimal tree structure obtained at the end of pruning is shown in Figure 3.5.

Table 3.4 : Cost complexity measures of all possible trees for period P2 dataset.

All possible tree	Terminal nodes numbers	CV cost	CV std. error	Resubsititution cost	Node complexity
1	6	0.277	0.071	0.000	0.000
2	5	0.277	0.071	0.035	0.035
3*	**4**	**0.305**	**0.074**	**0.107**	**0.071**
4	3	0.357	0.046	0.250	0.143
5	1	0.750	0.000	0.750	0.250

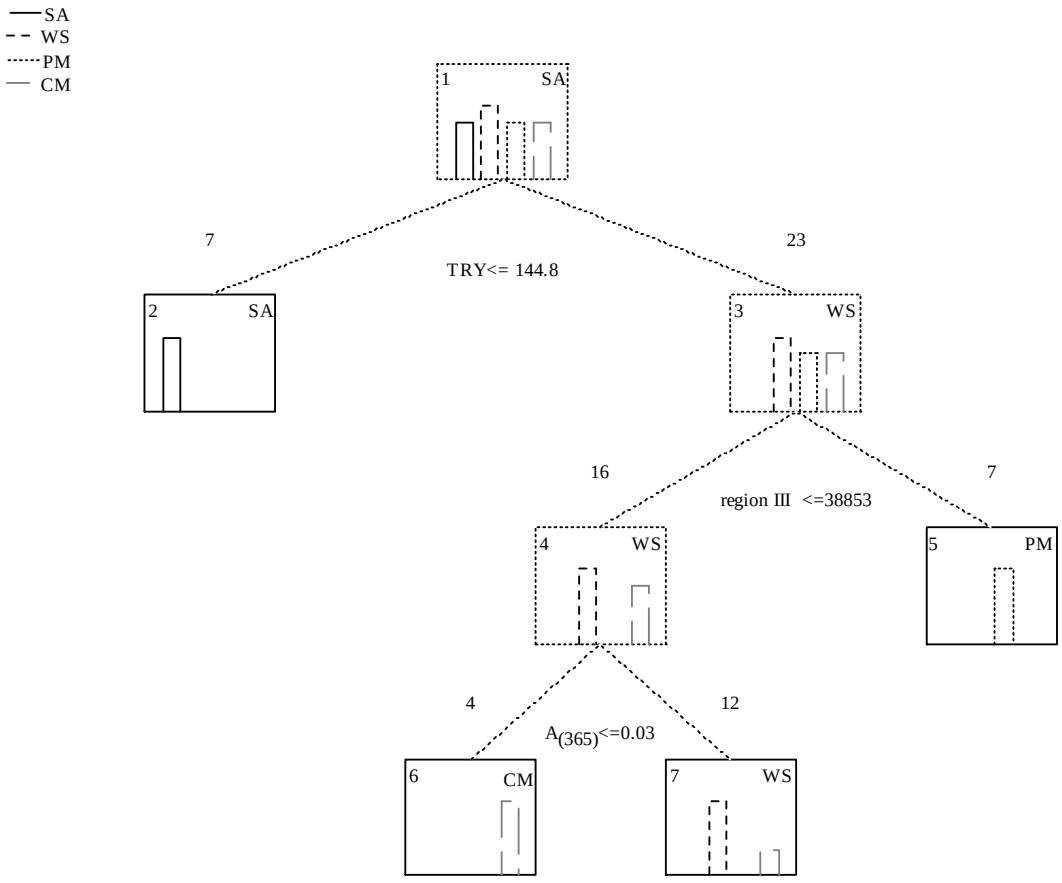

Figure 3.5 : Optimum tree for the fluorescence properties of DOC issued from the farm wastes during biodegradation for period P2 (8-56 days after incubation). Abbreviations are the integrated fluorescence intensities across tryptophan (TRY) zone and region III, spectral absorbance $A_{(365)}$.

First discriminator splitting the root node was the integrated fluorescence intensities across tryptophan (Try) zone which classified SA treatment from the farm wastes. Among the farm

waste treatments, integrated fluorescence intensities across region III discriminated PM treatment from CM and WS treatments. Spectral absorbance A_{365} discriminated WS treatment but CM was mostly misclassified with WS treatment.

Prediction accuracy assessment of optimum tree for the biodegradation period P2 was 66.7%. This tree had a high accuracy (100%) for predicting SA and WS treatments and fair accuracy (57.1%) for PM prediction but prediction accuracy for CM treatment was 0% as it misclassified with WS treatment (Table 3.5). During the biodegradation period P2, discrimination of PM treatment from cow manure was 100 % but 28.6% misclassified with WS treatment. The CM treatment was 100% misclassified with WS treatment while WS treatment is 100% correctly classified from the rest of the farm wastes treatments.

Table 3.5 : Confusion matrix of predicted versus observed treatment resulting from cross-validation procedure applied on optimum tree for period P2.

Predicted	Observed			
	SA	WS	PM	CM
	n = 7	n =9	n =7	n =7
SA n =8	**100%**	0%	14.28%	0%
WS n=18	0%	**100%**	28.57%	100%
PM n=4	0%	0%	**57.10%**	0%
CM n=0	0%	0%	0%	0%
Total accuracy rate (n =30)				66.67%

Discussion

Impact of farm wastes on DOM production during biodegradation

Significant higher DOM concentrations in the farm waste treatments throughout the incubation experiment confirm the impact of farm waste manuring on soil DOM concentrations. Previous studies had recognized similar trends of DOM in cultivated soil (Kalbitz et al., 2000; Shand and Coutts, 2006) as well as in the rivers draining farm waste fertilised catchments (Jardé et al., 2007a). In soil alone treatment, DOM peak on 15 days after incubation indicated the possible DOM release from dead microbial biomass that starved from the depletion of substrate.

Strong decrease in DOM concentrations in PM and CM treatments within 24 hours suggested the presence of a rapidly biodegradable fraction of DOM (23% to 41% decomposable soluble carbon in CM and PM respectively in our study) and this decrease could also be related to the preferential consumption of simple carbohydrate monomers, organic acids and protein fractions of DOM during initial phase of decomposition (Marschner and Kalbitz, 2003). DOM dynamics during both P1 and P2 periods suggested a more biodegradable DOM fraction in farm waste treatments compared to soil alone (Gregorich et al., 2003). DOM pool demonstrated stability against biodegradation up to 30 days in CM treatment and subsequent decline reflected its higher susceptibility to biodegradation compared to PM amended soil treatment after 30 days. In the end of experiment, significantly higher DOM in PM treatment compared to CM treatment ($p<0.05$) indicated higher DOM production potential of pig manures whereas others (Hunt and Ohno, 2007) found an opposite trend of increasing DOM concentration in the cow manure and decreasing in pig manure after decomposition. This reflects the variability of diet fiber contents that can have a great effect on wastes composition for a given type of animal (Shriver et al., 2003). Using only the DOM parameter, farm wastes were discriminated from soil alone with higher DOM concentrations and also PM discriminated from CM and WS in the start and only from CM in the end of biodegradation period.

Persistence of spectral indices of soil and farm waste using PCA analysis

Temporal variability of fluorescence properties of DOM released from PM and soil alone was detected using PCA analysis (Figure 3.3). Therefore spectral indices are not persistent in PM and soil alone treatments. From qualitative point of view, strong similarities were observed in DOM fluorescence indices from CM and WS soil extracts which reflects the same spectral composition of DOM. As a consequence, certain persistence of fluorescence signature is observed (Figure 3.3). After cancelling out the carbon rate differences among the farm waste input (4g C / kg dry soil) and DOM differences among all the treatments during fluorescence measurements (fluorescence intensities normalised at 5 mg L^{-1}), the distinction between soil and farm wastes along the PCA axes during both study periods reflected the DOM quality differences. PM could be discriminated from WS and CM treatments by biochemical integrated fluorescence across region IV and TRY zone during P1. This suggests heterogeneity in DOM quality among the farm wastes. The data illustrate the wide variation and dissimilar effects of decomposition on TRY zone and region IV among the farm wastes during period P1. Temporal shift of PM treatment from biochemical (region IV and TRY zone) to geochemical fluorescence (HL, FL, and geochemical region) properties from period

P1 to P2 confirm the biodegradation of biochemical fluorescence indices in P1. However, during period P2, presence of more condensed aromatic structures and humified fluorescent fraction of DOM in the PM treatment indicate the persistence in the biodegradation environment and can be related to high organic matter degradation. For SA treatment during P2, strong negative correlation between HIX and ratios TRY, TRY:HL, TRY:FL, bio:geo, IV:V suggests that it can be discriminated with higher HIX and lower ratios of TRY:HL, TRY:FL, bio:geo, IV:V during mid term biodegradation from farm wastes. Strong structural changes of DOM must have occurred during degradation process, leading to higher increase in carboxylic groups in soil and preferential consumption of protein contents that result in higher humification and as a consequence, HIX discriminates soil from the farm wastes. Zsolnay (Zsolnay et al., 1999) also calculated humification index to differentiate the microbial cell lysis products and more humified DOM. Biodegradation effects on DOM are not coherent among farm wastes studied as we observe an evolutionary trend in fluorescence indices of PM but lack of significant evolution of DOM fluorescence properties in CM. This reflects the variation in the chemical properties of feed materials as well as different digestive process of the animals (Hunt and Ohno, 2007).

Potential of CART analysis for discriminating the farm wastes during biodegradation

CART tree approach (Breiman et al., 1984) enabled to find the best predictor/tracer of various farm waste treatments during two biodegradation study periods P1 and P2. We hypothesize that farm wastes contamination can be short term (recent contact of farm wastes with water, 0-7 days) or mid term (through runoff from farm waste spreading on cultivated hillslopes after one or two months). Our results suggest that short term farm wastes pollution can be traced with higher ratio TRY:HL values (split value ≥0.013 RU) and average farm wastes pollution with higher TRY zone values (split value ≥144.8 RU) and qualify as potential tracers of farm wastes. Among the farm wastes treatments, FL zone is ranked as the most discriminant predictors of PM during period P1 and FL zone shows its positive correlation with biochemical region IV (r, 0.77) which suggest that FL zone and region IV can trace fluorescent fraction of PM during P1. Region III during period P2 is the best predictor of PM and its weaker correlation (r, 0.17) with TRY confirm the degradation of biochemical fluorescent fraction of PM. It also suggests that fluorescent fraction of PM treatment get more humified as we observe that region III discriminate the PM treatment during period P2. Spectral absorbance A_{365} qualifies as a potential tracer of wheat straw during period P2 which

identify the increasing chromomorphic fraction of DOM during wheat straw biodegradation. The ratio III:V is suggested as the only discriminator of WS which separates from CM treatment with 60% classification success. Misclassification rate of cow manure with wheat straw during both periods of biodegradation indicate the presence of common substrate quality i.e. residues of WS in CM treatment. The potential of CART analysis success for predicting the farm wastes treatments as well as soil alone was estimated by cross validation to be globally of 63% and 66% for both periods P1 and P2 respectively. We also tested the performance of CART analysis by using the same fluorescence properties of DOM from three incubated soil samples (test sample) (similar type of soil as used in current study) along with the dataset of period P1. CART tree correctly classified the test samples with soil with the same variable of TRY:HL. During period P2, we obtained globally the same tree structure but tree was less complex, easier to interpret as compared to the tree in period P1. Classification success for SA treatment (91% and 100% for P1 and P2 respectively) suggests the compositional differences in soil DOM compared to farm wastes.

Fluorescence spectroscopic characterisation in combination with PCA analysis reflected the degradation of biochemical fluorescence indices during short term contamination in PM and shifted towards geochemical integral intensities in mid term pollution with more condensed and humified geochemical structures of fulvic like, humic like substances which could persist in the degradation environment. CART analysis enabled us to trace farm waste contamination by considering stepwise the most discriminant variable selection and complexity reduction. Farm wastes were discriminated from soil alone with ratio TRY:HL and TRY zone during short and mid term pollution with prediction success of 90% and 100% respectively. Pig manure waste discriminated from cow manure and wheat straw by FL zone and region III with prediction accuracy of 72.7% and 57.1% respectively. Wheat straw classified from cow manure by A_{365} with 100% accuracy rate during P2. However, cow manure was generally found misclassified with wheat straw due to common substrate quality. This investigation underlines the potential of 3 dimensional excitation emission fluorescence spectroscopy in combination with CART analysis as a non-destructive innovative method for monitoring farm waste dissolved organic matter contamination.

This method was tested as an alternative method to PARAFAC with simple fluorescence index based on regional integration procedure. PARAFAC is a robust method which is very efficient in obtaining spectral images of DOM components and accounts for physical phenomena i.e. the lack of distinctly separated spectral areas and often-observed overlapping of emission peaks components isolated via PARAFAC.”

CART analysis is found useful as it extracts the most salient information from the large dataset and also gives misclassification probability for the classifier. CART tree procedure also gives easily interpreted information regarding the predictive structure of the data. However, potential of CART approach for discrimination of DOM has to be tested by another dataset with different type of soils and animal wastes.

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CHAPTER 4

Chapter 4

Impact of long term pig slurry or cow manure amendments on fluorescent dissolved organic matter properties

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Abstract

Most of the agricultural soils receive farm manures application for a long period of time, as organic amendments serve as valuable nutrients resource for crops and seem good alternative of chemical fertilizer. However, dissolved fraction of organic matter added by farm manures can pose water quality problems through diffused pollution at catchment scale. By applying fluorescence spectroscopy, we investigated long term impact on fluorescent dissolved organic matter composition of pig slurry and cow manure amendments in comparison with mineral fertilized soil. Two experimental fields located in Brittany (Western France) were considered with controlled agronomic dose applications of mineral, cow and pig slurry for a period of 7 years on a soil with 2.5% of organic C (Kerguehennec site) and of mineral and pig slurry during 14 years on a soil with (0.9% C) (Champ Noël site). Sampling was done one year after the last soil amendments. Pig slurry had a significant impact on the biochemical fluorescent fraction of dissolved organic matter (DOM) materials. Integral fluorescence intensities in the biochemical (bio) region, tryptophan zone and ratio tryptophan:(humic-like:fulvic-like) were qualified as the fluorescence discriminators of pig slurry in two types of soils fertilized for a long period of time under monoculture and polyculture cropping system. The difference was higher in soil with low organic matter content. Ratios biochemical to geochemical (ratio bio:geo) can be also a discriminant of pig slurry amendments in the soil with lower native soil carbon (0.9% C) at Champ Noël site. Supply of cow manure at agronomic dose does not significantly modify the fluorescence properties of soil DOM compare to mineral fertilization in the soil with 2.5% of organic C.

Key words: pig slurry, cow manure soil amendments, fluorescence properties, dissolved organic matter composition, cropping system

Introduction

Background level of stable organic matter in cultivated soils poses difficulty to assess the changes induced in soil organic matter by short term land management practices (Gregorich et al., 1994). But dissolved fractions of organic matter respond rapidly to changes in carbon supply (Bol et al., 2003) and therefore, can enable us to study the impact of management practices (organic or mineral fertilization) and cropping systems on dissolved organic matter (DOM) composition in cultivated soils. However, changes in DOM upon management practices are generally of short duration (Rochette et al., 2000) and the long term effects are more related to vegetation type and to the amount of plant residues returned to the soil (Chantigny, 2003).

In soils, crop residues undergo the aerobic degradation via enzymatic oxidation and depolymerisation of tissue components, resulting in initial formation of progressively smaller and more soluble molecules (Wershaw et al., 1999). A fraction of this carbon pool subsequently undergoes enzymatic mediated polymerization such that total soluble C pool represents a continuum of substances ranging from little modified plant oligosaccharides through recalcitrant lignin derived materials to fulvic like microbial resynthesis products.

Aliphatic, aromatic and amino acids in soluble pool of carbon are receiving attention as they are probably the building blocks of fulvic and humic like materials and play an important role in plant nutrient uptake, mineral weathering (Raulund-Rasmussen et al., 1998) and soil genesis. Distribution of these organic acids in the soil is largely influenced by vegetation, soil moisture level, clay contents, microbial activity (Flessa et al., 2000) and in agricultural systems, by the management practices like organic fertilization (Bolan et al., 1994). A great proportion of smaller molecules like fulvic acid, hydrophilic acids, carbohydrates and amino acids are present in agricultural soils (Delprat et al., 1997; Leinweber et al., 2001). Recent research on dissolved organic matter has focussed on its role as an immediately available carbon resource from decaying plant litter, its leaching through soils as a result of pedogenic process and its subsequent impact on the ground and stream water quality (Qualls and Haines, 1992; Christ and David, 1996; Qualls et al., 2000).

At the watershed level, Cronan et al. (1999) have shown the decreasing high molecular weight DOM molecules in streams with increasing proportion of agricultural land. In cultivated soils, increasing management intensity has been linked to the decreasing DOM concentrations and increased humification value of DOM (Kalbitz, 2001). Phenols, lignin polymers as well as

nitrogen containing aromatic compounds are suggested as the ecological indicators to link the topsoil effects on adjacent surface and ground waters (Leinweber et al., 2001)

Crop species can influence the amount and the nature of C input to the soil (Xu and Juma, 1993; Zsolnay, 1996) and crop rotations in agricultural soils may influence DOM concentration from year to year depending upon the changes in soil moisture, temperature, precipitation as well as in situ rhizodeposition (Campbell et al., 1999a; Campbell et al., 1999b). During two consecutive seasons, higher water extractable organic matter concentration in the top 20 cm of silty clay loam and clay loam type of soils were observed under legume than under gramineae species which also reflected different root exudation pattern among crop species (Chantigny et al., 1997). Overall, the existing literature suggests that in agricultural soils, plant species influences DOM production. But the question remains to be answered how plant species influences DOM concentration and composition.

Inorganic nitrogen fertilization has not been found to significantly influence the DOM production in agricultural soil (Zsolnay and Görlitz, 1994). In a long term study (16 yr), DOM production remained unchanged in chronic nitrogen fertilized plots (McDowell et al., 2004). In other studies, nitrogen fertilizers favour the production of DOM from biodegradation of solid organic matter (Guggenberger et al., 1994; Kalbitz et al., 2000). While comparing various cropping systems with or without nitrogen fertilization, increase in water extractable organic matter has been attributed to a greater crop residue input in fertilized soils than in unfertilized soils (Campbell et al., 1999a; Campbell et al., 1999b).

Among the organic fertilization practices, pig slurry cause a rapid increase in dissolved organic matter during first weeks of its amendment but its effect on soil microbial biomass for 19 consecutive years not remain long lasting (Rochette et al., 2000) . Pig slurry amendment cause rapid increase of soil microbial biomass that last for at least 4 months which can coincides with the extractable carbon concentrations. The dairy slurry derived carbon (labile) has been observed from the liquid phase during 0-48 hours of slurry application to grassland soil while in the second phase (beyond 48 hours), the slurry derived carbon is from less mobile particulate carbon (Bol et al., 2003). But in five consecutive years of pig slurry amended soil, increase in total organic carbon and water soluble organic carbon was non significant (Hernández et al., 2007) However, DOM production remains significant in pig slurry, cow manure and wheat crop residues soil treatments in an incubation study conducted in the laboratory for two months duration (Bilal et al., accepted).

Angers et al. (2006) have shown that there is little impact of dairy slurry and solid manures on the water extractable carbon of the soil in two consecutive years of application in silage corn

field. However, moderate impact on total organic carbon (6.5% increase) and microbial biomass (>25%) has been observed in long term (9 years) application of pig slurry (Dambreville et al., 2006). Chantigny (2002a) has observed that pig slurry and alfalfa accelerate the soil microbial activities more than cattle manure and maize crop as well as related these differences to the ratio of lignin to nitrogen contents of the various amendments. Studies have demonstrated that organic amendments can increase the production of dissolved organic carbon over two years (Zsolnay and Görlitz, 1994). In an incubation study (70 days), Kirchmann and Lundvall (1999) has observed an evolution of pig slurry carbon by 65 % in comparison to anaerobically fermented pig slurry (48%) and cattle slurry (42%).

Type of organic amendments can have impact on soil DOM composition. DOM from pig slurry rapidly decompose during first week of its application and the second linear phase of decomposition, probably involved more recalcitrant materials and it also cause rapid increase in microbial biomass (Rochette et al., 2000). Still, developments of analytical approaches are needed to provide the insights on the DOM composition in long-term soil amendments and land use change.

Recent developments in fluorescence spectroscopy have enabled to collect the fluorescence intensity data across a wide range of excitation and emission wavelengths. In the river systems, different fluorophores like tryptophan, tyrosine and humic like and fulvic like have been detected. Baker (2002) has identified animal wastes with higher protein like intensities and found higher tryptophan:fulvic/humic like ratios for animal wastes compared to stream waters. Naden et al. (2009) have demonstrated the relevance of fluorescence as an indicator of cow slurry in diffuse agricultural pollution by way of higher tryptophan:fulvic/humic like ratios. Hernandez et al. (2007) have also observed partial incorporation of fulvic acids fractions from pig slurry into native soil fulvic acids. In an incubation study, we have observed a temporal evolution of fluorescence intensities from biochemical (proteins like) to geochemical (fulvic and humic like) fluorescence (Bilal et al., accepted). Farm wastes could be discriminated from soil alone through higher tryptophan: humic like fluorescence ratios after one week and tryptophan zone after two months.

In the presented study, fluorescent tracers of farming wastes in DOM are measured in soils receiving inorganic or organic fertilisation since more than seven years in two different pedoclimatic situations. Fluorescent tracers were analysed in two soil types with different crop rotation because, these factors strongly influence the DOM production and composition.

Fluorescent tracers of farming waste recycling on soils are researched one year after last farming waste supply in soils which have been submitted to long term (more than 7 years)

supply. However, it will be difficult to assess the impact of 7 years compared to one year effect.

The study aims at identifying the long term (minimum one year) impact of farming wastes amendments on the properties of fluorescent dissolved organic matter (DOM) in two types of soils (i) one soil with less than 1% of C under corn monoculture (ii) the second one with 2.5% of C under polyculture cropping system.

Material and methods

Experimental fields and sampling

The experimental fields of Champ-Noël and Kerguehennec used in the study were located in Brittany (in western France).

Kerguehennec site

This experimental field was established in 2000 and was located close to Bignan, France (47° 52′ N; 2° 46′ W). Soil texture was loamy soils (clay = 17%, silt = 46%, sand = 37%) developed on alterite micaschist. Soil depth varied between 60cm to 80cm with total organic carbon content in the Ap horizon of 2.5%. Different crops were grown in rotation: canola seeds- corn- wheat. Pig slurry and cow manure have been in practice once a year in spring since 2000. Three plots were sampled i) reference (labelled KM) which receive only agronomic dose of a mineral fertilizer (ammonium nitrate), ii) a pig slurry amended specifically with pig slurry labelled (KPS) and a specific dairy manure amended (KCM). The agronomic doses of organic manures were calculated according to the nitrogen requirement of crop. Pig slurry represents a mean load of 1.3 t of OC ha^{-1} $year^{-1}$ and 2.8 t of OC ha^{-1} $year^{-1}$ for dairy manure. Soil samples for this study were collected in March 2007, one year after the last fertilization.

Champ-Noël site

This experimental field was established in 1993 and was located near to Rennes, France (48° 7′ N; 1° 40′ E). Soil texture was silt loam soils (clay = 16%, silt = 70%, sand = 14%) developed on alterite micaschist. Soil depth varied between 75cm to 1m with 0.9% of total organic carbon content in the Ap horizon. The plot sampled remained under continuous cultivation of corn crop since 1993. Agronomic doses of pig slurry have been in practice once a year in spring since 1993 on the experimental plot. Pig slurry dose on plot X (labelled

CNPS) had a mean load of 0.6 t of organic carbon (OC) ha^{-1} $year^{-1}$ and was calculated on the basis of N requirement by maize crop. A second untreated plot was used as control soil to study and quantify the impact of pig slurry application. On this control soil (CNM), recommended dose of ammonium nitrate commercial fertilizer was applied at the rate of 110 kg ha^{-1} $year^{-1}$ N-NH_4NO_3. Soil samples were collected in March 2007, one year after the last fertilization.

Extraction of microbial biomass

Fumigation extraction method (Vance et al., 1987) was used for the estimation of microbial biomass using 0.025-M solution of K2SO4 to extract relatively labile organic carbon from the fumigated and non fumigated samples. To estimate the microbial biomass, organic carbon extracted in the non-fumigated samples was subtracted from the organic carbon extracted in the fumigated samples and expressed as g C Kg^{-1} dry soil. Total organic C contents were determined by elemental analyser (Flash EA 1112, Thermofinningan, Milan, Italy). For microbial biomass determination at Champ Noël and Kerguehennec sites, soil samples were taken 7 months later of farm manures amendments in October 2006.

Sample preparation and DOM extraction

On each site, representative samples were obtained by gathering and mixing of 8 samples taken in the 0-20 cm soil depth and sieved at 2 mm. DOM extracts were obtained with 1:1 ultra pure water to soil ratio. Soil water suspensions were shaken mechanically on orbital shaker for 3h and then centrifuged at 4000 rpm for 20 minutes and filtered through 0.7 and 0.22 µm nitrocellulose filters. To avoid any contamination, all the filters were rinsed with ultra pure water before vacuum filtration and dried overnight. Chemical analysis was done on one replicate of soil water suspension.

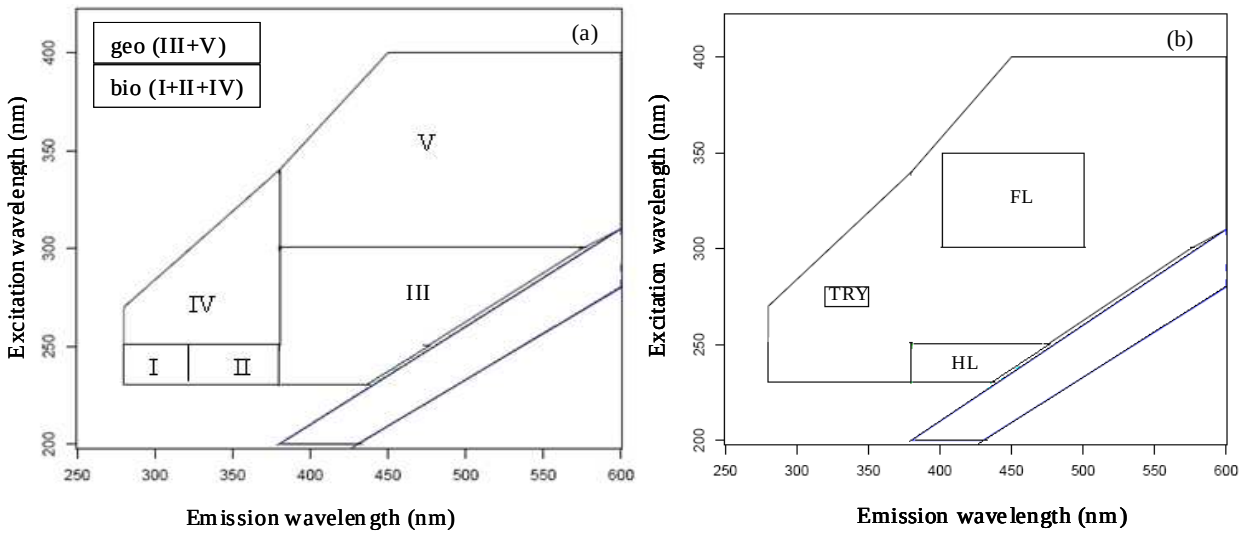
Chemical Analysis

Dissolved organic carbon (DOC) in soil solution extracts was measured on a Shimadzu TOC 5050 A total carbon analyzer. Accuracy on DOC measurements was ±5%, based on repeated measurements of standard solutions (K-phtalate). pH was determined on 20-ml filtered water samples using a digital pH-meter (WTW) calibrated with buffers (WTW) of pH 4 and 7. UV-Visible absorbance was measured on a Perkin Elmer Lambda 20 UV-Visible spectrophotometer across 200-600 nm excitation wavelengths range with data interval 0.5nm, slit width 2 nm and scan speed 120 nm/min.

Fluorescence measurements of DOM were performed using a Perkin-Elmer LS-55B luminescence spectrometer. The spectrophotometer uses a xenon excitation source and slits were set to 5 nm for both excitation and emission. To obtain excitation-emission matrix spectra, excitation wavelengths were incremented from 200 to 425 nm at steps of 5 nm and emission was detected from 250 to 600 nm with a 0.5 nm step. Scan speed was set at 1500 nm/min, yielding an EEM in 22 minutes with 45 total scans. To minimise the temperature effect, samples were allowed to equilibrate with room temperature (20±2°C) prior to fluorescence analysis. A Raman normalised integrated EEM spectrum of ultra pure water was subtracted from the data sample to eliminate the water Raman scatter peak. To eliminate the second order Raleigh light scattering, excitation and emission cutoff filters were applied at 230-310 nm and 380-600 nm respectively on the lower side of three dimensional plots (Figure 4.1). Inner filter effects were removed with the formula of (Ohno, 2002). To maintain the consistency of measurements and standardise the whole fluorescence dataset, all the integrated fluorescence intensities were normalized to average Raman emission intensity units of 30 for ultra pure water samples at excitation and emission wavelengths of 350 nm and 397 nm respectively. The fluorescence dataset presented in this study was normalised at 5 mg L^{-1} DOC.

Regional integration of excitation emission matrix (EEM)

An internal program was developed in the laboratory using the R® software (http://www.r-project.org) for the integration of fluorescence intensities across the whole EEM landscape Figure 4.1. Here peaks at shorter wavelengths (<250 nm) and shorter emission wavelengths (<380 nm) are related to simple aromatic proteins such as tyrosine and tryptophan (Regions I and II). Peaks at intermediate excitation wavelengths (250–340 nm) and shorter emission wavelengths (<380 nm) are related to soluble microbial by-product-like material (Region IV) while peaks located at the excitation wavelengths (230–300 nm) and the emission wavelengths (380-575 nm) represent humic acid-like substances (Region III). Peaks at longer excitation wavelengths (>300 nm) and longer emission wavelengths (>380 nm) are related to fulvic acid-like organics (Region V). With this technique, EEM was divided into biochemical (bio) (I, II, IV) and geochemical (geo) (III, V) fluorescent regions (Figure 4.1a) (Table 4.1) and three peak intensity zones of tryptophan (TRY), fulvic like (FL) and humic like (HL) fluorescence (Figure 4.1b). 45 spectral loadings were used to reproduce three-dimensional plots of fluorescence intensity as a function of excitation and emission wavelengths.



Erreur ! Des objets ne peuvent pas être créés à partir des codes de champs de mise en forme.

Figure 4.1 : Integration of fluorescence intensities across biochemical and geochemical regions (a) and Tryptophan (TRY), Fulvic like (FL) and humic like (HL) zones (b).

Table 4.1 : Definition of excitation (Ex) - emission (Em) wavelengths (nm) ranges for each region and zones

	Ex (nm)	Em (nm)		Ex (nm)	Em (nm)
Regions			**Zones**		
region I	230-250	280-330	Tryptophan	270-280	320-350
region II	230-250	330-380	Fulvic like	300-350	400-500
region III	230-300	380-575	Humic like	230-250	360-420
region IV	250-340	280-380			
region V	300-400	380-600			
region bio	230-340	280-380			
region geo	230-400	380-600			

Statistical analysis

Numerous replicates of fluorescent measurement on soil extraction in previous studies conducted with the same apparatus showed 5% coefficient of variation (unpublished data). Therefore we imposed this dispersion parameter to simulate three replicates per treatment in order to integrate potential analytical errors in the treatment comparison. We did not take into

account the variability arising from sampling. Statistical analysis of the treatment means were run by one way ANOVA with STATISTICA 7.1 (Statsoft).

For the coefficient of variation (CV_R) of the ratio X/Y of two variables, we applied the approximation suggested by Holmes and Buher (2007): **Erreur ! Des objets ne peuvent pas être créés à partir des codes de champs de mise en forme.**, where CV_X and CV_Y are CVs of X and Y variables respectively.

Results

The study aims at identifying the impact of farming wastes amendments on the properties of fluorescent dissolved organic matter (DOM) in two types of soils (i) one having low soil carbon level under corn monoculture (ii) the second with high carbon level under polyculture cropping system.

Dissolved organic carbon differences between treatments at the two sites

At Kerguehennec site, there was no significant difference of Dissolved Organic Carbon concentration [DOC] on pig slurry amended soil with respect to mineral and cow manure amended soils (Figure 4.2). [DOC] was statistically lower ($p<0.05$) in cow manure amended soil than mineral fertilized plot. At Champ Noël site, pig slurry did not show any DOC difference to mineral fertilizer soil. However, Champ Noël soil showed significant higher [DOC] concentration level compared to Kerguehennec soil.

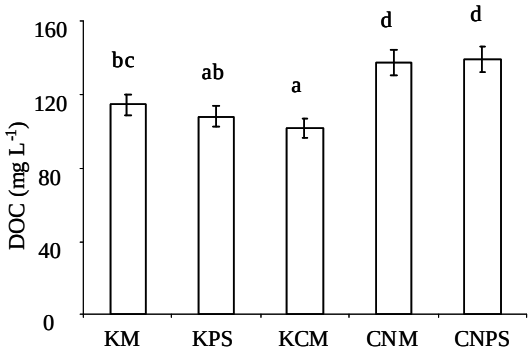


Figure 4.2 : Dissolved organic carbon concentration at Kerguehennec (K) and Champ Noël (CN) under mineral (M), pig slurry (PS) and cow manure (CM) soil application. Confidence intervals were estimated assuming coefficient of variation of 5%. Bars with the same letter indicate non significant mean differences (one way ANOVA) ($p<0.05$).

Biochemical and geochemical fluorescence of dissolved organic matter

At Kerguehennec site, significant higher bio fluorescence intensities were observed in pig slurry (PS) amended field (KPS) (3889 RU) compared to cow manure (CM) (3462 RU) and mineral fertilized fields (3451 RU) (Figure 4.3a). Geo fluorescence was significantly higher in pig slurry amended plot (43530 RU) compared to mineral fertilized plot (38625 RU) (Figure 4.3b). However, impact of CM (40392 RU) on geo fluorescence was not significant in comparison with mineral and PS amended treatments.

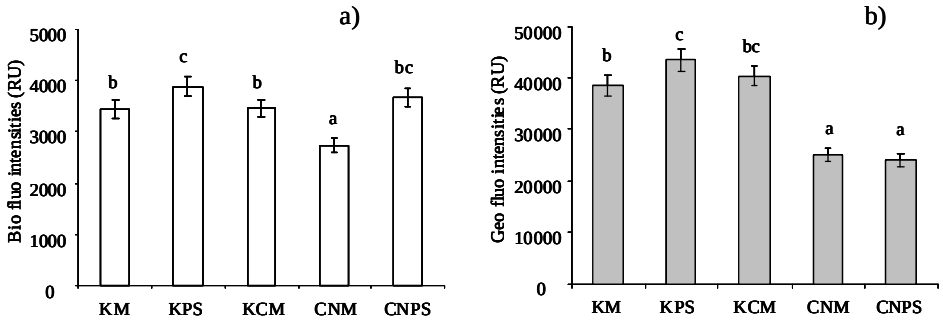


Figure 4.3 : Biochemical (bio) (a) and geochemical (geo) (b) fluorescence intensities at Kerguehennec (K) and Champ Noël (CN) with mineral (M), pig slurry (PS) and cow manure (CM) amendments. Bars with the same letter indicate non significant mean differences (one way ANOVA) ($p<0.05$).

Similarly at Champ Noël, pig slurry amended plot (CNPS) (3680 RU) showed significant higher bio fluorescence compared to mineral fertilized plot (CNM) (2746 RU). But there was no difference of geo fluorescence between CNM and CNPS treatments as well as in bio fluorescence at CN and Kerguehennec sites under pig slurry amendment.

Ratios in regions of EEM

At Kerguehennec site, ratios bio:geo did not discriminated pig slurry and cow manure amended plots from mineral fertilized plot. At Champ Noël site, pig slurry modality showed significant higher bio:geo (0.19) ratio compared to mineral fertilized plots with 0.14 (Figure 4.3). Ratios bio:geo was significantly higher at Champ Noël (0.14 to 0.19 in CNM and CNPS respectively) compared to Kerguehennec site in all the modalities of soil amendments (0.12 in KM, KCM, KPS treatments) (Figure 4.4a).

Significant higher fluorescence intensities in region III vs V were measured in Kerguehennec site, KM (19832,18792 RU), KPS (20274,20117 RU), KCM (22139,21392 RU) compared to CNM (11424,13672 RU) and CNPS (11303,12751 RU) at Champ Noël site (Figure 4.4b).

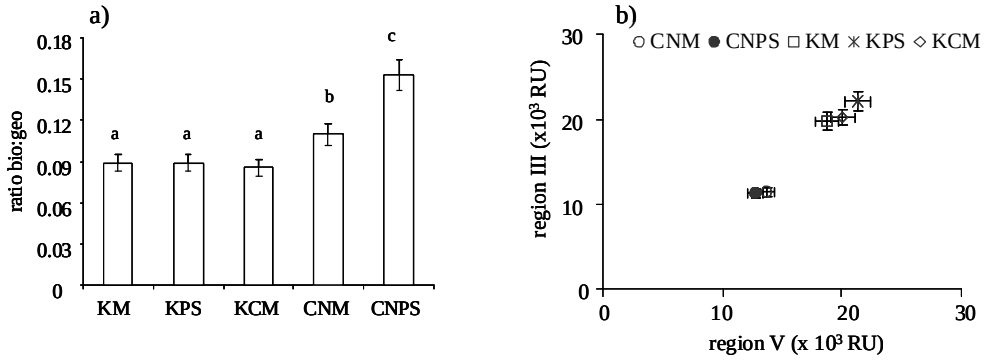


Figure 4.4 : Ratio of biochemical (bio) to geochemical (geo) fluorescence (ratio bio:geo) (a), integral fluorescence intensities of region III vs V (b) at Kerguehennec (K) and Champ Noël (CN) sites with mineral (M), pig slurry (PS) and cow manure (CM) amendments. Confidence intervals were estimated assuming coefficient of variation of 5%. Bars with the same letter indicate non significant mean differences (one way ANOVA) ($p<0.05$).

Fluorescence in the zones of EEM.

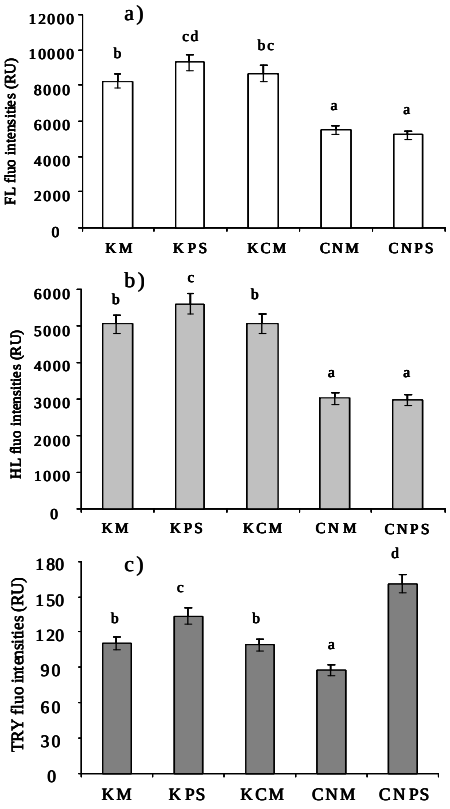


Figure 4.5 : Fluorescence intensities across fulvic like (FL) (a), humic like (HL) (b) and TRY (c) areas at Kerguehennec (K) and Champ Noël (CN) sites with mineral (M), pig slurry (PS) and cow manure (CM) amendments. Confidence intervals were estimated assuming coefficient of variation of 5%. Bars with the same letter indicate non significant mean differences (one way ANOVA) ($p<0.05$).

At Kerguehennec site, FL fluorescence intensities were statistically higher in PS fertilized (9284 RU) compared to mineral plot (8263 RU) ($p<0.05$). But FL fluorescence in cow manure treatment was not different from mineral and pig slurry treatment (Figure 5.5a and 5.5b). HL fluorescence intensities were significantly higher in PS amended plot (5585 RU) compared to mineral (5049 RU) and CM amended soil (5061 RU). At Champ Noël site, in pig slurry treatment, FL and HL fluorescence intensities were not significantly different (5210 RU and 2971 RU respectively) from mineral fertilized plot (5496RU and 3025 RU respectively). However, Kerguehennec soil was differentiated with significant higher fulvic like (FL) and humic like (HL) fluorescence intensities compared to Champ Noël soil.

At Kerguehennec site, tryptophan fluorescence intensities (TRY) were significantly higher in PS amended soil (133 RU) compared to mineral (110 RU) and CM fertilized soil (110) (Figure 5.5c). At Champ Noël site, impact of pig slurry amendment was prominent with significantly higher TRY fluorescence (161 RU) compared to mineral fertilized plots (87 RU). Contrary to FL and HL fluorescence intensities, Champ Noël soil amended with pig slurry showed significantly higher TRY fluorescence than Kerguehennec soil under pig slurry application.

Ratios in the zones of EEM

At Kerguehennec site, TRY:HL ratio is not significantly higher in the three modalities. At Champ Noël site, ratio TRY:HL discriminated the pig slurry treatment (0.054) from mineral fertilized plot (0.028) (Figure 46a).

At Kerguehennec site, ratio TRY:(HL:FL) discriminated the pig slurry amendment (221) from mineral and cow manure fertilized fields with values 180 and 186 respectively. However ratio TRY:(HL:FL) was unable to discriminate the cow manure and mineral fertilizer amended fields. At Champ Noël site, ratio TRY:(HL:FL) significantly discriminated the pig slurry modality (283) from the mineral fertilized plot (158) (Figure 4.6b). Overall, TRY:(HL:FL) was found good discriminants of fluorescent DOM composition in the pig slurry amended fields at both sites.

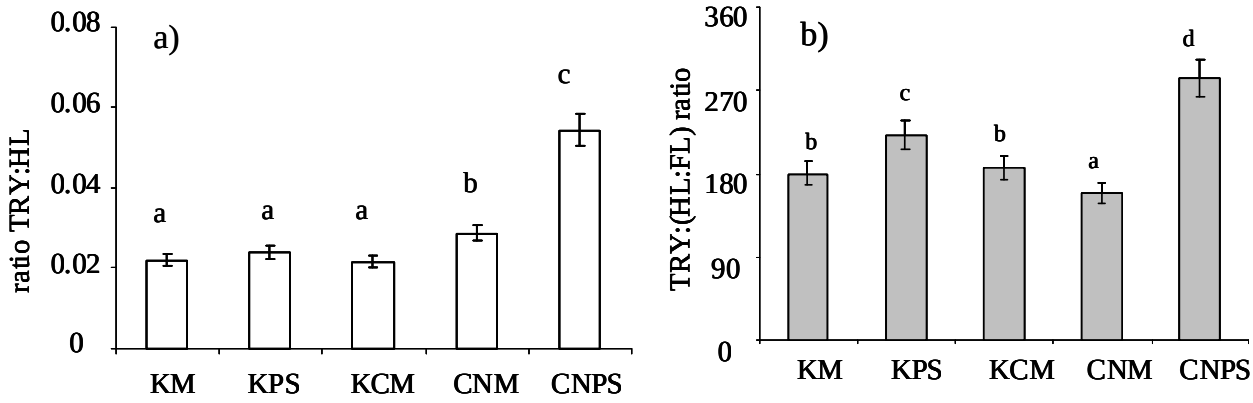


Figure 4.6 : Ratio of tryptophan (TRY) to fulvic like (FL) (a), humic like to fulvic like (HL:FL) (b) ratio at Kerguehennec (K) and Champ Noël (CN) sites with mineral (M), pig slurry (PS) and cow manure (CM) amendments. Confidence intervals are estimated assuming coefficient of variation of 5%. Bars with the same letter indicate non significant mean differences (one way ANOVA) ($p<0.05$).

Microbial biomass and organic carbon contents in two soils

Dambreville et al.,(2006) had observed significant impact of pig slurry amendment on the microbial biomass compared to control in top surface horizon at Champ Noël site (Table 4.2). The microbial biomass in mineral cultivated soil at Champ Noël site was significantly higher compared to mineral soil at Kerguehennec site.

Organic carbon contents were significantly lower at Champ Noël site than Kerguehennec site. At Champ Noel site, pig slurry amendment differentiated significantly from mineral soil. But Kerguehennec site, there was no significant difference in organic carbon contents among the mineral, pig slurry and cow manure amended treatments.

Table 4.2 : Microbial biomass and organic carbon contents

Site	Treatment	Date	Microbial biomasse ($mgC.kg^{-1}$soil)	C total ($g.kg^{-1}$)	Reference
Champ-Noël	Mineral	2002	144(4)^{a}	9.2 ^{a}	Dambreville et al. 2006
	Pig slurry	2002	239 (4)^{b}	9.8 ^{b}	
Kerguehennec	Mineral	2006	117(10)^{c}	20.3^{c}	Not published
	Pig Slurry		N.D	20.0^{c}	
	Cow manure		N.D	20.7^{c}	

Numbers in parentheses are standard errors (n=8)

Similar letters in the same column showed non significance difference ($p<0.05$)

N.D (not determined)

Discussion

Impact of farm wastes on DOM production

In the current study, the impact of farm wastes on DOM production in long term (14 years at CN and 7 years at Kerguehennec sites) field experiments under pig slurry and cow manure soil fertilization was investigated. The effects are observed one year after the last organic fertilization supply. No significant difference was measured in the soil water extractable DOC concentrations of the organic and mineral fertilized soils at Kerguehennec and Champ Noël sites. Results are in agreement with (Hernández et al., 2007) who show no increase in water soluble organic carbon in pig slurry amended soil for five consecutive years. While Rochette et al. (2000) observed rapid increase in dissolved organic matter during first week of pig slurry amendment. The dairy slurry derived carbon has been observed from the liquid phase during 0-48 hours of slurry application to grassland soil while in the second phase (beyond 48 hours), the slurry derived carbon is from less mobile particulate carbon (Bol et al., 2003). Moreover, Angers et al. (2006) have shown little impact of dairy slurry and solid manures on the water extractable carbon in the soil in two consecutive years of application in silage corn field. In the present study, as sampling was done one year later after farm wastes amendments, it was not possible to see the immediate impact of organic amendments on DOM production at each site. However, DOM production differences were significant between two sites of Kerguehennec and Champ Noël. The values of pH were not significantly different between the two sites (data not shown).

Inspite of higher background level of soil carbon (2.5%) at Kerguehennec site, lower DOM production is observed. Perhaps at Kerguehennec site, DOM released from the farm wastes amendments serves as readily available source for microbial biodegradation of higher return of crop residues. So the concentrations of DOC were expected to be lower at Kerguehennec site. Furthermore, microbial biodegradation reduce the DOM concentrations and the presence of aromatic rings in organic molecules can be adsorbed on the soil (McKnight et al., 1992) and leading to the compositional changes. The expected increase in DOM production is possible with climate change and higher return of litter input in forest soils (Kalbitz et al., 2007) and higher level of DOM can be aggravated in the farm wastes amendments in agricultural soils.

We hypothesize that continuous pig slurry and dairy manure amendments can impact the DOM composition and we applied fluorescence spectroscopy to search possible explanations

for the change of fluorescent DOM composition that can be resulted from farm applications under different cropping systems.

Impact of farm manures amendments on the fluorescent DOM properties

Absence of DOM concentration differences among the organic and mineral fertilized soils at both sites raises a question. Is there any change in fluorescent DOM composition by pig slurry and cow manure after 7 (Kerguehennec) to 14 (Champ Noël) years of specific cow manures or pig manure fertilization?

Research have demonstrated that livestock faeces typically comprises 15-25% proteins in wet manure (poultry, cattle and pigs and within this 3g/16g true protein N is tryptophan and tyrosine) (Day, 1977). Hence protein fluorescence is also expected. High values of TRY intensities were measured in extract of pig or cattle slurry (Baker, 2002). The fluorescence properties of farming wastes are characterized by strong bio and TRY intensity (results in chapter one of this thesis).

Biochemical (proteinacious) fluorescence was higher in pig slurry modality than in mineral at Champ Noël and from mineral or cow manure at Kerguehennec. TRY as well as TRY:(HL:FL) are ranked as pertinent indicators of pig slurry supply for both sites.

However, at Kerguehennec site, cow manure soil amendment was not discriminated from mineral amendments which reflect the biodegradation of fluorophores present in the cow manures. It can suggest that DOM materials in cow manures serve as readily available energy sources for microbial community in the soil and with the passage of time, degraded and becomes part of native soil carbon. Increase of the geochemical fluorescence intensities after addition of pig slurry was only observed in Kerguehennec site and not in Champ Noël. Even after 14 years of supply of organic matter in low OM content soil, the DOM fraction was not more humified than in a soil which received only mineral fertilization. But in a soil with higher OM content, the supply of pig slurry modifies the fluorescence properties of the DOM fraction and revealed a more humified DOM. So the quality of the DOM and perhaps its functions of pollutant transport or biodegradability are modified. The supply of cow manure has no impact on the DOM fluorescence properties, which is quite surprising since cow manure is supposed to modify the organic matter properties like with pig slurry. Studies on the modification of DOM properties of soil with different amendments are scarce. Many references have focused on soil organic matter composition (Plaza et al., 2003). Humic acid of soil organic matter after pig slurry amendment, or generally farming waste amendments, compared to control soil are characterized by higher contents of S- and N-containing groups

and polysaccharide components, lower organic free radical contents, a prevalent aliphatic character, and lower degrees or aromatic polycondensation, polymerization and humification (Brunetti et al., 2007).. On the other hand, during the maturation and stabilization of any organic amendment, organic matter mineralization and humification occur. In particular, the chemical, physico-chemical and spectroscopic characteristics tend to approach those typical of native soil humic substances which indicates the occurred partial decomposition of aliphatic, polypeptidic and polysaccharide-like components and increase of the degrees of aromatic ring polycondensation and polymerization.(Senesi et al., 1996).

However, the fact that the biochemical signature was maintained unexpectedly in both soils (elevated TRY) under pig slurry application since these labile products should be rapidly decomposed. This biochemical signature can result from the microbial activity which can be favoured in soil under organic amendment. Results of microbial biomass in mineral modality compare to pig slurry modality at CN site (Table 4.2) reflect that micro-fauna is more active under pig slurry amendment at Champ Noël site. But at Champ Noël site, Jarde et al; (2009) observed the significant impact of pig slurry on steroids persistence steroid after nine years of application which also suggest the persistence of biomarkers of pig origin.

Impact of soil type and cropping system on the fluorescent DOM composition

Now, we consider only the mineral modality of the two sites. Significantly higher fluorescence in bio and region IV compared to Champ Noël (CNM) site can be related to the rapid turn over of fresh crop residues of canola seed, corn and wheat in rotation at Kerguehennec soil as compared to monoculture corn crop. But in contrast, higher microbial biomass in CN than KM site reflects that microbial by products is more aromatic that released during the microbial degradation process of crop residues.

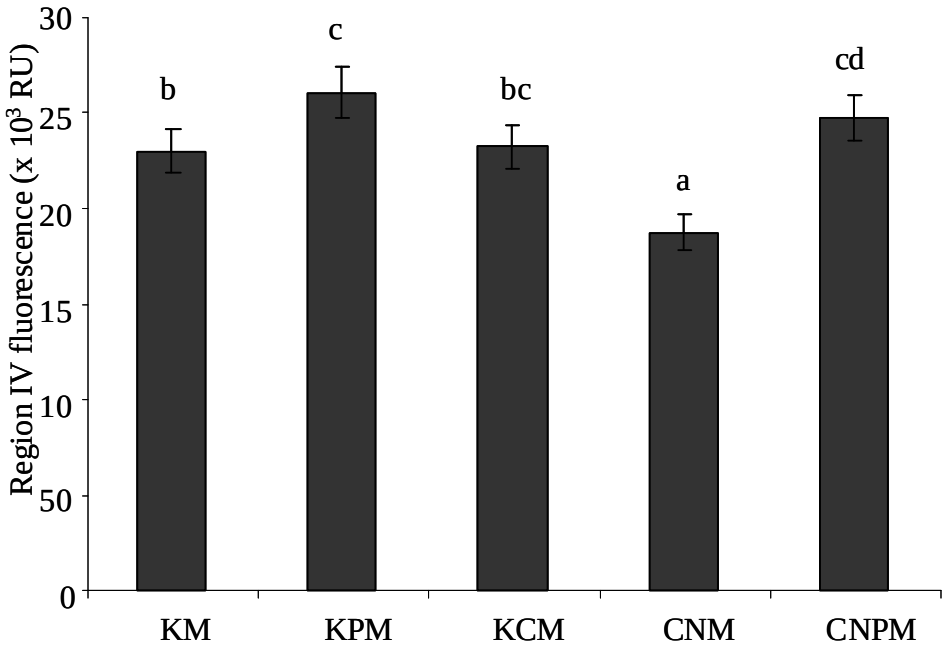


Figure 4.7 : Fluorescence intensities across in region IV (related to microbial activity) at Kerguehennec (K) and Champ Noël (CN) sites with mineral (M), pig slurry (PS) and cow manure (CM) amendments. Confidence intervals were estimated assuming coefficient of variation of 5%. Bars with the same letter indicate non significant mean differences (one way ANOVA) ($p<0.05$).

Furthermore, due to higher return of crop residues at Kerguehennec site, additional proteinacious material can accumulate in the Ap horizon which is susceptible to contribute to higher biochemical fluorescence. Distribution of these organic acids in the soil is largely influenced by vegetation, soil moisture level, clay contents, microbial activity (Flessa et al., 2000) and in agricultural systems, by the management practices like organic fertilization (Bolan et al., 1994).

At Kerguehennec site, geochemical (geo) fluorescence is significantly higher compared to Champ Noël mineral soil. It reflects that higher biomass generated at Kerguehennec soil from crops can release higher refractory compounds like lignin and carboxylic groups at different stages of degradation in the natural soil environment compared to CN mineral soil. It also reflects a difference in native organic matter composition. Humification is an ongoing process in which the polymerization of originally monomeric plant breakdown products and plant material decomposition leads to structural complexity of soluble carbon pool (Merritt and Erich, 2003) that can increase the geochemical fluorescence at Kerguehennec site.

At Kerguehennec site, native total organic carbon (2.5%) in loamy soils is higher compared to 0.9% at Champ Noël silt loam soils. This difference in organic content between sites can result in different DOM composition.

At CN site, although the quantity of pig slurry carbon at CN site was one half of that applied onto Kerguehennec site (0.6 t vs 1.3 t ha^{-1} $year^{-1}$) yet fluorescence properties of DOM substance are capable to discriminate the pig slurry amended soil compared to mineral fertilized fields. Moreover, integrated fluorescence intensities in the geochemical (geo) region and in the zones of FL, HL discriminated the two soil types with higher fluorescence intensities at Kerguehennec site than Champ Noël site.

Persistence of fluorescence tracers of pig slurry waste after soil amendments

Even one year after last farming waste application, our results demonstrate an obvious modification of the fluorescence properties of DOM on pig slurry treatment compared to mineral treatment with higher TRY, TRY:HL and bio:geo ratio in Champ Noël. In Kerguehenec, the difference between pig slurry treatment and mineral treatment is less important than CN site. This shift has been measured in soil receiving pig slurry since 7 years in Kerguehennec site and since 14 years in Champ Noel. The better discrimination between pig slurry and mineral modality observed in CN can be due to the cumulative effect rather than a soil type effect. Measurements on experimental devices with same period of supply should be carrying out to test this effect.

The results reflect that integrated fluorescence intensities in the biochemical (bio) region, TRY zone and the ratio TRY:(HL:FL) can be used as tracers of pig slurries in every type of soil under monoculture or polyculture cropping systems. The impact of cow manure soil amendment on the fluorescence properties of DOM does not persist and no longer remains in the soil environment and seems to be biodegraded.

Conclusion

Our results reflect the following conclusions:

In the low organic matter soil (0.9 % of C):

Fourteen years of pig slurry recycling on soils increase the soil organic matter compared to mineral fertilization but no effect on Dissolved Organic Carbon concentration is observed one year after the last spreading.

The DOM humic/fulvic-like fluorescence of pig slurry treatment is not significantly different from mineral fertilization.

However, biochemical fraction of fluorescent DOM is significantly increased and appears as a tracer of DOM produced in soil receiving pig slurry. TRY, biochemical region, ratio TRY:HL are the proposed fluorescent indices. These important biochemical fluorescence

characteristics could be due to higher microbial biomass or to preservation of proteinacious fluorescent markers in DOM.

In the highest organic matter soil (2.5% of C):

Seven years of pig slurry and cow manure recycling on soil do not modify the organic matter content of the soil and has no effect on Dissolved Organic Carbon concentration one year after the last spreading.

The biochemical fraction of fluorescent DOM is slightly modified after pig slurry recycling on soil but is not modified after cow manure recycling. TRY and ratio TRY(HL:FL) are higher on the pig slurry treatment.

The evidence of change in biochemical DOM remained unclear whether this modification of biochemical fluorescent DOM was due to the cumulative effect of long term recycling of pig slurry on soils or its the persistence of organic compounds after one year of spreading.

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CHAPTER 5

Chapter 5

Use of fluorescence to trace DOM sources in a headwater agricultural catchment

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Abstract

High Dissolved Organic Carbon concentration [DOC] in stream modifies the physical, biological and chemical quality of natural waters, particularly via the transport of mineral or organic pollutants in agricultural catchments. In headwater agricultural catchment, in French Brittany, Valley Bottom Wetlands (VBW) are the main contributors of dissolved organic matter (DOM) during storm events. Most of the area in VBW is intensively cultivated under maize, wheat crops or meadows with farming waste spreading in spring season or serve as intensively grazing pastures. In this study, the potential of EEM fluorescence spectroscopy for the determination of farming waste impact on the quality of DOM in the streams was studied. Recent studies showed that ratio tryptophane/fulvic-humic like can be a good tracer of DOM from animal waste. In headwater agricultural catchments, two sources of DOM can be transferred to the stream during storm events: DOM from soil and DOM from farming waste recently applied on soil or in intensively grazing pastures. 15 headwater catchments streams were studied during 2007. Land occupation (forest, agricultural surface), linear of hedges, and agricultural practices (organic fertilization, crop rotation) were characterized in the Valley Bottom Wetlands by remote detection analysis and farming survey. [DOC] was analyzed during three storm events between February and June 2007. EEM fluorescence spectroscopy with regional integration approach was carried out on water samples. EEM was divided into biochemical (bio) and geochemical (geo) fluorescent regions and the fluorescence were also integrated in reference wavelengths range of fluorophores of tryptophan (TRY), fulvic like (FL) and humic like (HL). The quantitative analysis included the integration of fluorescent volume beneath each region and zone. Ratios TRY:HL and bio:geo were also calculated. The fluorescence DOM quality differences, among three storm events, were analysed by principal component analysis (PCA) on the DOC normalised fluorescence dataset with R® software (package ade4). Ratio TRY:HL and bio:geo were always higher in one catchment with important maize production and cow manure fertilization in the Valley Bottom Wetlands. In all the other catchments, the DOM fluorescence properties during Storm S2 showed a major contribution of highly humified DOM probably due to flushing of soils. The DOM fluorescence properties shift towards less humified and higher ratio Bio/Geo or TRY/HL during S1 in six catchments suggested a contribution from DOM issued directly from recent farming wastes. During S3, a small increase of the ratio bio:geo and TRY/HL suggested also a transfer of DOM from farming waste but less pronounced than during S1. In four catchments,

the ratio were very low during all the events and these catchments were the less impacted by intensive agricultural practices in the Valley Bottom Wetlands. Results demonstrate that fluorescence spectroscopy, by coupling with regional integration approach, are capable to reveal chemical changes in the DOM quality and depict the anthropogenic loads versus contribution from soils on fresh water streams.

Key words : Agricultural headwater catchment, farm wastes supply, excitation emission fluorescence spectroscopy, land occupation, storm events

Introduction

Higher concentrations of Dissolved Organic Carbon [DOC] in streams modify the physical, biological and chemical quality of natural waters, particularly via the transport of mineral or organic pollutants in agricultural catchment (Muller et al., 2007; Pedrot et al., 2008). Water treatment is becoming increasingly complex, and the formation of trihalomethane is enhanced (Galapate et al., 1999). In Brittany, 80% of the water resources come from superficial resources (river), so the transfer of DOM (dissolved Organic Matter) is an important water quality concern.

A high proportion of the [DOC] is exported during storm events in small catchments (Grieve, 1984; Hinton et al., 1997; Dalzell et al., 2005; 2007). High [DOC] exports are typically associated with near-surface hydrological flow paths that intercept DOC-rich organic horizons (Boyer et al., 1997). Thus, wetland and riparian areas in the catchment represent the main sources of DOC (Hinton et al., 1998; Inamdar et al., 2006).

In Brittany, Valley Bottom Wetlands (VBW) are the main contributors of dissolved organic matter (DOM) in agricultural catchment (Morel et al., 2009). These areas can be under intensively maize or wheat crop with farming waste spreading in spring season or serve as intensively grazing pastures.. These areas, located near the stream, can contribute to the DOM stream contamination by two ways either direct transfer of DOM issued from farming waste sources in intensively grazing areas or from recent farming waste supply on soils during a storm event. Excess load of farm manure application to cultivated hillslopes can contaminate the stream waters through excess nitrate and phosphorous as well as soluble phase of organic matter fluxes (Plaza et al., 2002; Chantigny, 2003; Vadas et al., 2007). These wastes an increase indirectly the water-extractable organic carbon of soil (Gregorich et al., 1998; Chantigny et al., 2002b) which can be flushed to the rivers when the groundwater level reached the surface horizon.

Agricultural practices can also modify the water pathways by creating surface runoff on compacted soils or preferential flow via tile draining, thus facilitating DOM transport (Royer et al., 2007a; Hernes et al., 2008). Changes in the DOM chemical characteristics are related to agricultural land use, nitrogen loading and wetland loss (Wilson and Xenopoulos, 2009). Furthermore, Sanderman et al., (2009) has demonstrated a shift old and recalcitrant DOM in deeper horizon with young and fresh DOM in the surface horizon and the hydrological connectivity of DOM rich riparian source influence on stream DOM composition and it

reflects the influence of soil biogeochemical cycling of organic matter and hydrological routing of water through landscape. Many authors have pointed out that DOM can enhance the transport of many pollutants (trace elements, pesticides, viruses, etc.) applied on cultivated soils towards natural water resources (Williams et al., 2005; Foppen et al., 2006; Chen et al., 2008; Song et al., 2008). Hence, to restore water quality, it is essential to understand DOM sources in agricultural catchments and to investigate intensive farming practices in the Valley Bottom Wetlands.

DOC is of limited use as environmental tracer and more information on the nature of DOM is required. Techniques used in the field of DOM sources tracking studies include gas chromatic analysis for the separation and identification of sterols in faecal detection (Saim et al., 2009), capillary electrophoresis to identify two or more electropherogram peaks of DOM decomposition (He et al., 2008), ^{13}C Nuclear Magnetic Resonance and Pyrolysis Field Ionization Mass Spectrometry for functional group investigation of fulvic acids and their molecular subunits (Leinweber et al., 2001). While spectroscopic techniques, UV-Visible spectroscopic ratios for DOM distribution in lake water (Regina et al., 2003), Fourier Transform Infrared (FTIR) spectroscopy applied in polysaccharides and carboxyl groups identification during biodegradation by (Kalbitz et al., 2003).

There are growing needs to control chemical quality of water in short time of analysis and on-line water quality monitoring in water treatment industry, which lead to replace the existing more expensive and time consuming techniques with some reliable, less expensive techniques. In this aspect, 3-dimensional excitation emission matrix (3DEEM) fluorescence spectroscopy seems to be a good candidate due to its high sensitivity to physicochemical changes in DOM materials (Thacker et al., 2005). Excitation emission (EEM) fluorescence captures many spectral features by scanning over a wide range of excitation and emission wavelengths and generating a landscape surface defined by the fluorescence intensity over excitation emission wavelength pairs (Wu et al., 2003; Sierra et al., 2005; Xie et al., 2008).

In river waters, different fluorescence peaks are reported from EEM and ascribed to protein like (tryptohan, tyrosine), fulvic like and humic like fluorophores in DOM in the aquatic and soil environment (Baker, 2002; Henderson et al., 2009; Naden et al., 2009; Bilal et al., sumitted). Relative strength of protein like and humic like fluorophores as well as their ratios has been used to differentiate various sources of DOM (Baker and Inverarity, 2004; Cumberland and Baker, 2007). Baker (2002) has indicated higher protein like fluorescence in animal wastes and demonstrate higher peak intensity ratio of tryptophan:fulvic like for animal wastes than stream water. Lapworth et al., (2009) have used maximum peak fluorescence

intensities of tryptophan like and fulvic like and observed more attenuation in tryptophan like compared to fulvic like fluorescence in hyporheic zone (0.5 meter below river bed) with changing surface waters inputs from upstream processes in riparian areas. Fellman et al., (2009a) has showed change in chemical quality of DOM in spring and fall wet season in bog, forested wetland and upland forests. He further showed the contribution of DOM from upland watersheds during stormflows and the contribution of humic like fluorescence increased and protein like fluorescence decreased (Fellman et al., 2009b).

Instead of using fluorophores peak fluorescence intensities, a chemometric approach of fluorescence regional integral integration proposed by Chen et al., (2003b) is getting popularity among the fluorescence users community. In drain flows, Naden et al., (2009) has adopted regional overlap of the anticipated fluorophores and proposed the TI:FI ratio (Tryptophan-like and fulvic/humic-like fluorescence) as tracer of cow slurry incidental losses in drain flow after slurry spreading. In soil, Bilal et al., (accepted) has used fluorescence regional integration of fluorophores in the EEM and proposed integrated fluorescence intensities in tryptophan zone and tryptophan:humic like ratio as tracers of farm wastes in farm waste amended soil during 56 days of biodegradation.

In order to trace the exogenous DOM loads in the stream, we applied fluorescence spectroscopy by coupling with regional integration approach and principal component analysis and the spatial and temporal variability of fluorescence tracers of DOM is observed in 15 agricultural minicatchments during three storm events. Moreover, in this catchment network, the agricultural practices (crop rotation, fertilization) in the Valley Bottom Wetlands area were characterized with a farm survey. This study aimed to analyze the relation between fluorescence tracers and intensification of agricultural practices in Valley Bottom Wetlands.

Material and Methods

Study site

Principal agricultural catchment of Haut Couesnon located in French Brittany, north western France, was divided into four sub-watersheds (7, 11, 15, 19) and these four sub-watersheds were subdivided into 15 subcatchments.

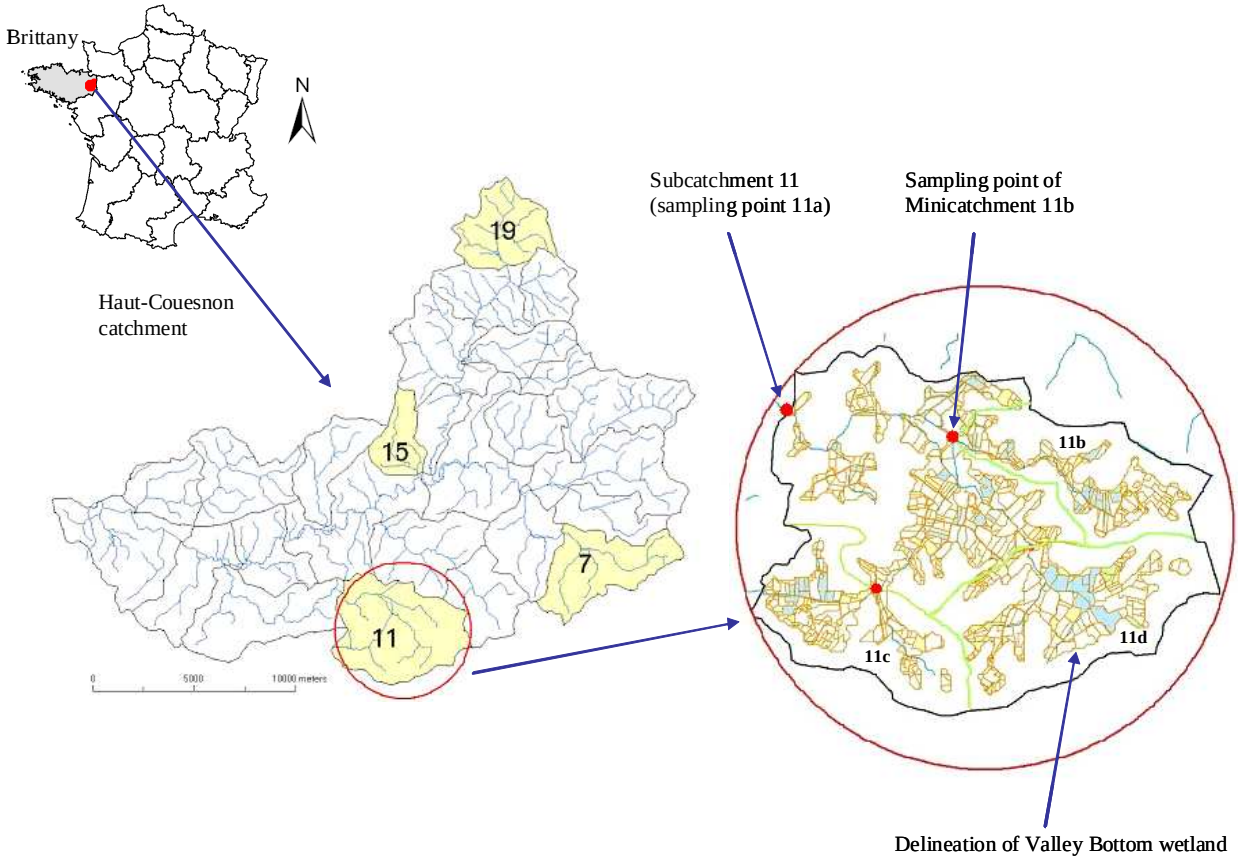

Figure 5.1 : .Location of 4 subcatchments of a principle agricultural headwater catchment at Haut Couesnon (HC) site, Britany. Subcatchment 11 is divided into 4 “minicatchments (MC) 11a, 11b, 11c and 11d. Red dots represents sampling points. Similarly subcatchments 7 (7a, 7b, 7c, 7d), 15 (15a, 15b, 15c) and 19 (19a, 19b, 19c, 19d) were also divided into minicatchments.

Delineation of the Valley Botton Wetlands (VBW)

Due to the lack of field characterization of Valley Bottom Wetlands (VBW) on the Haut Couesnon Basin, we applied here the method proposed by (Merot et al., 1995) and (Merot et al., 2006) for predicting wetland delineation in small catchments. VBW were defined in two steps: first step predicts the potential VBW distribution, i.e. wetlands derived from catchment geomorphologic and climatological features. The second step extracts the existing VBW (VBWe) i.e. wetlands unmodified by anthropic activity among the set of potential VBW. The potential VBW were defined using a climato-topographic index (ICT), taking into account the downhill slope (β_l) and upslope effective rainfall volume (Vr), following an approach first proposed by (Beven and Kirkby, 1979) and modified by (Merot et al., 2003).

Catchment characteristics and agricultural practices in the Valley Bottom Wetland

The minicatchment surface areas ranged from 272 ha (7b) to 2598 ha (11a) (Table 5.1). 20 to 40 % of the surface area of the catchment was composed of hydromorphic soils and is temporarily or permanently saturated with water.

Table 5.1 : Catchment area (ha) and Valley Botton Wetland of the 15 minicatchments

Minicatchments	Surface area (ha)	VBW (ha)	% of VBW catchment
7a	1768	384	22
7b	370	83	22
7c	272	57	21
7d	288	48	17
11a	2598	1039	40
11b	466	164	35
11c	655	278	42
11d	417	151	36
15a	700	140	20
15b	351	71	20
15c	129	39	31
19a	1447	363	25
19b	507	114	23
19c	337	63	19
19d	172	59	34

The VBW were dominated by meadows (Table 5.2). The catchment 7b showed the lowest meadows superficies but the highest maize occupation percentage. Forest was important (between 15% and 17% of the VBW) in 11d and 11b respectively. In other catchments, the percentage was lower than 7%. The VBH in the catchments 7 and 15 showed the lowest forest occupation and are essentially occupied by maize and wheat. The maize occupied between 7 and 53 % of the wetland for 7c and 7b respectively. Organic fertilization in the Valley Bottom Valley is important in the catchment 11a, 15c, 7b and 11c (Figure 5.2).

Table 5.2 : Wetland (% of catchment area), land occupation and fertilization practices of mineral fertilizer (MF) ($KgN.ha^{-1}$), cow manure (CM) ($KgN.ha^{-1}$) and pig slurry (PS) ($KgN.ha^{-1}$) in the wetland area.

Site	% wetland	Hedge (m/ha)	Meadows (%)	Forest (%)	Wheat (%)	maize (%)	MF	CM	PS
7a	22	159	59	3	5	27	61	32	17
7b	22	169	27	0	0	53	25	66	26
7c	21	137	81	2	2	7	54	16	9
11a	40	125	62	7	13	24	66	40	22
11b	35	202	83	17	0	17	47	37	0
11c	42	98	72	1	7	20	65	57	69
11d	36	104	57	15	10	30	60	39	11
15a	20	154	62	2	18	16	50	35	1
15b	20	164	55	1	26	11	58	28	1
15c	31	119	47	0	10	25	10	69	0
19a	25	154	53	6	12	29	63	29	16
19c	19	181	64	2	7	29	59	48	1
19d	34	172	61	4	10	29	33	40	2

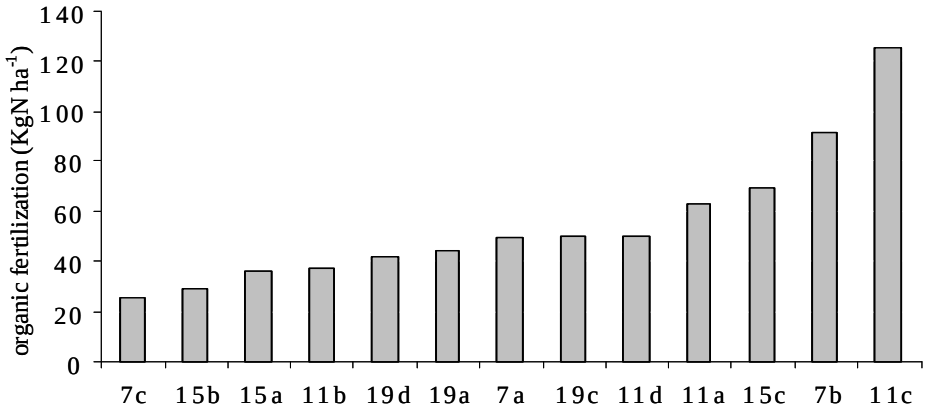

Figure 5.2 : Organic fertilization in Valley Bottom Wetlands ($kgN.ha^{-1}$))

Stream water sampling

Two winter storms (S1, S2) and one spring storm (S3) events were studied to analyse the DOM fluorescence properties differences among the 15 mini-watersheds. Principal agricultural catchment of Haut Couesnon was equipped a stream gauge station at the stream outlet. 30ml water samples were filtered with syringe driven mounted hydrophilic filter (0.22µm) (Millipore Millex-GV) at sampling place. To avoid any microbial transformation, filtered water samples were kept at 4°C in pre-acid washed polypropylene 30ml plastic bottles and returned to the laboratory for chemical analysis.

Chemical analysis

Dissolved organic carbon (DOC) was measured on a Shimadzu TOC 5050 A total carbon analyzer. Accuracy on DOC measurements is ±5%, based on repeated measurements of

standard solution (K-phtalate). The stream water samples having higher DOC concentrations were diluted with ultra pure water and brought in the range of 5mg$L^{-1.}$ Absorbance was measured in the diluted sample at 254nm and specific ultraviolet absorbance (SUVA) was calculated by multiplying the absorbance at 254nm with a factor of 100 and divided by the DOC concentration. Orthophosphate were analysed by colorimetry after reaction of the sample with Molybdate acid solution and Antinomy Potassium Tartrate. After reduction with ascorbic acid, the blue colour is detected at 660 nm.

Fluorescence spectroscopic measurements of DOC were performed using a Perkin-Elmer LS-55B luminescence spectrometer. Due to conditioning error, the water samples from mini catchments 7a, 7b and all the MCs of 15 and 19 during storm S1 could not be analysed in fluorescence. The spectrophotometer uses a xenon excitation source and slits were set to 5 nm for both excitation and emission. To obtain excitation-emission matrix spectra, excitation wavelengths were incremented from 200 to 425 nm at steps of 5 nm and emission was detected from 250 to 600 nm with a 0.5-nm step. Scan speed was set at 1500 nm/min, yielding an EEM in 22 minutes with 45 total scans. To minimise the temperature effect, samples were allowed to equilibrate with room temperature (20±2°C) prior to fluorescence analysis. Excitation emission matrix (EEM) were reproduced by subtracting Raman normalized distilled water blank spectra and the water Raman scatter peak was eliminated. Resonance peak (Fig. 3) on the lower side of three dimensional plots was also removed. The whole fluorescence dataset presented in this study was normalised at 5 mg L^{-1} DOC. To maintain the consistency of measurements and standardise the whole fluorescence data set, water blank corrected fluorescence spectra were normalised with 29.31 Raman emission intensity units of ultra pure water sample at 350 nm and 397 nm of excitation and emission wavelengths respectively.

Regional integration of excitation emission matrix (EEM)

An internal program was developed in the laboratory using the R® software (http://www.r-project.org) for the integration of fluorescence intensities across the whole EEM landscape. Here peaks at shorter wavelengths (<250 nm) and shorter emission wavelengths (<380 nm) are related to simple aromatic proteins such as tyrosine and tryptophan (Regions I and II) Peaks at intermediate excitation wavelengths (250–340 nm) and shorter emission wavelengths (<380 nm) are related to soluble microbial by-product-like material (Region IV) while peaks located at the excitation wavelengths (230–300 nm) and the emission wavelengths (380-575 nm) represent humic acid-like substances (Region III). Peaks at longer excitation wavelengths

(>300 nm) and longer emission wavelengths (>380 nm) are related to fulvic acid-like organics (Region V). With this technique, EEM is divided into biochemical (bio) (I, II, IV) and geochemical (geo) (III, V) fluorescent regions (Figure 5.3a) and three peak intensity zones of tryptophan (TRY), fulvic like (FL) and humic like (HL) fluorescence (Figure 5.3b(Table 5.3). The quantitative analysis included the integration of fluorescent volume beneath each region and zone. Moreover, ratios TRY:HL, TRY:FL, HL:FL, bio:geo, IV:V and III:V were also calculated. Humification index (HIX) was determined according to Ohno (2002). 45 spectral loadings were used to reproduce three-dimensional plots of fluorescence intensity as a function of excitation and emission wavelengths.

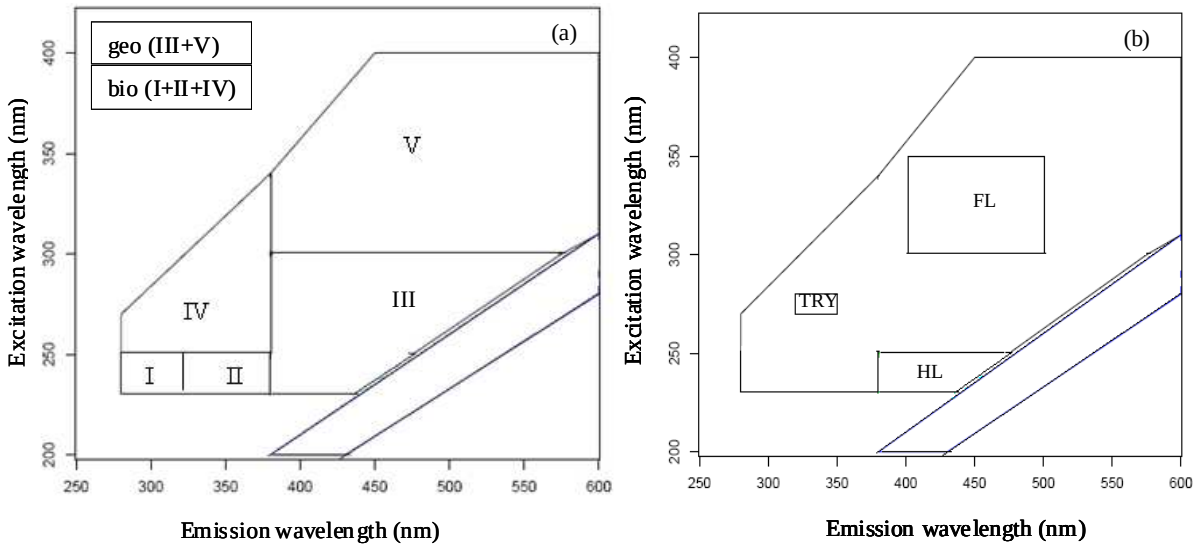

Figure 5.3 : Integration of fluorescence intensities across regions (a) and maximum peak intensity zones (b).

Table 5.3 : Definition of excitation (Ex) - emission (Em) wavelengths (nm) ranges for each region and zones

Regions	Ex (nm)	Em (nm)	Zones	Ex (nm)	Em (nm)
region I	230-250	280-330	Tryptophan	270-280	320-350
region II	230-250	330-380	Fulvic like	300-350	400-500
region III	230-300	380-575	Humic like	230-250	360-420
region IV	250-340	280-380			
region V	300-400	380-600			
region bio	230-340	280-380			
region geo	230-400	380-600			

Statistical analysis

The DOM quality differences, among three storm events, were observed by applying principal component analysis **(**PCA) on the DOC normalised fluorescence dataset with R® software (package ade4). Significant differences among the three storms and sub-watersheds were tested one way ANOVA ($p<0.05$) with STATISTICA (version 7.1). Among the fluorescence variables, Pearson's product moment correlation was calculated using R® software (package Rcmdr).

Results

Storms hydrology

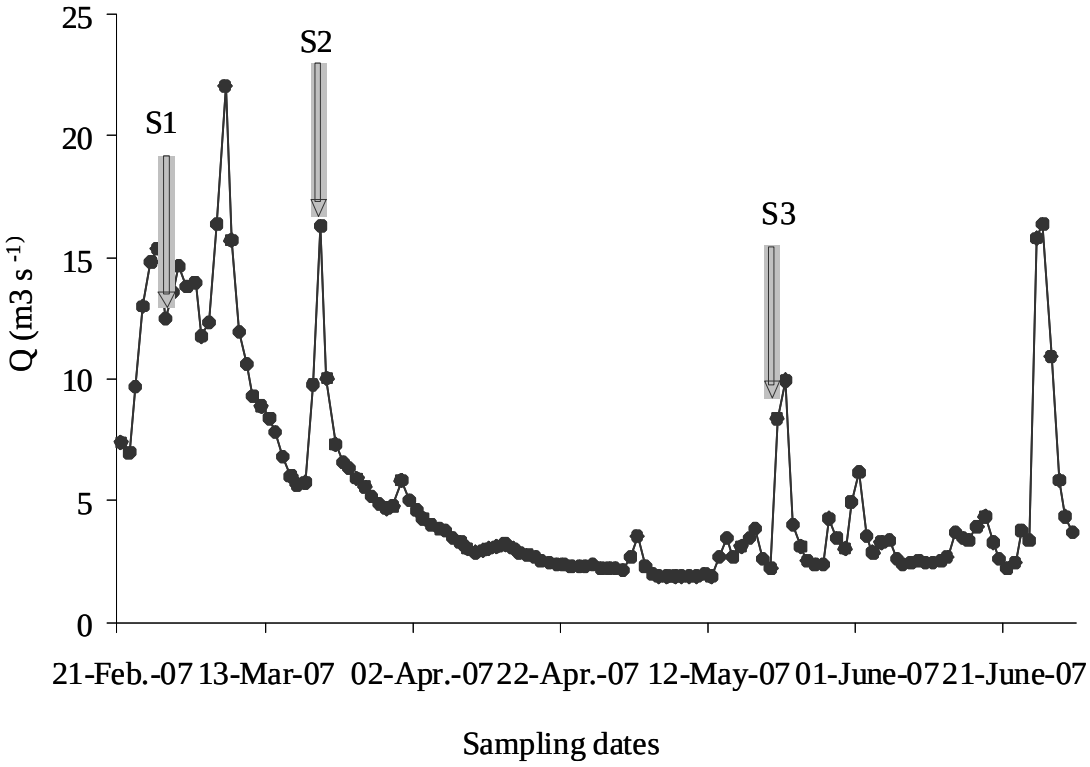

Figure 5.4 : Daily water discharge (Q) during between February and June 2007 and sampling date of the three storm events S1, S2 and S3 in Haut Couesnon catchment.

Water discharge rate during the three storm events (S1, S2 and S3) was $12m^3s^{-1}$, 16 $^3s^{-1}$ and $8m^3s^{-1}$ respectively (Figure 5.4). During S2, discharge rate was two fold higher than S3 and a little higher than storm S1. S1 and S2 occurred during winter with one month interval whereas S3 occurred during the spring after a dry period in the month of April. The sampling time for three storms S1, S2 and S3 was 27 February, 20 March and 21 May 2007 respectively.

Dynamics of dissolved organic carbon (DOC) and orthophosphate

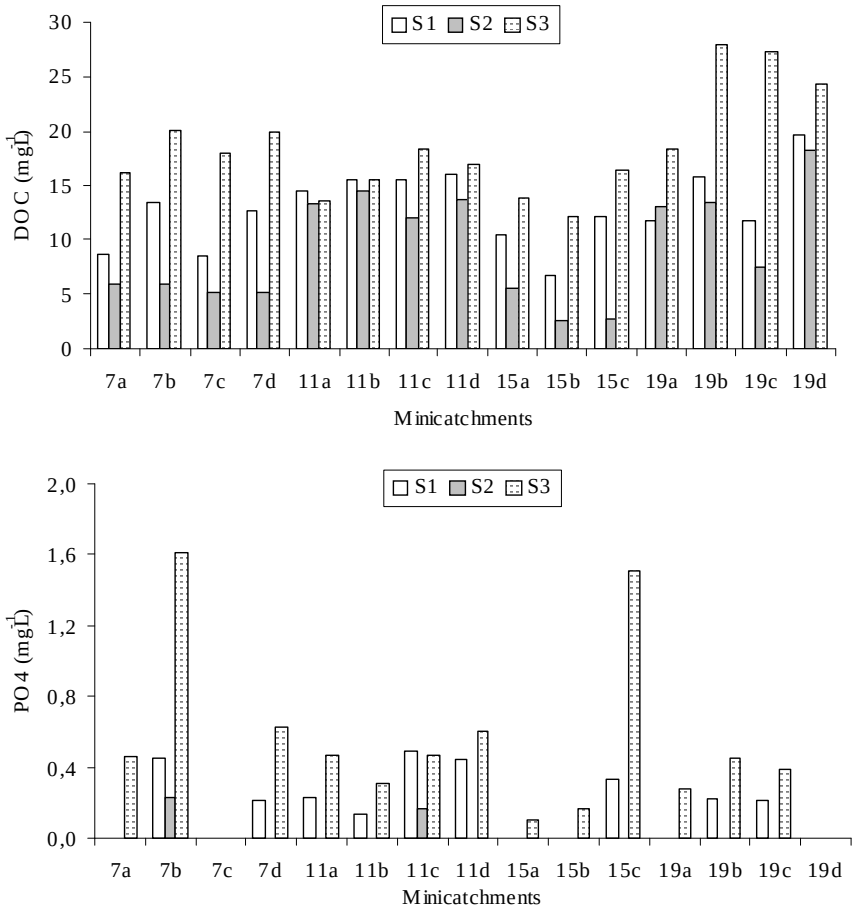

Figure 5.5 : a) Dissolved organic carbon (DOC) and b) orthophosphate dynamics during three storms S1, S2 and S3 among the 15 agricultural minicatchments (MC) at Haut Couesnon (HC) site.

During S1, the lower [DOC] were measured in 7a, 7c (8.7 $mg.L^{-1}$) and 15b (6.7 $mg.L^{-1}$) (Figure 5.5a). In the other MC, the [DOC] ranged between 8 and 15 $mg.L^{-1}$. The [DOC] is important and stable in all the MC 11 (between 14,5 and 16 $mg.L^{-1}$). The highest concentration was measured in 19d (19.6 $mg.L^{-1}$). Most of the mini catchments exceeded the limits of 10mgL^{-1} DOC. In France, the legislation authorities required a [DOC] lower than 10mg L^{-1} in <95% of the water samples collected per year for potable drinking water supplies. Hence most of these MCs are important DOC contributors.

During storm S2, 15b and 15c showed lowest [DOC] (2.6 $mg.L^{-1}$ and 2.7 $mg.L^{-1}$ respectively). In 15a and 7a, b, c, d, the [DOC] was more important and ranged between 5 and 6 $mg.L^{-1}$. Whereas concentration in 11 (11a, 11b, 11c and 11d) were still stable and important (12 to 14 mg L^{-1}). 19a, 19b, 19c ranged between 7 $mg.L^{-1}$ and 13 $mg.L^{-1}$ and the most important [DOC] was measured in 19d as in S1.

Higher [DOC] were measured in all the catchments during S3. In 7, its concentration ranged from 16 (7a) and 20 mg L^{-1} (7b and 7d). In 11, [DOC] was between 13 and 18 mg L^{-1}. The maximum [DOC] for this event were measured in 19 b, c, d (22 to 27 mg.L^{-1}).

In storm S1, DOC concentrations were always higher than storm S2 except in 19a. But difference of [DOC] between S1 and S2 was variable between mini catchments. In 7 b, d and 15c, [DOC] variations were important and greater than 6 mg L^{-1} whereas in others catchments, differences were less important (7a, 7c, 15b, 19c). All the MCs of 11 showed almost constant [DOC] during three storms except 11c where [DOC] fluctuation was observed with higher values (18 mg L^{-1}) in S3 and lower values (12 mg L^{-1}) in S2. Almost no variation was observed in (19a,d). The response to a climatic event was different from one sub-watershed to another. Some minicatchments appeared as constant high contributors of DOM during storm event (11 and 19d), others showed important [DOC] variations between storm events.

During S1 event, the orthophosphate concentrations (PO4) concentration was detected only in 7b, d, 11 a,b,c,d and 15c as well as 19b,c (Figure 5.5b). During S2, phosphate concentrations were only detected in 7b and 11c. While during S3 event, orthophosphates were detected in 7a,b,d and 11 a,b,c,d. There was also a contribution from 15 a,b,c and 19 a,b,c during S3 event. 7b and 11c showed the phosphate transfer during all the three storms. Overall, we observed higher PO4 concentrations in S3 event compared to S1, S2. In S3, minicatchments 7b and 15c were the principal contributor of orthophosphates in the streams. The minicatchments 7b and 15c were thus more impacted by phosphorous transfer than the other catchments. During S3, 7a,d and 11 a,b,c,d showed almost similar values of PO4 transfer.

During S3, hydrological processes seemed to be different from S1 and S2 with evidence of surface transfer which induced an increase of dissolved P in the streams.

Pertinent fluorescent tracers of DOM at agricultural catchment scale

Ratio of biochemical to geochemical (ratio bio:geo) regions

Ratio bio:geo discriminated all the MCs of sub-watershed 19 with lower values (0.06 to 0.08) compared to rest of the MCs of sub-watersheds of 7, 11 and 15 with higher values during three storms S1, S2 and S3 as shown in Figure 5.6. During S1, MCs of sub-watersheds 7 (7c, 7d) and 11 (11a, b, c, d) discriminated with higher values of ratio bio:geo (0.16, 0.14) and (0.15, 0.14, 0.14, 0.15) respectively compared to the values during S2 and S3 storms.

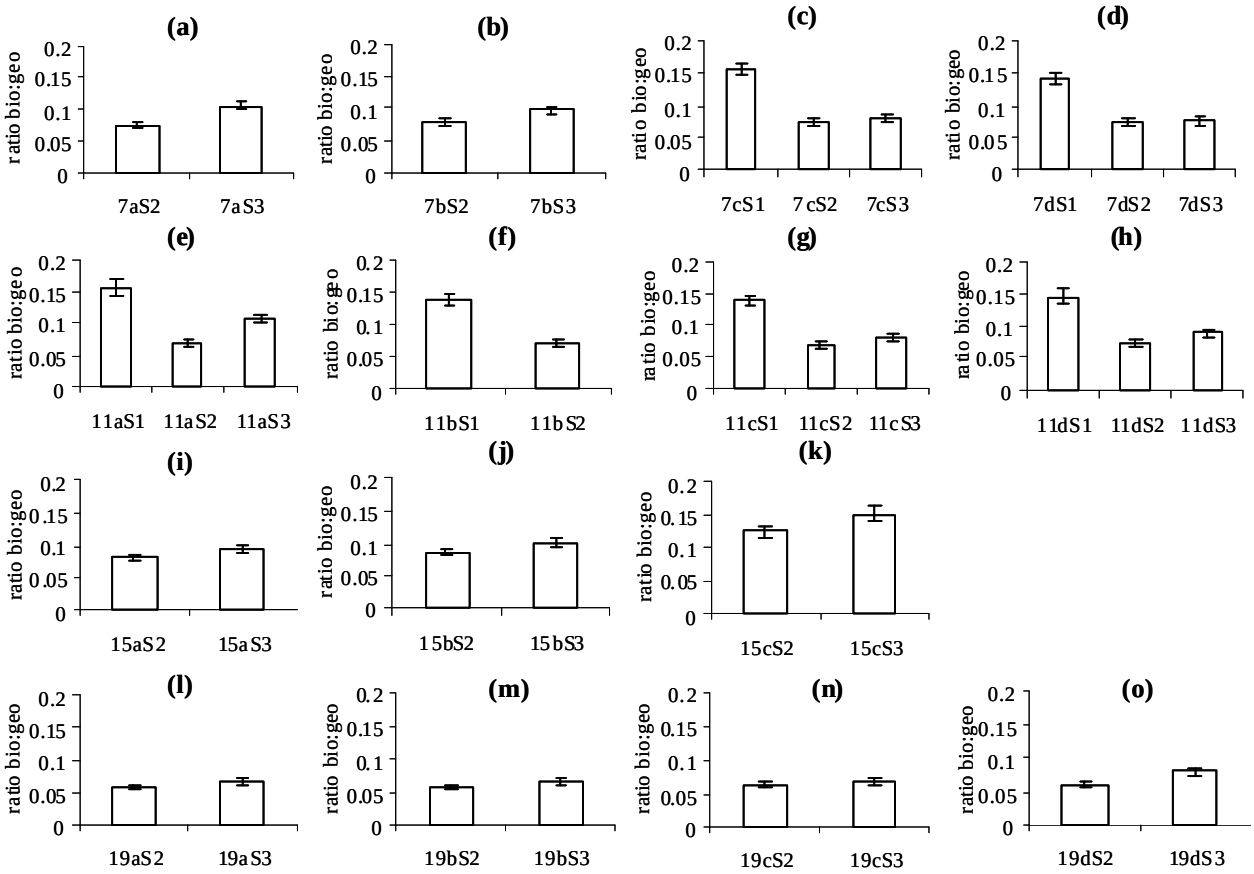

Figure 5.6 : Discrimination of DOM quality among 15 agricultural mini catchments (MCs) on the basis of ratio biochemical to geochemical regions (ratio bio:geo) during three storms S1, S2 and S3 at Haut Couesnon (HC) site.

Storm S2 showed lower values of ratio bio:geo in all the MCs of sub-watersheds (7, 11, 15) compared to storm S3 as increasing values of ratio bio:geo were observed in S3. However, response of 15c was discriminated all other MCs with higher values of ratio bio:geo (0.13 and 0.15) during S2 and S3 respectively.

Ratio of tryptophan to humic like (ratio TRY:HL) zones in EEM optical space

Ratio TRY:HL well discriminated the MCs of sub-watershed 19 with lower values (0.01) compared to all the MCs of sub-watersheds 7 (7a,b,c,d) with values 0.03-0.04, 0.02, 0.02-0.03 during S1, S2, S3 respectively and 15 (15a,b,c) with values 0.02-0.05, 0.03-0.06 during storms S2, S3 respectively and to all the MCs of sub-watershed 11 during S1 (0.02-0.03) and S3 (0.02-0.03) only (Figure 5.7).

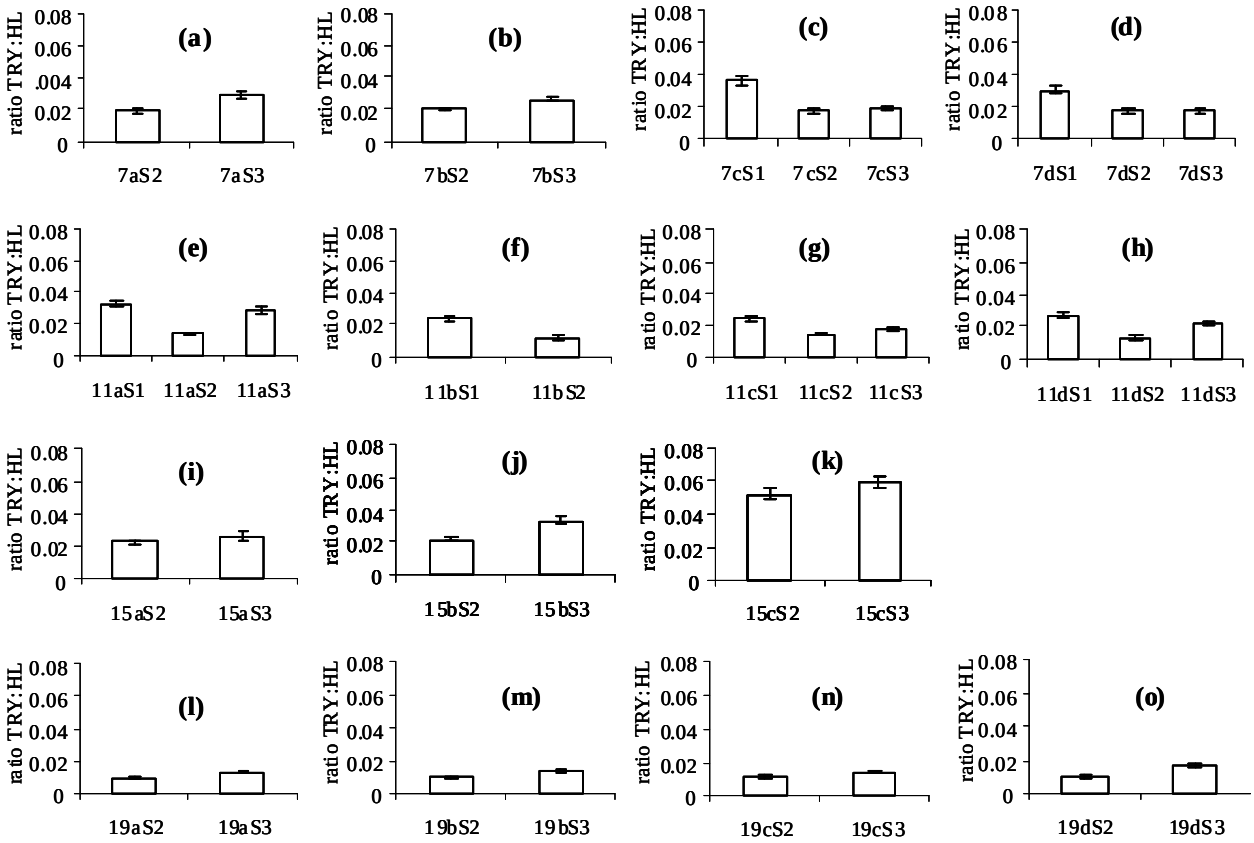

Figure 5.7 : Discrimination of DOM quality among 15 agricultural mini catchments (MCs) on the basis of ratio tryptophan to humic like (ratio TRY:HL) during three storms S1, S2 and S3 at Haut Couesnon (HC) site.

While, during S2, all the MCs of sub-watershed 11 showed similar values of ratio TRY:HL (001) to the MCs of sub-watershed 19. Mini catchment 15c showed discrimination with respect to all MCs of sub-watersheds of 7, 11 and 15a,b with higher ratios of 0.05 to 0.06 during storms S2 and S3 respectively. During S1, mini catchment 7c, 7d and 11a, 11d showed higher values of ratio TRY:HL (0.04, 0.03 and 0.03, 0.03 respectively) compared to 11a, 11c with value of 0.02 respectively. During S2, TRY:HL ratios for all the MCs were lower compared to S3 as increasing TRY:HL ratios were observed in S3. During S3, only mini catchment 15c demonstrated highly discriminant value ratio TRY:HL (0.06).

Temporal shift of DOM fluorescent properties among three storms events

The DOM spectroscopic properties were analysed by principal component analysis (PCA) to retrieve the additional information on temporal shift of the observed indices during three successive storm events S1, S2 and S3. The axis 1 and axis 2 of the PCA explained 56% and 30%, respectively, of variability in 17 spectroscopic indices of DOM (Figure 5.8). Axis 1 of PCA explained the variability in biochemical fluorescence and ratios among various

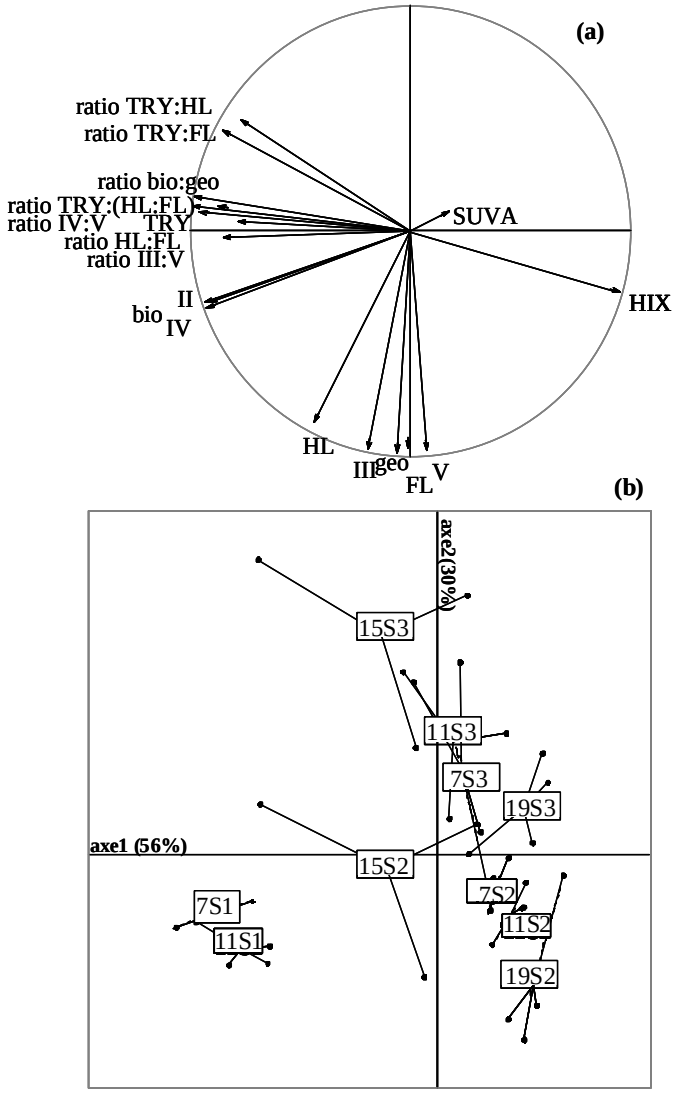

Figure 5.8 : Principal component analysis (PCA) of spectroscopic dataset: integral fluorescence in biochemical (bio), geochemical (geo), region II, III, IV, V and tryptophan (TRY), fulvic like (FL), humic like (HL) zones. Ratios of bio:geo, III:V, IV:V, TRY:FL, TRY:HL, TRY:(HL:FL), HL:FL. and SUVA (specific ultra violet absorbance) (a).Variability in DOM fluorescence among the 15 mini catchments of sub-watersheds of 7, 11, 15 and 19 during three storm events S1, S2 and S3 (b).

biochemical and geochemical regions and zones while axis 2 explained the variability in geochemical fluorescence and humification indices of DOM.

During S1, all the MCs of sub-watersheds 7 and 11 were clearly separated from the rest of two storms S2 and S3 with negative scores on axe 1 and axe 2. Biochemical (bio), region II, IV, TRY zone as well as ratio bio:geo, region IV:V and TRY:(HL:FL) had strong negative weightings on axis1 (Figure 5.7a) and separated the MCs of sub-watersheds 7 and 11 during S1. The spectral differences in DOM chemical characteristics, during S1, of sub-watersheds 7

and 11 were significant ($p<0.05$) with average axe1 scores -1.90 and -1.67 respectively compared

to S2 (0.54, 0.68 respectively) and S3 (0.24, 0.18 respectively). During S2, all the MCs of sub-watersheds 7 and 11 were projected 'in opposite quadrant to that in S1' in PCA space with positive scores on axe1 and negative scores on axe2. However, during S3, MCs of sub-watersheds 7 and 11 were located in opposite quadrants with positive scores on both axes 1 and 2.

During S2, all the MCS of sub-watersheds 19 had positive scores on axe1 and negative scores on axe2. During S3, it had positive scores on both axes 1 and 2. HIX had strong positive weighting on axe1 and it discriminated sub-watershed 19 from 7 and 11 during S3. Region III, V, geo and FL, HL zones had strong negative weightings on axe2 and separated 7, 11, 19 during storm S2 from S3 except 19S3 which showed resemblance of fluorescence properties to 7, 11 during S2.

More dispersion was observed in sub-watershed 15 during S2 and S3 compared to other MCs of sub-watersheds 7, 11 and 19. During S2, MCs of sub-watershed 15 had negative scores -0.40 and -0.14 on axes 1 and 2 respectively while during S3, these minicatchments had negative scores on axe1 (-0.46) and positive scores on axe2 (1.85). Sub-watershed 15, during S3, differentiated with negative weightings of ratios TRY:HL and TRY:FL on both axes. Axis1 was incapable to discriminate 15 from sub-watershed 7 and 11 during S3 while axis 2 showed similarities between MCs of sub-watersheds 15 and 11 during S3.

Variability in land occupation and agricultural activities in catchments

PCA was applied on the dataset of land occupation, soil fertilization practices in all MCs of sub-watersheds 7, 11, 15 and 19 at Haut Couesnon site (Figure 5.9). Three principal axes (axi1, axis2, axis3) of PCA explained the variability in the dataset by 32% and 25%, 17% respectively. By considering the axis1 and axis2 of PCA, minicatchments of sub-watershed 11 demonstrated positive scores on axis2 except 11b showing highly negative scores on axis1.

All the MCs of sub-watersheds 7, 15 and 19 showed dispersed position in PCA space. Mini catchment 15c had highly positive scores on axis1 and discriminated from rest of the MCs with cow manure land spreading and largely influenced with maize crop cultivation. While 15a and 15b were projected in opposite quadrant to 15c in PCA with negative scores on both axes and discriminated with higher influence of hedges and the cultivated area of both MCs

was under wheat crop cultivation. Mini catchment 7b had highly positive scores on axe1 and differentiated with cow manure and maize crop. While 7c had highly negative scores on axe1 and meadow (grazing) dominated in the Valley Bottom wetlands.

Table 5.4 : PCA weightings of three principal axes for the land use and land spread variables during biodegradation study periods P1 and P2.

Variables	axe1	axe2	axe3
PWL	-0.04	0.85	0.24
Hedges	-0.04	-0.72	0.49
Meadows	-0.85	0.21	0.24
Forest	-0.40	0.24	0.72
Wheat	-0.23	-0.21	-0.72
Maize	0.84	0.05	0.24
Mineral fertlizer	-0.71	0.27	-0.22
Cow manure	0.85	0.30	0.05
Pig slurry	0.15	0.81	-0.24

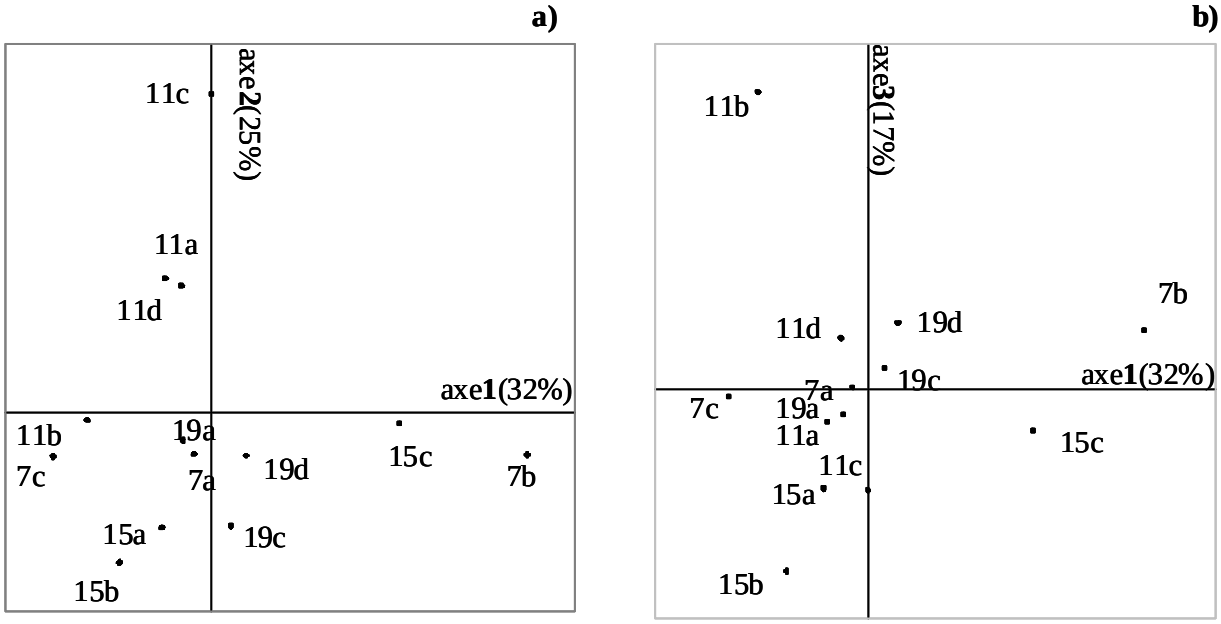

Figure 5.9 : Principal component analysis (PCA) (axe1, axe2 in (a) and axe3 (b) explaining the variability of land occupation (cultivated area under wheat, maize crops, meadows, forest, hedges, potential wetland area (PWL)) and soil fertilization practices (pig slurry, cow manure, mineral fertilizer) among the mini catchments of sub-watersheds of 7, 11, 15 and 19 during three storm events S1, S2 and S3.

Mini catchment 11b was mainly differentiated from 11a, c, d with dominant influences meadows and mineral fertilizers. However, there was dominant influence of potential wetlands (PWL) and pig slurry soil amendments on mini catchment 11c. Overall, all the MCs of sub-watershed 11 were characterized by the most important VHB area but under

heterogeneous land occupation by meadows, forests. With respect to axe3 of PCA, 11b showed highly positive scores on axe3 and discriminated this MC with forest occupied soils and hedges. Although minicatchments of 19 showed average occupation of wetlands, hedges, maize cultivation and mineral and cow manure amended fertilization practices (Table 5.2) but these minicatchments showed poor projection in PCA space along three axes.

Discussion

Spatial and temporal variability of DOM export from the catchments during storms

Storm S2 was marked with higher spatial variability between subcatchments 7, 11, 15 and 19 than during S1 and S3. Overall, DOC concentrations remained lower in S2 compared to S1 and S3 especially in 7, 15. This is not a dilution effect due to higher discharge rate as chloride concentrations are not diluted (data not shown) Discharge rates are mean daily data, It is possible that sampling occurred at the beginning of the storm event and not at the maximum peak discharge flows. Manual sampling in during storm S2 is not really satisfactory and an automatic sampling at the peak discharge would have been far better option.

Although discharge was not higher in S3 yet DOM concentrations were the highest during the three events. It is contradictory to Clark et al (2007) who showed that magnitude of rainfall and discharge could be important in controlling DOM fluxes (Clark et al., 2007). It could be reason that as storm S3 occurred after a period of base flow and the period of farming waste supply to soil (April and May), we suspected that crop residues and farming wastes were at the early stage of decomposition and DOM released might flushed into the stream water channel. Also likely that during baseflow period, the lower precipitation prevents the transport of DOM to the stream channel. So that organic material remain in the upper soil surface and can be flushed into the streams with subsequent intense rainfall and cause sharp peaks of DOC concentrations. Worrall et al., (2008) showed that runoff from the catchments was associated with increasing DOC concentrations.

Many studies demonstrated that a high proportion of the DOC is exported during storm events in small catchments (Grieve, 1984; Hinton et al., 1997; Dalzell et al., 2005; 2007; Fellman et al., 2009a). Our results are in agreement with these studies. But we observed a great variability in the DOC concentration for the three events in each catchment and between catchments for one event. The origin of this variability may depend on land use and agricultural practices in the Valley Bottom Wetland. Buffer land cover (in 90-m along all

stream banks) were identified as best predictor of DOC concentration variations (Molinero and Burke, 2009b). Moreover, DOC concentrations increased even at short time scale (one to two months time intervals) during storm flows. We also suspect that biological activity coupled with geochemical control over the increased production of DOC (Lumsdon et al., 2005). However, DOC itself is of limited use as environmental tracer and more information on the nature of DOM (biological or geochemical origin as well as freshly produced vs highly humified) is required. Therefore, some molecular techniques are required to explore the DOM sources.

Fluorescence tracers of DOM in large network of agricultural catchments

Fluorescence properties have enabled us to explore variation in quality of fluorescent DOM exported from a large network of headwater minicatchments in time and space at Haut Couesnon catchment. Ratio bio:geo, TRY:HL, geo and HIX were ranked (PCA results, Figure 5.8) prominent discriminators of DOM chemical characteristics variations among the storms as well as in various studied minicatchments. We consider that ratio bio:geo and TRY:HL trace the recently originated DOM (Chapter 3). Moreover we also suppose that fluorescence intensities in region geo and HIX indicate the humified organic matter either part of native soil organic matter or highly humified organic materials. In the present study, all minicatchments of 19 showed positive Pearson product moment correlation between HIX and geochemical fluorescence intensities (geo) (0.71, $p<0.04$) while rest of the subcatchments did not show this correlation. The existence of this correlation marked the variability in DOM fluorescence and probably related to the contribution from soil or highly humified dissolved organic matter that originated as a result of degradation process. In chapter 3 of the present thesis, we found that HIX was the prominent discriminator of soil DOM. While in chapter 2, runoff simulation experiment, we marked that runoff DOM from control plots exhibited strong correlation (r) between HIX and geochemical signatures (0.98, $p<0.0001$) in 6L and 0.76 ($p<0.001$) in 14L runoff DOM material. Besides this, there was significant correlation between HIX and geo fluorescence in cow manure soil amendments (Chapter 3 and chapter 2 of the present thesis). It reflects that existence of this correlation contradict our hypothesis about only soil originated DOM. So significant correlation between HIX and geo fluorescence may give the proxy of DOM released either from cow manure amended soils or from native soil carbon. As in case of pig slurry, this correlation did not exist. So in subcatchment 19, DOM either originated from soil or from cow manure wastes and it reflects the use of geochemical fluorescence and HIX as tracers of geochemical signatures in fluorescent DOM

in complex network of watersheds. It marked the spatial variability in biogeochemistry of fluorescent DOM materials in among the subcatchments 7, 11, 15 and 19.

There is heterogeneous responses of ratio bio:geo to farm wastes supply at soils. Normally ratio bio:geo and ratio TRY:HL or FL increase upon farm wastes amendments. But we also found (chapter 3), Kerguehennec soils do not discriminated with any farm wastes amendments compared to Champ Noël soil that marked the increasing ratios in pig slurry amended soils.

Minicatchment 15c is marked with increasing bio:geo and TRY:HL ratios and we suspect the contribution of farm wastes DOM S2 and S3 compared to the rest of minicatchments in the same events. Particularly during S3, as DOC concentration increased compared to S2 event and there is high probability of farm wastes DOM contribution in the stream. Besides this, mini catchments 7c, 7d and 11 a, b, c and d also showed the increasing bio:geo and TRY:HL rations particularly in first winter storm S1.

Temporal variations of chemical characteristics

In storm S1, there is strong probability of overland flow and rapid transfer of DOM originated from farm wastes practices and possibly discriminated with higher bio:geo, TRY:HL ratios. While in S2, DOC concentrations are not increased and most probably, the chemical characteristics of DOM remain close to the soil as it marked with higher HIX values in S2 (0.97-0.98) compared to S1 (0.89-0.91) and 0.93-0.96 in S3. Higher values of HIX indicate the DOM contribution from soil origin.

Subcatchments 7, 11 and 15 has shown increasing ratios bio:geo and TRY:HL after one month dry period and after the season of farming waste supply on soils. Sanderman et al., (2009) in a headwater coastal catchment detected a pulse of fresh DOM disproportionate to the magnitude of the flow. He attributed the release of DOM to the turnover by microbial community, as a consequence of wetting and drying cycles on organic matter in soils. The DOM pool can be replenished after a drying period. This DOM could have a biochemical signature more important than in February. But the modification of fluorescence properties under wetting and drying cycle has still to be studied, so we can not conclude whether fluorescence modifications observed during S3 is a result of an increase of microbial soluble soil DOM or a direct transfer of DOM from farming waste.

Despites [DOC] stability in all the MCs of sub-watershed 11 except 11c, fluorescence properties are different during the three events. S1 was marked by high bio:geo ratio, S2 differentiated with lower ratios of bio:geo and TRY:HL and higher HIX values (0.97) and S3

with increasing ratios of TRY:HL which depicted different chemical quality of DOM materials. There is no significant difference between the subcatchments 7 and 11 during S3 that mark the presence of common DOM quality (Figure 5.8).

In mini catchment 15c, during S2, DOM substance demonstrated impact of agricultural intensification as we observed increasing ratios of bio:geo and TRY:HL compared to MCs 15a, 15b that means there is always active source of farm waste DOM that contribute to the DOM flushing in this minicatchment.

Evidence of DOM pollution by farming waste contribution

During S1, a contribution of farming waste is suspected in catchment 7 and 11. The ratio bio:geo and TRY:HL are high. The P concentrations are also elevated in the catchment 7b, c, d and 11a and b. P was not detected in the other catchments. The presence of soluble phosphorus corroborate the hypothesis of a contamination either directly by transfer of faeces or pig slurry from the soil surface, or by export of DOM issued from farming waste freshly biodegraded.

PCA analysis of VBW agricultural practices showed that 7b and 15c were highly associated with cow manure land spreading and maize crop cultivation. During S2 and S3 storms, the values of ratios bio:geo and TRY:HL were ranged from 0.12 to 0.15 and 0.05 to 0.06 respectively in the 15c which reflected the impact of cow manure land spreading. Results are in agreement with Naden et al., (2009) who has found increasing values of T1:F1 in drain flow after cow slurry spreading. 7b was highly impacted by cow manure fertilization ad maize crop (Figure 5.9) but there was no marked increase in ratio bio:geo and TRY:HL although orthophosphate concentrations were very high in 7b during S3. Mini catchment 7c was associated with higher occupation of meadows and we suspect the contribution by cow faeces too probably by direct transfer in storm S1. Higher values of bio:geo (0.4 to 0.5) and ratio TRY:HL (0.18 to 0.22) were measured on pure cow faeces wastes but this source can contaminate the stream and increase the ratio of the stream. So there is possibility of diffuse contamination of stream water by cow faeces and impact of farming wastes are marked with increasing values of ratio bio:geo and TRY:HL in S1 and S3.

During S1, 11b was associated with meadows and showed the ratio bio:geo (0.14) similar to composted manures with 4 to 6 months composting times. The similar values reflect the presence of dungs excreted by cows which undergo biotransformation and the values approaches to cow manure composts. But there is strong temporal variability in subcatchments 7 and 11 and ratio bio:geo, TRY:HL remains higher in S1 followed by S3.

While 15c did not show temporal variability between S2 and S3 and higher ratios of bio:geo, TRY:HL reflect the constant sources of cow manure wastes supply on the soil.

All the MCs of subcatchment 11 showed buffering impact on DOM production and export except in 11c. It is because of heterogeneous land occupation sources. Mini catchment 11b seems to be reactive to DOM export and probably the DOM contribution in the stream is from meadows and forest litter. MCs 11c was largely under the influence of potential wetlands and pig slurry fertilization and also showed increasing with increasing bio:geo ratio. As the soil occupation sources are very complex, definitely, impact on DOM is clear with increasing fluorescence bio:geo and TRY:HL ratios when we compared with sub-watershed 19. Although we observed increasing bio:geo and TRY:HL ratios in 19d during S3 but it reflects contribution highly degraded proteins of microbial origin from peatland lake which is located alongside the stream bank.

So in S2, in most of the catchments, contribution of natural DOM was prominent and DOM was probably issued from soil origin. While during S1 and S3, impact of agricultural practices was marked. On the whole, the impact of agricultural practices such as grazing meadows or cow manure and pig slurry land spreads along with wheat and maize crop cultivation was more clear in MCs 15b,c, 7c and 11c in the Valley Bottom Wetlands.

Fluorescence tracers as a support to water quality monitoring and policy

To meet the growing needs of best quality of drinking water, strict policy measures have to be adopted. In this regards, fluorescence spectroscopy seems to be the best compromise for the detection of farm wastes impact on fresh water bodies. The fluorescence technique can benefit water industry as on line sensor of water quality monitoring of DOM pollution because of relatively low cost and these tools has to be included in the regular analysis of DOM quality in fresh water supplies. It can also be applied in fundamental research to observe DOM fluorescence response to various agricultural inputs.

This study also reflects the necessity of DOM quality monitoring during baseflow time and in this we may put in evidence the diffuse water pollution during storm flows. Moreover, soil sampling should be done in catchments in order to associate the DOM chemical properties measured stream storms. Morel et al., (2009) has pointed out the contribution of 80% DOM from riparian wetlands during storm events in Brittany. While Fellman et al., (2009b) have shown the possible DOM contribution from upland and wetland watersheds. To observe the possible sources and variability in DOM fluorescence properties during one storm, several samples should be taken along the ascending and descending limbs of hydrograph.

Conclusion

In this research study, the potential of EEM fluorescence spectroscopy for the determination of farming waste impact on the increasing DOM in the streams was studied. The agricultural practices and crop rotation were characterized in the Valley Bottom Wetland which is recognized as the main source of DOM in French Brittany during storm event. So agricultural indicators (organic fertilization, crop rotation) are detailed and their relation with fluorescence indicators is discussed. A heterogeneous optical response of DOM substance was observed in 15 mini-watersheds as well as among the storm events. Our results reflect the following conclusions:

- Mini catchment 15c was discriminated from rest of the catchments with higher ratios of bio:geo and TRY:HL and depicted the impact of cow manure land spreading. The intense grazed meadows (MC, 11b) by cows showed their impact of DOM export in stream during stormflows with higher bio:geo ratio (0.14).
- Humification index (HIX) was found a good tracer of highly humified DOM exported from peatland during stormflows.
- Significant correlation between integral fluorescence in regions geo in the EEM and HIX reflected the use of geochemical fluorescence as tracers of highly aromatic and complex DOM structures.
- Storm S2 was differentiated from S1 and S3 with humified DOM material, probable contribution was of natural humified DOM of soil origin and reverse was the case in S1 and S2.

Results demonstrate that fluorescence spectroscopy, by coupling with regional integration approach, are capable to reveal chemical changes in the DOM quality and depict the farm wastes load on the increasing DOM in fresh water streams.

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GENERAL CONCLUSION

General conclusion

Objectives and strategy

The general objective of the thesis was to assess excitation emission matrix fluorescence as a tracer of dissolved organic matter (DOM) issued from farm waste recycling in soils and transferred to stream water in agricultural headwater catchments.

We developed an approach at different scales:

- Characterization of the fluorescence tracers directly on a diversity of farm wastes recycled in cultivated soils (pig slurry, cow faeces, crop residues, cow manure and composted cow manure).
- Detection of fluorescence tracers of DOM issued from farming wastes first researched in a laboratory simulation of natural water and farming waste mixing and then in a runoff experiment on 1m² plots receiving cow manure and pig slurry at agronomic rate.
- Short term incubation study (two months) to observe evolution of fluorescence parameters of pig slurry and cow manures in soils.
- Assessment of long term persistence of fluorescence tracers in soils under pig slurry and cow manure wastes recycling. First experiment was conducted on an experimental site with 14 years of application of mineral and pig slurry amendments on a loamy soil with low organic carbon content (0.9%) and under maize crop cultivation. Second experiment was characterized by 7 years of application of mineral, pig slurry and cow manure wastes on experimental site having higher soil organic carbon content (2.5%) and polyculture cropping system.
- Storm stream water sampling was carried out in fifteen headwater agricultural catchements of Haut Couesnon between February to June. A farming survey was conducted on agricultural practices (crop rotation, fertilization) in the Valley Bottom Wetland (VBW). These areas are the non limiting source of DOM during storm events in Brittany. The relation between farming waste management and presence of fluorescence tracers in stream water was explored during three storm events.
- Fluorescence dataset was obtained by integrating the whole excitation emission matrix (EEM) of aqueous extracts of dissolved organic matter (DOM) from pure agricultural

farm wastes and soil amended with pig slurry and cow manure wastes. The whole EEM was divided into biochemical (bio) and geochemical (geo) regions. Fluorescence intensities were also integrated in region III, V as well as tryptophan (TRY), humic like (HL) and fulvic like (FL) zones. Ratios of bio:geo, TRY:HL, TRY:(HL:FL) were calculated.

Synthesis of results

Different questions were considered:

- What are the fluorescence tracers of different pure farm wastes?
- How are the fluorescence properties in natural streams impacted by known direct transfer of pig slurry and cow manure composts?
- Do fluorescence properties discriminate pig from cow manure contamination in runoff water?
- How evolve the fluorescence parameters of DOM in soils receiving farming waste in the days following the spreading? What is the persistence of the fluorescent tracers two months after the spreading?
- Does long term application (7-14 years) of pig slurry and cow manure wastes on cultivated soils significantly modify the fluorescence properties of DOM?
- Can we detect influence of farming waste recycling on soil or impact of grazing pasture in stream water in a network of 15 agricultural headwater catchments? Is there any relation between farming waste management (intensive recycling on soil or grazing pasture) in Valley Bottom Wetland and presence of fluorescence tracers in stream waters?

Fluorescence tracers of farm wastes

Fluorescence tracers have the potential to discriminate the farming wastes DOM from soil DOM. DOM produced from pig slurry, cow faeces and cow manures are discriminated from soil DOM with significant higher ($p<0.05$) bio:geo, TRY:HL, TRY:(HL:FL) ratios as well as TRY fluorescence (Table1). Significant lower fluorescence in region V and III also discriminate the farm wastes from soil (Table1). Among the pure farming wastes, cow faeces resemble to pig slurry with fluorescence tracers of ratio bio:geo and region V but both pig slurry and cow faeces differentiated well from cow manures with significant higher values of fluorescence tracers of ratio bio:geo, TRY, ratio TRY:HL and ratio TRY:(HL:FL) (Table1).

Detection of direct transfer of farming waste DOM to natural stream

Direct transfer of pig slurry wastes into the stream water modifies the biochemical and geochemical quality of DOM. As a consequence, pig slurry contamination in the stream water can be detected with increasing TRY fluorescence as well as ratios bio:geo, TRY:HL, TRY:(HL:FL) (Table1). Moreover, geochemical fluorescence of DOM in regions III and V decrease with direct input of pig slurry wastes into the stream (Table1).

Persistence of fluorescence tracers of farm wastes in soils

Short term (week to month)

Farm wastes recycling in soil modifies the chemical quality of native soil DOM. At Kerguehennec site (2.5% Carbon contents), ratio bio:geo qualify as pertinent fluorescence tracer of pig slurry and cow manure wastes during one week after soil amendments and discriminates farm wastes amended soils from mineral fertilized soil. At the same soil, TRY fluorescence, TRY:HL and TRY:(HL:FL) discriminate the pig slurry and cow manure amended soils from mineral fertilized soil for two months after spreading.

Long term (one year later) (7-14 years farm wastes recycling)

At Kerguehennec (2.5% carbon contents, 7 years of pig slurry and cow manure wastes recycling) and Champ Noël (1% carbon contents, 14 years of pig slurry recycling) sites, only TRY fluorescence qualified as tracer of pig slurry after one year of soil amendment (Table 2). But we do not know that either TRY fluorescence persists in both soils for one year after pig slurry soil amendment or it is the cumulative effect of long term (7 to 14 years) pig slurry recycling on both soils.

At Champ Noël site, ratio bio:geo, ratio TRY:HL, TRY:(HL:FL) discriminated the DOM in pig slurry amended soil from mineral fertilized soil after one year of soil amendment but these tracers do not work well at Kerguehennec site (Table 2). It reflects the impact of soil type which should be explored.

Discrimination of pig slurry and cow manure contamination in runoff water

Significant higher values of TRY fluorescence, ratios bio:geo, TRY:HL, TRY:(HL:FL) discriminate the DOM in the runoff from pig slurry and cow manure amended soils than soil runoff. However, similarly to soils, all these fluorescence tracers do not permit to discriminate the pig slurry from of cow manure DOM sources (Table 2).

One fluorescence tracer is not enough to detect the farm wastes DOM pollution; therefore, there is necessity to use multivariate analysis. Classification and Regression Tree (CART) approach was applied to track the DOM origin from pig slurry and cow manure amended soils than mineral fertilized soil DOM. CART approach showed almost 100% prediction accuracy in predicting the DOM of farm wastes origin than soil during two months of soil amendment. Although CART analysis made the distinction between DOM origin in pig slurry and cow manure soil contamination but prediction accuracy was limited to 72% (one week) which reflect the 30% chance of poor prediction for pig slurry wastes.

Farming waste fluorescence tracers in agricultural headwater stream

A general shift in fluorescence properties of DOM in stream was observed from biochemical (Storm1) to geochemical (Storm2) and then a slight shift towards to biochemical (Storm3). In Brittany, Valley Bottom Wetlands (VBW) are identified as main contributing areas to DOM fluxes during storms. With the help of farm survey data in VBW and fluorescence spectroscopy, we detected possible contamination of cow manure land spreading in the small catchment 15c. While in 19d, DOM contribution in stream was of soil origin.

During storm 3, 15c shows increasing TRY fluorescence, bio:geo, TRY:HL, TRY:(HL:FL) ratios compared to 19d showing the impact of farming wastes spreaded at soils in Valley Bottom Wetlands (VBW). In contrast, 19d resemble to most of soil extracts. Region III and V fluorescence in 19d shows similarity to surface transfer runoff sample from control soil (Table1). However, baseflow characterization as well as soil sampling in small agricultural catchment in VBW should be explored to study the difference between baseflow and highflow streams as well as spatial variability of soil type.

Table 1 : Discrimination of fluorescence tracers of farm wastes DOM from soil DOM as well as between pig slurry and cow manure

			Kerguehennec site (2.5% C)					**Champ Noël 1% C**	**Stream water during storm events**		
Tracers	Treatment	Farm wastes/soil extraction	Runoff water		Biodegradation		7 years of recycling (1 year after spreading)	14 years of recycling (1 year after spreading)	Impacted pig slurry	Catchment 15c (16.3 $mg.L^{-1}$ DOC)	Catchment 19d (24.2 $mg.L^{-1}$ DOC)
			6L	14L	7 days	2 months					
Ratio bio:geo	Cow faeces	0.471±0.071^{b}								0.15	0.08
	Pig slurry	0.470±0.002^{b}	0.277±0.049^{a}	0.214±0.057^{a}	0.093±0.002^{a}	0.085±0.008^{a}	0.089±0.004^{a}	0.152±0.007	0.177±0.015		
	Cow manure	0.267± 0.030^{a}	0.248±0.091^{a}	0.240±0.070^{a}	0.095±0.007^{a}	0.091±0.007^{a}	0.085±0.004^{a}				
	Control (soil / water)	0.083±0.003	0.118±0.022	0.128±0.028	0.077±0.005	0.083±0.005	0.089±0.004	0.109±0.005	0.121±0.006		
Region V	Cow faeces	8167±2820^{a}								15534	23094
	Pig slurry	18568±1819^{a}	11866±3075^{a}	13746±3362^{a}	39486±4287^{b}	38342±3245^{b}	21391±1069^{a}	12751±637	10873±545		
	Cow manure	5314±870^{b}	10440±3220^{a}	10482±2959^{a}	32696±6197^{a}	29988±3496^{a}	20117±1005^{a}				
	Control (soil / water)	53214±1748	25311±7581	23827±5502	43711±7719	30458±3455	18792±939	13672±683	16905±845		
Region III	Cow faeces	13883±4455^{b}								15724	23431
	Pig slurry	22538±2180^{c}	16303±3834^{a}	17894±4033^{b}	44726±4648^{b}	44335±3803^{b}	22139±1106^{a}	11303±565	12370±618		
	Cow manure	10606±1149^{a}	12807±2681^{a}	12838±2624^{a}	35955±7050^{a}	33901±4222a	20274±1013^{a}				
	Control (soil / water)	62351±2381	30129±8576	28814±6199	50446±9996	35953±4785	19832±991	11424±571	18604±930		
TRY	Cow faeces	742±168^{b}								237	99
	Pig slurry	1507±153^{c}	520±155^{b}	397±89^{a}	296±40^{b}	217±40a	133±6^{b}	161±8	168±8		
	Cow manure	366±51^{a}	354±121^{a}	351±99^{a}	226±41^{a}	187±33a	109±5^{a}				
	Control (soil / water)	210±37	230±52	269±92	162±51	126±17	110±5	87±5	92±5		
Ratio TRY:HL	Cow faeces	0.201±0.014^{b}								0.06	0.02
	Pig slurry	0.251±0.001^{c}	0.115±0.025^{a}	0.083±0.029^{a}	0.025±0.005^{a}	0.017±0.003^{a}	0.024±0.001^{a}	0.054±0.003	0.048±0.002		
	Cow manure	0.127±0.011^{a}	0.110±0.051^{a}	0.118±0.041^{a}	0.024±0.007^{a}	0.019±0.002^{a}	0.021±0.001^{a}				
	Control (soil / water)	0.011±0.001	0.028±0.011	0.033±0.016	0.009±0.000	0.011±0.001	0.021±0.001	0.028±0.001	0.018±0.000		
Ratio TRY : (HL:FL)	Cow faeces	806±231^{b}								427	186
	Pig slurry	2073±191^{c}	673±203^{b}	526±114^{a}	458±68^{b}	314±50^{a}	221±11^{b}	283±14	258±12		
	Cow manure	563±76^{a}	491±130^{a}	502±117^{a}	351±56^{a}	279±44^{a}	186±9^{a}				
	Control (soil / water)	267±44	315±66	358±109	205±43	162±18	180±9	158±8	145 ±7		

Color indicate the significant difference of farm wastes DOM from soil ($p<0.05$, one factor ANOVA)

different letters represent significant difference between pig slurry and cow manure (one factor ANOVA; $p<0.05$, ± standard deviation)

Table 2 : Persistence of fluorescence tracers in surface transfer and in soils

Tracers	**Rapid surface transfer**		**Persistence in Kerguehennec site (2.5% C)**			**Persistence in Champ Noël site (1% C)**	
	Discrimination pig and cow / soil	Discrimination pig / cow	Short term	Long term: 7 years of recycling (one year later)	Discrimination pig / cow	Short term (not studied)	Long term: 14 years of recycling (one year later)
Ratio bio:geo	yes	no	week	no	no	-	yes
Region V	yes	no	no	no	no	-	no
Region III	yes	no	no	no	no	-	no
TRY	yes	no	2 months	yes	no	-	yes
Ratio TRY:HL	Yes	no	2 months	no	no	-	yes
Ratio TRY:(HL:FL)	yes	no	2 months	no	no	-	yes

Limitations and perspectives of this study

Long term monitoring of farm wastes soil amendments

A biodegradation experiment should be carried out in the laboratory for one year on pig slurry amended soil at agronomic dose. If TRY fluorescence maintained in the biodegradation environment for one year, then it means the persistence otherwise it will be cumulative effect of long term pig slurry waste recycling.

At Kerguehennec site, there is no differentiation between pig slurry and cow manure amended soils. But discrimination between pig slurry and cow manure soil amendment at loamy soil of Champ Noël site rest to be quantified.

Stream water sampling in agricultural headwater catchments

Long term monitoring of dissolved organic matter sources in stream have to be carried out in baseflow periods and highflow storms. Although dissolved organic matter concentrations remained low during baseflow periods but the chemical characterization of dissolved organic matter during baseflow and highflow will enable us to differentiate the functionality of fluorescence tracers in stream bed during baseflow and hillslope contribution during storms. Temporal variability has to be studied through fluorescence characterization of baseflow period and highflow storms. Soil extractions have also to be started in small agricultural catchments of 15c and 19d to study spatial variability of DOM in Valley Bottom Wetlands.

Management actions

Fluorescence spectroscopy permits to identify the animals DOM origin from soil. To restore stream water quality, management actions have to be taken at small agricultural catchment scale in Valley Bottom Wetlands. To avoid possible direct transfer of DOM from animal faeces in the areas of intensive grazing pastures, buffered zones and embankments should be developed along the bank stream.

Besides this, possible human waste contribution should also be considered as domestic wastes also show the tryptophan fluorescence.

Need for statistical analysis

Classification and Regression Tree (CART) analysis, although, discriminated well the farm waste soil contamination from mineral fertilized soils but it was tested in dataset of two

months soil amendments and same soil type. Therefore, we encourage to apply CART tree approach on a large dataset with different soil types and on long term monitoring devices. However, the functionality of this approach limited to large number of observations (3:1; observation / predictors) per treatment.

Another statistical approach Parallel Factor Analysis (PARAFAC) also has to be included in the tracing studies as it is advance stage of principal components analysis and decomposes the excitation emission matrix (EEM) fluorescence landscape and identify well the chemical suit of dissolved organic matter via principal components.

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Annexes

Chapter 1

	ratio bio:geo	ratio III:V	ratio IV:V	ratio TRY:FL	ratio TRY:HL	ratio HL:FL	ratio TRY:FL
ratio codes	r b:g	r III:V	r IV:V	r T:F	r T:H	r H:F	r T:F

Farm wastes	I	II	III	IV	V	bio	geo	r b:g	r III:V	r IV:V	F	H	TRY	r T:F	T:H	r H:F	r T:(H:F)	SUVA	HIX
CF1	690	1323	10912	7583	6683	9596	17594	0,545	1,633	1,135	3322	3112	683	0,206	0,220	0,937	729	1,230	0,469
CF1	842	1347	11388	7980	6905	10170	18293	0,556	1,649	1,156	3479	3231	716	0,206	0,222	0,929	771	1,007	0,444
CF1	766	1335	11150	7782	6794	9883	17944	0,551	1,641	1,145	3400	3171	700	0,206	0,221	0,933	750	1,118	0,456
CF2	609	1335	10696	6118	5664	8062	16361	0,493	1,888	1,080	2870	2996	577	0,201	0,193	1,044	553	0,072	0,524
CF2	544	1229	10726	5892	6007	7665	16733	0,458	1,786	0,981	2969	2857	572	0,193	0,200	0,962	595	0,077	0,547
CF2	576	1282	10711	6005	5836	7864	16547	0,475	1,836	1,029	2920	2927	575	0,197	0,196	1,002	573	0,074	0,536
CMF	353	1173	11064	4399	8612	5925	19676	0,301	1,285	0,511	4064	2970	394	0,097	0,133	0,731	539	0,073	0,671
CMF	410	1313	12396	5040	9291	6763	21687	0,312	1,334	0,542	4508	3275	455	0,101	0,139	0,727	626	0,072	0,665
CMF	382	1243	11730	4719	8952	6344	20682	0,307	1,310	0,527	4286	3123	424	0,099	0,136	0,729	583	0,072	0,668
CMC4	72	1637	29996	5735	30027	7444	60023	0,124	0,999	0,191	14500	8077	385	0,027	0,048	0,557	691	3,072	0,873
CMC4	297	1531	28806	5466	29069	7294	57876	0,126	0,991	0,188	13993	7638	364	0,026	0,048	0,546	666	2,915	0,871
CMC4	149	1622	30142	5767	30167	7538	60309	0,125	0,999	0,191	14388	8127	382	0,027	0,047	0,565	676	3,011	0,873
CMC6	468	2381	39252	8069	37944	10918	77197	0,141	1,034	0,213	18628	10891	500	0,027	0,046	0,585	856	3,373	0,876
CMC6	260	2038	36155	7215	34871	9512	71025	0,134	1,037	0,207	17234	9910	454	0,026	0,046	0,575	789	3,107	0,883
CMC6	328	1995	35056	6976	33922	9300	68977	0,135	1,033	0,206	16652	9833	431	0,026	0,044	0,591	730	3,417	0,884
CMC1	230	879	10974	2650	9837	3759	20811	0,181	1,116	0,269	4909	2976	209	0,043	0,070	0,606	344	3,212	0,874
CMC1	213	855	10617	2588	9440	3656	20057	0,182	1,125	0,274	4719	2899	195	0,041	0,067	0,614	318	3,055	0,885
CMC1	222	867	10795	2619	9639	3708	20434	0,181	1,120	0,272	4814	2937	202	0,042	0,069	0,610	331	3,134	0,879

Farm wastes	I	II	III	IV	V	bio	geo	r b:g	r III:V	r IV:V	F	H	TRY	r T:F	T:H	r H:F	r T:(H:F)	SUVA	HIX
CFd	695	2045	19366	9379	11692	12119	31058	0,390	1,656	0,802	5746	5058	933	0,162	0,185	0,880	1060	1,206	0,617
CFd	718	2152	20750	9828	12374	12697	33124	0,383	1,677	0,794	6058	5268	999	0,165	0,190	0,870	1149	1,255	0,627
CFd	661	2057	19256	9249	11555	11967	30811	0,388	1,666	0,800	5677	4902	932	0,164	0,190	0,864	1079	1,094	0,622
CM1	222	959	11588	4175	11183	5356	22772	0,235	1,036	0,373	5158	2946	375	0,073	0,127	0,571	656	2,831	0,721
CM1	259	882	10564	4022	10228	5162	20791	0,248	1,033	0,393	4721	2693	371	0,079	0,138	0,570	651	2,990	0,636
CM1	145	749	9389	3492	9197	4387	18586	0,236	1,021	0,380	4313	2369	326	0,076	0,138	0,549	594	2,676	0,705
CM2	256	1006	9979	3619	8754	4881	18733	0,261	1,140	0,413	4339	2932	333	0,077	0,114	0,676	493	3,263	0,696
CM2	249	989	9456	3402	8117	4640	17573	0,264	1,165	0,419	4042	2856	315	0,078	0,110	0,707	446	2,503	0,697
CM2	210	883	9294	3275	8561	4368	17855	0,245	1,086	0,383	4232	2638	304	0,072	0,115	0,623	488	2,115	0,707
PS1	479	2535	19577	12828	14777	16090	34354	0,468	1,325	0,868	7284	5200	1297	0,178	0,249	0,714	1817	0,981	0,511
PS1	502	2983	22365	14649	16640	18464	39005	0,473	1,344	0,880	8093	5982	1509	0,186	0,252	0,739	2042	2,205	0,524
PS1	455	2759	20971	13739	15709	17277	36679	0,471	1,335	0,875	7688	5591	1403	0,182	0,251	0,727	1929	1,593	0,517
PS2	727	2914	22503	14746	16986	18496	39489	0,468	1,325	0,868	8372	5977	1491	0,178	0,249	0,714	2088	0,730	0,696
PS2	832	3429	25708	16838	19127	21224	44835	0,473	1,344	0,880	9303	6877	1735	0,186	0,252	0,739	2347	0,978	0,694
PS2	779	3172	24106	15792	18057	19860	42162	0,471	1,335	0,875	8838	6427	1613	0,182	0,251	0,727	2218	0,854	0,695
WS	835	683	5284	2968	4415	3916	9700	0,404	1,197	0,672	2631	1280	299	0,114	0,234	0,487	614	0,848	0,478
WS	956	657	5300	2945	4475	3845	9774	0,393	1,184	0,658	2665	1270	298	0,112	0,235	0,476	625	1,294	0,480
WS	896	657	5311	3047	4473	3972	9784	0,406	1,187	0,681	2682	1263	309	0,115	0,245	0,471	656	0,979	0,477
Soil	266	3173	60746	6010	51668	9702	112414	0,086	1,176	0,116	24236	19238	192	0,008	0,010	0,794	242	3,495	0,958
Soil	243	2940	61221	5793	52865	9091	114086	0,080	1,158	0,110	24741	18984	186	0,008	0,010	0,767	243	3,580	0,961
Soil	269	3300	65087	6625	55112	10318	120199	0,086	1,181	0,120	25731	20405	253	0,010	0,012	0,793	320	3,687	0,952

Farm Wastes Mixtures	I	II	III	IV	V	bio	geo	r b:g	r III:V	r IV:V	F	H	TRY	r T:F	T:H	r H:F	r T:(H:F)	SUVA	HIX
CMC3	328	1995	35056	6976	33922	9300	68977	0,135	1,033	0,206	16652	9833	431	0,026	0,044	0,591	730	3,458	0,884
CMC3	345	2095	36808	7325	35618	9765	72426	0,142	1,085	0,216	17484	10325	453	0,027	0,046	0,620	767	3,631	0,928
CMC3	312	1896	33303	6627	32225	8835	65528	0,128	0,982	0,195	15819	9341	410	0,025	0,042	0,561	694	3,286	0,840
NW (Stream 2)	327	2972	61039	6872	57625	10171	118664	0,086	1,059	0,119	28067	17167	254	0,009	0,015	0,612	415	4,035	0,918
NW (Stream 2)	344	3121	64091	7215	60506	10680	124597	0,090	1,112	0,125	29470	18026	266	0,009	0,016	0,642	435	4,236	0,964
NW (Stream 2)	311	2824	57987	6528	54744	9663	112730	0,081	1,006	0,113	26664	16309	241	0,009	0,014	0,581	394	3,833	0,872
50% mix	167	2184	45940	6793	44340	9144	90279	0,101	1,036	0,153	21851	12591	335	0,015	0,027	0,576	582	2,704	0,954
50% mix	175	2293	48237	7133	46557	9602	94793	0,106	1,088	0,161	22943	13220	352	0,016	0,028	0,605	611	2,839	1,002
50% mix	159	2075	43643	6454	42123	8687	85765	0,096	0,984	0,146	20758	11961	318	0,015	0,025	0,547	553	2,569	0,906
25% CMC3	605	4689	95418	11727	90403	17022	185821	0,092	1,055	0,130	43844	26906	480	0,011	0,018	0,614	783	3,108	0,939
50% CMC3	167	2184	45940	6793	44340	9144	90279	0,101	1,036	0,153	21851	12591	335	0,015	0,027	0,576	582	2,704	0,954
75% CMC3	873	4291	77833	12927	74828	18091	152660	0,119	1,040	0,173	36556	22017	712	0,019	0,032	0,602	1183	3,182	0,900
PS	479	820	7013	2742	5766	4041	12779	0,316	1,216	0,476	2823	2022	223	0,079	0,110	0,716	311	0,270	0,733
PS	502	861	7364	2879	6054	4243	13418	0,332	1,277	0,499	2964	2123	234	0,083	0,116	0,752	327	0,283	0,769
PS	455	779	6663	2605	5477	3839	12140	0,300	1,156	0,452	2682	1921	212	0,075	0,105	0,680	296	0,256	0,696
NW (Stream 1)	796	1042	18604	2458	16905	4296	35509	0,121	1,101	0,145	8221	5236	93	0,011	0,018	0,637	146	1,655	0,867
NW (Stream 1)	836	1094	19534	2581	17750	4510	37285	0,127	1,156	0,153	8632	5498	98	0,012	0,019	0,669	153	1,738	0,910
NW (Stream 1)	756	990	17674	2335	16060	4081	33734	0,115	1,045	0,138	7810	4974	88	0,011	0,017	0,605	139	1,572	0,823
50% mix	596	901	12371	2620	10874	4118	23244	0,177	1,138	0,241	5355	3484	168	0,031	0,048	0,651	259	0,781	0,815
50% mix	626	946	12989	2751	11417	4324	24407	0,186	1,195	0,253	5622	3658	177	0,033	0,051	0,683	272	0,820	0,856
50% mix	567	856	11752	2489	10330	3912	22082	0,168	1,081	0,229	5087	3309	160	0,030	0,046	0,618	246	0,742	0,774

Chapter 2

Chapter 2 (Annex 1)

	Runoff Water	ratio bio:geo	ratio III:V	ratio IV:V	ratio TRY:FL	ratio TRY:HL	ratio HL:FL	ratio TRY:FL
Codes	RW	r b:g	r III:V	r IV:V	r T:F	r T:H	r H:F	r T:F

Annex 1

Treatment	RW(L)	DOC (mg/L)	I	II	III	IV	V	bio	geo	r b:g	r III:V	HIX
R1 Control	0,77	5,1	306	1487	27597	3423	23602	5217	51199	0,103	1,1693	0,90
R1 Control	4,77	3,8	388	2020	41586	4831	35629	7240	77215	0,094	1,1672	0,94
R1 Control	5,55	3,2	397	1996	40794	4635	34967	7028	75760	0,094	1,1666	0,93
R1 Control	9,68	3,3	389	1968	38066	4571	32572	6928	70638	0,100	1,1687	0,93
R1 Control	10,47	4,1	364	1775	30670	4077	25499	6216	56169	0,111	1,2028	0,92
R1 Control	11,27	3,9	292	1798	34238	4158	28403	6248	62640	0,101	1,2054	0,93
R1 Control	13,02	4,1	399	2020	32973	4326	27331	6744	60305	0,112	1,2064	0,92
R2 control	0,78	2,8	501	1730	27850	4056	22996	6287	50845	0,126	1,2111	0,90
R2 control	4,74	2,1	586	1827	30706	4806	24836	7219	55542	0,131	1,2363	0,89
R2 control	5,51	2,5	489	1418	23272	4062	19239	5969	42511	0,141	1,2096	0,88
R2 control	9,47	2,2	519	1430	24506	3870	19970	5819	44476	0,138	1,2272	0,88
R2 control	10,25	2,2	568	1473	23988	4000	19639	6042	43627	0,141	1,2215	0,88
R2 control	11,83	1,9	896	1885	26711	5859	20877	8640	47588	0,182	1,2795	0,82
R3 Control	0,77	3,8	409	928	13787	2607	11137	3944	24924	0,158	1,2380	0,86
R3 Control	4,75	3,5	448	1722	31485	4138	26587	6308	58072	0,111	1,1842	0,91
R3 Control	5,52	3,1	531	1926	34088	4716	28808	7173	62896	0,115	1,1833	0,91
R3 Control	9,62	3,4	431	1528	27503	3718	23221	5677	50724	0,114	1,1844	0,91
R3 Control	10,39	2,3	660	2182	36179	5539	30095	8380	66274	0,127	1,2022	0,89
R3 Control	11,96	2,9	452	1397	24672	3584	20637	5433	45309	0,122	1,1955	0,89
R3 Control	16,85	3,2	505	1267	17448	3885	13862	5658	31310	0,177	1,2587	0,83

Treatment	RW(L)	DOC (mg/L)	I	II	III	IV	V	bio	geo	r b:g	r III:V	HIX
R1 Pig slurry	0,75	70,8	522	1466	16276	5884	11794	7871	28070	0,281	1,3800	0,71
R1 Pig slurry	4,55	40,7	733	2086	23656	8454	17840	11273	41496	0,280	1,3260	0,73
R1 Pig slurry	5,34	32,8	351	1358	18473	4473	14081	6182	32554	0,188	1,3119	0,82
R1 Pig slurry	9,39	28,1	444	1482	19810	4975	15397	6902	35206	0,196	1,2866	0,82
R1 Pig slurry	10,17	22,7	399	1313	19627	4143	16166	5854	35793	0,162	1,2141	0,85
R1 Pig slurry	11,76	23,7	356	1299	18416	4186	14013	5841	32428	0,176	1,3142	0,84
R1 Pig slurry	16,82	21,5	434	1477	21297	5082	16313	6993	37610	0,182	1,3055	0,83
R2 Pig slurry	0,77	64,6	535	1492	15668	6013	11277	8040	26946	0,300	1,3894	0,69
R2 Pig slurry	4,71	28,3	382	1170	15421	4630	11510	6182	26931	0,229	1,3397	0,77
R2 Pig slurry	5,49	20,2	468	1419	17892	5725	13135	7612	31027	0,256	1,3622	0,76
R2 Pig slurry	9,55	17,2	427	1612	25143	5920	19231	7959	44374	0,173	1,3074	0,80
R2 Pig slurry	10,33	14,3	530	1642	20650	5371	15571	7543	36222	0,209	1,3262	0,81
R2 Pig slurry	11,9	13,5	540	1579	19619	5078	15040	7197	34659	0,200	1,3045	0,81
R2 Pig slurry	16,37	9,6	243	997	17629	2508	14738	3747	32367	0,105	1,1962	0,87
R3 Pig slurry	0,78	65,1	642	1842	17163	7618	11723	10102	28886	0,353	1,4640	0,66
R3 Pig slurry	4,78	86,6	381	1069	10880	4434	7666	5884	18546	0,313	1,4192	0,66
R3 Pig slurry	5,565	75,3	392	1151	11305	4503	7949	6046	19254	0,310	1,4222	0,69
R3 Pig slurry	9,565	65,2	407	1202	11958	4687	8520	6295	20479	0,300	1,4035	0,69
R3 Pig slurry	10,335	53,5	404	1210	12179	4501	8808	6115	20987	0,290	1,3827	0,71
R3 Pig slurry	11,905	50,6	431	1274	13160	4774	9766	6478	22926	0,274	1,3475	0,71
R3 Pig slurry	17,265	37,5	470	1369	15251	4884	11401	6724	26652	0,251	1,3377	0,75

Treatment	RW(L)	DOC (mg/L)	I	II	III	IV	V	bio	geo	r b:g	r III:V	HIX
R1 Cow manure	0,76	23,5	373	1009	10989	3492	8657	4874	19645	0,244	1,2694	0,76
R1 Cow manure	4,64	93,7	456	1116	9346	4514	7076	6085	16421	0,369	1,3208	0,64
R1 Cow manure	5,4	88,6	410	1023	9334	4087	7143	5520	16476	0,330	1,3068	0,66
R1 Cow manure	9,31	71,2	369	1068	10065	3786	8528	5223	18593	0,271	1,1802	0,71
R1 Cow manure	10,08	66,8	387	1053	10126	3821	7906	5261	18032	0,283	1,2808	0,70
R1 Cow manure	11,61	63,6	400	1141	10992	4010	8586	5550	19577	0,282	1,2803	0,72
R1 Cow manure	15,81	52,0	372	1113	11883	3934	9148	5420	21031	0,258	1,2990	0,75
R2 Cow manure	0,78	11,9	502	1329	14757	4571	11292	6402	26050	0,244	1,3068	0,76
R2 Cow manure	4,71	25,6	533	1434	13423	4990	9245	6957	22668	0,306	1,4518	0,73
R2 Cow manure	5,51	34,9	439	1159	10900	4281	7504	5879	18404	0,317	1,4525	0,72
R2 Cow manure	9,48	38,1	384	1055	9889	3829	7508	5268	17397	0,302	1,3171	0,71
R2 Cow manure	10,26	35,0	361	1063	10655	3747	7477	5172	18132	0,284	1,4250	0,74
R2 Cow manure	11,79	27,3	476	1380	13370	4914	9839	6769	23209	0,291	1,3590	0,73
R2 Cow manure	15,56	24,7	517	1428	13166	4937	9579	6882	22745	0,303	1,3746	0,73
R3 Cow manure	0,77	29,4	249	1003	16014	2858	15144	4110	31158	0,133	1,0575	0,88
R3 Cow manure	4,57	17,6	264	930	15038	2672	13889	3866	28927	0,130	1,0827	0,88
R3 Cow manure	5,34	15,7	300	1025	15467	3016	14015	4341	29483	0,146	1,1036	0,87
R3 Cow manure	9,16	15,8	317	1068	15991	3191	14283	4576	30275	0,149	1,1196	0,86
R3 Cow manure	9,94	15,2	306	1021	15385	3064	13787	4391	29172	0,149	1,1159	0,86
R3 Cow manure	11,52	13,2	267	1049	17401	3203	15705	4519	33106	0,132	1,1080	0,87
R3 Cow manure	15,145	13,8	259	973	15133	3022	13444	4254	28577	0,145	1,1257	0,86

Chapter 2 (Annex 2)

Annex 2

6L		I	II	III	IV	V	bio	geo	r b:g	r III:V	r IV:V	FL	HL	TRY	r T:F	T:H	r H:F	r T:(H:F)	HIX
CR1	Average	364	1835	36659	4296	31399	6495	68058	0,096	1,168	0,138	15321	10870	184	0,012	0,017	0,710	259,826	0,92
PSR1	Average	535	1637	19468	6270	14571	8442	34040	0,247	1,339	0,430	7294	5484	570	0,078	0,103	0,754	757,391	0,76
CMR1	Average	413	1049	9889	4031	7625	5493	17514	0,318	1,297	0,538	3752	2708	396	0,107	0,149	0,722	548,783	0,69
	Standard error	29	174	4537	440	3903	642	8440	0,003	0,001	0,004	1875	1307	11	0,001	0,001	0,002	16,330	0,01
	Standard error	111	227	2188	1165	1762	1497	3946	0,029	0,024	0,057	875	583	123	0,012	0,016	0,014	168,981	0,03
	Standard error	24	33	550	296	516	350	1066	0,036	0,015	0,070	243	182	37	0,016	0,022	0,004	50,559	0,04
6L																			
CR2	Average	526	1659	27276	4308	22357	6492	49633	0,131	1,214	0,194	10664	7915	286	0,027	0,037	0,741	385,332	0,89
PSR2	Average	462	1361	16327	5456	11974	7278	28301	0,258	1,366	0,457	6010	4577	475	0,079	0,104	0,762	622,108	0,74
CMR2	Average	491	1307	13027	4614	9347	6413	22374	0,291	1,401	0,505	4552	3534	462	0,104	0,133	0,780	592,973	0,73
	Standard error	30	124	2165	249	1647	375	3810	0,005	0,007	0,010	797	707	24	0,003	0,004	0,011	29,913	0,01
	Standard error	44	97	786	421	584	562	1363	0,021	0,017	0,039	281	206	49	0,010	0,012	0,007	58,884	0,03
	Standard error	28	80	1131	206	1095	311	2212	0,023	0,049	0,051	523	336	24	0,011	0,012	0,020	25,283	0,01
6L																			
CR3	Average	462	1525	26453	3821	22177	5808	48631	0,127	1,201	0,184	10599	7839	223	0,024	0,032	0,742	300,583	0,89
PSR3	Average	472	1354	13116	5519	9113	7344	22229	0,327	1,436	0,598	4628	3716	516	0,109	0,137	0,801	642,207	0,67
CMR3	Average	271	986	15507	2849	14349	4106	29856	0,138	1,074	0,199	6802	4205	206	0,030	0,049	0,619	332,900	0,88
	Standard error	36	304	6377	629	5557	965	11935	0,016	0,018	0,025	2683	1947	18	0,006	0,008	0,006	26,732	0,02
	Standard error	85	245	2027	1050	1308	1380	3335	0,011	0,016	0,026	671	570	111	0,007	0,008	0,006	132,698	0,01
	Standard error	15	39	752	81	649	130	1400	0,012	0,015	0,025	314	224	11	0,004	0,005	0,006	18,491	0,01

Annex 2																			
14L		**I**	**II**	**III**	**IV**	**V**	**bio**	**geo**	**r b:g**	**r III:V**	**r IV:V**	**F**	**H**	**TRY**	**r T:F**	**T:H**	**r H:F**	**r T:(H:F)**	**HIX**
CR1	Average	361	1890	33987	4283	28451	6534	62438	0,105	1,198	0,151	13910	10215	211	0,015	0,021	0,735	287,048	0,92
PSR1	Average	408	1393	19787	4597	15472	6398	35259	0,181	1,277	0,297	7530	5488	385	0,051	0,070	0,729	527,722	0,84
CMR1	Average	382	1094	10766	3888	8542	5364	19308	0,278	1,403	0,456	4213	2923	384	0,091	0,132	0,694	553,762	0,72
	Standard error	24	61	1547	109	1499	179	3043	0,004	0,008	0,005	732	451	3	0,001	0,001	0,009	5,783	0,00
	Standard error	20	50	591	251	526	318	1073	0,007	0,022	0,015	235	147	24	0,002	0,003	0,009	33,141	0,01
	Standard error	7	20	428	51	254	75	657	0,007	0,167	0,012	127	118	5	0,002	0,005	0,013	12,766	0,01
14L																			
CR2	Average	661	1596	25069	4576	20162	6834	45230	0,150	1,224	0,226	9543	7317	347	0,036	0,047	0,766	448,483	0,86
PSR2	Average	435	1457	20761	4719	16145	6611	36905	0,178	1,280	0,290	7941	5989	369	0,046	0,061	0,754	486,840	0,82
CMR2	Average	435	1232	11770	4357	8601	6023	20371	0,296	1,383	0,506	4180	3114	441	0,105	0,142	0,745	592,600	0,73
	Standard error	118	145	835	642	370	905	1204	0,016	0,002	0,027	134	302	75	0,007	0,008	0,021	83,888	0,02
	Standard error	69	154	1590	758	1043	967	2612	0,022	0,031	0,041	553	417	76	0,009	0,012	0,007	98,115	0,02
	Standard error	37	100	880	329	642	465	1515	0,004	0,014	0,004	298	222	34	0,001	0,003	0,011	47,090	0,01
14L																			
CR3	Average	512	1593	26450	4182	21954	6287	48404	0,135	1,209	0,200	10491	7832	271	0,028	0,037	0,748	361,684	0,88
PSR3	Average	428	1264	13137	4711	9624	6403	22761	0,283	1,368	0,495	4818	3738	438	0,092	0,118	0,777	563,754	0,71
CMR3	Average	287	1028	15978	3120	14305	4435	30282	0,147	1,113	0,219	6761	4330	231	0,034	0,053	0,641	359,884	0,86
	Standard error	52	203	3873	457	3355	700	7226	0,016	0,016	0,027	1616	1196	40	0,007	0,009	0,006	51,614	0,02
	Standard error	15	29	283	99	399	137	671	0,005	0,015	0,008	201	95	10	0,002	0,002	0,008	13,236	0,01
	Standard error	14	21	507	45	498	72	1005	0,003	0,003	0,005	226	134	1	0,001	0,001	0,002	1,686	0,00

Chapter 3

Chapter 3 (Annex 1)

Annex1	Dissolved organic carbon concentrations (DOC), mg L^{-1}				
	Days after incubation	soil alone	wheat straw	Pig manure	Cow manure
Average	0	12	29	74	43
Average	1	13	26	43	32
Average	3	15	25	51	31
Average	7	17	37	38	39
Average	15	23	41	38	37
Average	30	17	33	43	41
Average	56	15	35	47	30
Standard error	0	0,249	2,939	5,696	3,655
Standard error	1	0,416	3,838	6,286	5,380
Standard error	3	0,863	0,861	2,284	0,605
Standard error	7	0,937	3,217	0,891	2,804
Standard error	15	0,817	2,566	0,295	0,460
Standard error	30	0,879	1,410	8,691	7,153
Standard error	56	0,260	2,989	7,550	2,100

Chapter 3 (Annex 2)

Treatments	
SA	Soil alone
WS	Wheat straw
PM	Pig manure
CM	Cow manure
P1	Period 1 (0-7 days)
P2	Period 1 (8-56 days)

	ratio bio:geo	ratio III:V	ratio IV:V	ratio TRY:FL	ratio TRY:HL	ratio HL:FL
codes	r b:g	r III:V	r IV:V	r T:F	r T:H	r H:F

Annex 2

Treatment	III	IV	V	geo	**r b:g**	**r III:V**	**r IV:V**	**FL**	**HL**	**TRY**	**r T:F**	**r T:H**	**r H:F**	A365	HIX
SAP1	59725	5611	39791	109437	0,085	1,201	0,113	23289	19933	179	0,008	0,009	0,856	0,051	0,958
SAP1	58446	5439	39618	108216	0,081	1,174	0,109	23266	18828	175	0,008	0,009	0,809	0,057	0,961
SAP1	65718	6429	43705	119715	0,087	1,217	0,119	25165	22012	250	0,010	0,011	0,875	0,052	0,952
SAP1	56385	4836	39342	105759	0,070	1,142	0,098	27859	27258	261	0,009	0,010	0,978	0,034	0,969
SAP1	51528	4795	36241	97383	0,074	1,124	0,105	23136	17043	146	0,006	0,009	0,737	0,044	0,963
SAP1	58312	5503	39951	108374	0,080	1,165	0,110	21411	15287	161	0,008	0,011	0,714	0,037	0,960
SAP1	46071	4356	32955	88050	0,074	1,097	0,104	19430	13116	140	0,007	0,011	0,675	0,033	0,960
SAP1	40989	3892	28529	77667	0,076	1,118	0,106	17224	12461	125	0,007	0,010	0,723	0,023	0,962
SAP1	43661	3986	30333	82611	0,073	1,121	0,102	18248	13328	124	0,007	0,009	0,730	0,017	0,965
SAP1	38354	3659	26254	71388	0,079	1,161	0,111	15514	12100	129	0,008	0,011	0,780	0,020	0,944
SAP1	35722	3240	25152	67140	0,072	1,137	0,103	14758	10570	99	0,007	0,009	0,716	0,007	0,969
SAP2	32018	3290	22377	59312	0,081	1,173	0,121	12948	9641	115	0,009	0,012	0,745	0,073	0,955
SAP2	32477	3195	22740	60517	0,077	1,158	0,114	13439	9737	116	0,009	0,012	0,725	0,050	0,966
SAP2	30152	2868	21101	56359	0,075	1,151	0,109	12572	9052	98	0,008	0,011	0,720	0,062	0,964
SAP2	35258	3885	24571	65844	0,088	1,153	0,127	14657	10687	144	0,010	0,013	0,729	0,019	0,945
SAP2	41383	4158	27291	75496	0,089	1,213	0,122	16411	14092	142	0,009	0,010	0,859	0,029	0,955
SAP2	37925	3806	25354	69677	0,087	1,194	0,120	15298	12571	132	0,009	0,011	0,822	0,019	0,957
SAP2	42461	4143	27985	77681	0,086	1,206	0,118	16951	14476	137	0,008	0,009	0,854	0,029	0,957

Annex 2															
Treatment	III	IV	V	geo	**r b:g**	**r III:V**	**r IV:V**	**FL**	**HL**	**TRY**	**r T:F**	**r T:H**	**r H:F**	A365	HIX
WSP1	36570	4626	25992	68898	0,097	1,131	0,143	15595	10579	243	0,016	0,023	0,678	0,022	0,931
WSP1	36143	5251	25915	68254	0,108	1,126	0,164	15595	10425	290	0,019	0,028	0,668	0,018	0,916
WSP1	39728	4487	28341	75545	0,087	1,109	0,125	17099	11387	209	0,012	0,018	0,666	0,036	0,941
WSP1	41224	5009	28980	77656	0,095	1,132	0,137	17386	12244	256	0,015	0,021	0,704	0,047	0,939
WSP1	32125	4485	23087	60505	0,106	1,132	0,158	13873	9039	267	0,019	0,030	0,651	0,034	0,912
WSP1	36545	3716	26456	70067	0,076	1,090	0,111	15912	10089	151	0,009	0,015	0,634	0,044	0,955
WSP1	33272	3588	23840	63469	0,081	1,102	0,119	14451	9432	155	0,011	0,016	0,653	0,031	0,948
WSP1	37798	4709	26968	71454	0,099	1,123	0,140	16190	10830	223	0,014	0,021	0,669	0,033	0,922
WSP1	34135	4781	24741	64336	0,108	1,130	0,158	14437	9393	253	0,018	0,027	0,651	0,032	0,913
WSP1	29882	4601	22030	56305	0,115	1,131	0,174	13001	7852	291	0,022	0,037	0,604	0,027	0,898
WSP2	31411	4143	23041	58796	0,098	1,147	0,151	13187	8370	227	0,017	0,027	0,635	0,036	0,925
WSP2	28355	3615	20960	53648	0,092	1,121	0,143	12195	7395	201	0,016	0,027	0,606	0,036	0,930
WSP2	27791	3092	20330	52428	0,082	1,128	0,125	12209	7461	146	0,012	0,020	0,611	0,032	0,941
WSP2	37172	4152	27125	71000	0,082	1,099	0,123	16140	10047	193	0,012	0,019	0,622	0,029	0,939
WSP2	34061	4024	24919	64860	0,086	1,106	0,131	14645	9141	203	0,014	0,022	0,624	0,043	0,935
WSP2	34139	3838	24799	64824	0,083	1,113	0,125	14721	9340	174	0,012	0,019	0,634	0,032	0,941
WSP2	35694	4303	25370	66667	0,097	1,152	0,139	14806	10323	177	0,012	0,017	0,697	0,029	0,937
WSP2	37152	4669	26679	69557	0,099	1,146	0,144	15629	10473	213	0,014	0,020	0,670	0,035	0,928
WSP2	36065	4441	26015	67500	0,095	1,147	0,141	15169	10050	203	0,013	0,020	0,663	0,030	0,936

Annex 2

Treatment	III	IV	V	geo	**r b:g**	**r III:V**	**r IV:V**	**FL**	**HL**	**TRY**	**r T:F**	**r T:H**	**r H:F**	A365	HIX
PMP1	35631	4734	26227	66589	0,099	1,151	0,153	14644	9403	270	0,018	0,029	0,642	0,003	0,928
PMP1	46265	6196	33882	85774	0,100	1,171	0,157	18611	12383	348	0,019	0,028	0,665	0,085	0,913
PMP1	38799	5750	28686	73306	0,107	1,124	0,167	16256	10112	364	0,022	0,036	0,622	0,013	0,921
PMP1	51962	6352	37986	97890	0,092	1,131	0,138	21881	13976	336	0,015	0,024	0,639	0,002	0,938
PMP1	49474	5621	36465	94153	0,084	1,107	0,126	20824	13009	283	0,014	0,022	0,625	0,020	0,945
PMP1	42860	5790	31212	80273	0,099	1,146	0,155	17896	11648	327	0,018	0,028	0,651	0,028	0,928
PMP1	45699	5204	33493	86713	0,083	1,114	0,127	19350	12206	266	0,014	0,022	0,631	0,032	0,944
PMP1	43140	5226	31770	81760	0,087	1,117	0,135	18075	11315	281	0,016	0,025	0,626	0,028	0,938
PMP1	44880	5423	32212	84354	0,093	1,137	0,137	18990	12668	271	0,014	0,021	0,667	0,008	0,935
PMP1	44820	5118	32089	84346	0,088	1,134	0,129	18970	12731	237	0,013	0,019	0,671	0,021	0,945
PMP1	48459	5611	34692	91180	0,089	1,134	0,131	20419	13768	271	0,013	0,020	0,674	0,013	0,944
PMP2	39674	4656	28410	74045	0,088	1,154	0,135	16539	11264	231	0,014	0,020	0,681	0,022	0,941
PMP2	38892	3838	27785	73031	0,075	1,139	0,112	16534	11107	146	0,009	0,013	0,672	0,020	0,961
PMP2	43931	5369	30922	81141	0,096	1,181	0,144	17926	13008	254	0,014	0,020	0,726	0,026	0,935
PMP2	44707	5341	31579	83298	0,093	1,158	0,138	18699	13128	255	0,014	0,019	0,702	0,030	0,939
PMP2	47248	4903	33939	88185	0,080	1,154	0,120	19628	13309	210	0,011	0,016	0,678	0,033	0,953
PMP2	48460	4712	34932	90657	0,075	1,148	0,112	20344	13528	185	0,009	0,014	0,665	0,035	0,961
PMP2	47440	5297	33304	88396	0,087	1,158	0,129	19797	14136	244	0,012	0,017	0,714	0,023	0,950

Annex 2

Treatment	III	IV	V	geo	**r b:g**	**r III:V**	**r IV:V**	**FL**	**HL**	**TRY**	**r T:F**	**r T:H**	**r H:F**	A365	HIX
CMP1	32067	4379	23035	61475	0,104	1,090	0,149	13975	9032	228	0,016	0,025	0,646	0,025	0,919
CMP1	33431	4753	24138	63931	0,106	1,096	0,156	14532	9293	251	0,017	0,027	0,640	0,073	0,920
CMP1	40008	5081	28626	76952	0,098	1,083	0,138	17418	11382	239	0,014	0,021	0,653	0,040	0,931
CMP1	40928	4496	28959	78197	0,086	1,098	0,121	17543	11969	179	0,010	0,015	0,682	0,039	0,948
CMP1	54359	5451	39837	102666	0,075	1,125	0,113	22655	14522	227	0,010	0,016	0,641	0,047	0,958
CMP1	34459	4124	24661	66055	0,090	1,091	0,131	15018	9799	199	0,013	0,020	0,652	0,042	0,935
CMP1	33790	3878	24326	65160	0,085	1,077	0,124	14893	9464	187	0,013	0,020	0,635	0,034	0,942
CMP1	32360	3728	23237	62136	0,086	1,087	0,125	14146	9124	173	0,012	0,019	0,645	0,036	0,941
CMP1	34178	4383	24724	65077	0,096	1,106	0,142	14695	9453	241	0,016	0,025	0,643	0,033	0,928
CMP1	30678	4275	22219	58340	0,103	1,109	0,155	13227	8459	255	0,019	0,030	0,640	0,022	0,916
CMP2	29255	4680	21306	55186	0,117	1,128	0,181	12383	7949	316	0,026	0,040	0,642	0,028	0,896
CMP2	31801	3487	22847	59639	0,083	1,142	0,125	13503	8954	149	0,011	0,017	0,663	0,031	0,945
CMP2	27628	3192	19886	52715	0,085	1,101	0,127	12247	7742	147	0,012	0,019	0,632	0,017	0,937
CMP2	30084	3524	21652	56838	0,087	1,124	0,132	13071	8432	171	0,013	0,020	0,645	0,026	0,944
CMP2	34546	4537	24668	65120	0,101	1,130	0,148	14586	9878	222	0,015	0,023	0,677	0,060	0,923
CMP2	36209	4591	25778	68219	0,096	1,131	0,143	15318	10430	232	0,015	0,022	0,681	0,028	0,929
CMP2	38231	4609	27045	71697	0,096	1,142	0,138	16185	11186	200	0,012	0,018	0,691	0,026	0,940
CMP2	38814	4496	27241	73009	0,091	1,135	0,131	16427	11573	194	0,012	0,017	0,705	0,032	0,942

Chapter 4

Site / Treatment	Code
kerguehennec mineral	KM
kerguehennec cow manure	KCM
kerguehennec pig manure	KPM
champ Noël mineral	CNM
Champ Noêl pig manure	CNPM

Annex 1	**Dissolved organic carbon (mg/L)**		
Minicatchments	Storm 1	Storm 2	Storm 3
7A	8,78	6,02	16,16
7B	13,47	6,03	20,12
7C	8,62	5,13	17,98
7D	12,62	5,09	19,87
11A	14,48	13,33	13,59
11B	15,53	14,5	15,53
11C	15,59	12,13	18,3
11D	15,97	13,78	16,95
15A	10,57	5,56	13,9
15B	6,73	2,64	12,22
15C	12,21	2,72	16,35
19A	11,76	13,06	18,23
19B	15,81	13,48	27,82
19C	11,69	7,52	27,29
19D	19,66	18,16	24,22

Treatments	**I**	**II**	**III**	**IV**	**V**	**bio**	**geo**	**r b:g**	**r III:V**	**r IV:V**	**F**	**H**	**TRY**	**r T:F**	**T:H**	**r H:F**	**r T:(H:F)**	HIX
KM	281	867	19833	2304	18793	3451	38625	0,089	1,055	0,123	8264	5050	110	0,013	0,022	0,611	180,547	0,90
KM	295	910	20825	2419	19732	3624	40557	0,094	1,108	0,129	8677	5302	116	0,014	0,023	0,642	189,574	0,95
KM	267	823	18841	2189	17853	3279	36694	0,085	1,003	0,116	7851	4797	105	0,013	0,021	0,580	171,520	0,86
KCM	288	851	20274	2323	20118	3462	40392	0,086	1,008	0,115	8664	5061	109	0,013	0,022	0,584	186,878	0,91
KCM	302	894	21288	2439	21123	3636	42412	0,090	1,058	0,121	9097	5314	115	0,013	0,023	0,613	196,222	0,95
KCM	274	809	19261	2207	19112	3289	38372	0,081	0,957	0,110	8231	4808	104	0,012	0,020	0,555	177,534	0,86
KPM	312	972	22139	2605	21391	3889	43531	0,089	1,035	0,122	9284	5586	134	0,014	0,024	0,602	221,940	0,91
KPM	328	1021	23246	2735	22461	4084	45707	0,094	1,087	0,128	9748	5865	140	0,015	0,025	0,632	233,037	0,95
KPM	297	923	21032	2475	20322	3695	41354	0,085	0,983	0,116	8820	5306	127	0,014	0,023	0,572	210,843	0,86
CNM	276	603	11424	1867	13672	2746	25097	0,109	0,836	0,137	5497	3025	87	0,016	0,029	0,550	158,870	0,87
CNM	289	634	11995	1960	14356	2884	26351	0,115	0,877	0,143	5772	3177	92	0,017	0,030	0,578	166,813	0,91
CNM	262	573	10853	1774	12989	2609	23842	0,104	0,794	0,130	5222	2874	83	0,015	0,027	0,523	150,926	0,82
CNPM	381	826	11304	2473	12752	3680	24055	0,153	0,886	0,194	5211	2971	161	0,031	0,054	0,570	283,104	0,84
CNPM	400	868	11869	2597	13389	3864	25258	0,161	0,931	0,204	5472	3120	169	0,033	0,057	0,599	297,259	0,88
CNPM	362	785	10738	2350	12114	3496	22852	0,145	0,842	0,184	4950	2823	153	0,029	0,052	0,542	268,949	0,80

Chapter 5

Annex 2																	
minicatchments / storm	II	III	IV	V	bio	geo	r b:g	r III:V	r IV:V	F	H	TRY	r T:F	T:H	r H:F	r T:(H:F)	HIX
7S1	2178	28508	5971	26522	8637	55030	0,157	1,075	0,225	10732	6551	236	0,022	0,036	0,610	386	0,891
7S1	1639	23530	4661	23028	6610	46558	0,142	1,022	0,202	13481	7802	240	0,018	0,031	0,579	415	0,907
11S1	2082	27181	5551	24773	8042	51954	0,155	1,097	0,224	11334	7103	224	0,020	0,032	0,627	358	0,904
11S1	1913	26272	4874	25111	7083	51383	0,138	1,046	0,194	12285	7478	182	0,015	0,024	0,609	300	0,917
11S1	1880	27111	5085	25685	7243	52795	0,137	1,056	0,198	12581	7493	183	0,015	0,024	0,596	307	0,918
11S1	2013	27105	5304	25605	7673	52710	0,146	1,059	0,207	12890	7837	216	0,017	0,028	0,608	355	0,908
7S2	785	22562	2648	24590	3498	47152	0,074	0,918	0,108	11435	4998	96	0,008	0,019	0,437	219	0,966
7S2	872	23345	2835	25258	3805	48603	0,078	0,924	0,112	11703	5203	101	0,009	0,019	0,445	227	0,963
7S2	905	24511	2877	26130	3889	50641	0,077	0,938	0,110	12230	5541	97	0,008	0,018	0,453	215	0,963
7S2	894	24498	2879	26122	3881	50620	0,077	0,938	0,110	12195	5533	97	0,008	0,018	0,454	215	0,962

Annex 2

minicatchments / storm	II	III	IV	V	bio	geo	r b:g	r III:V	r IV:V	F	H	TRY	r T:F	T:H	r H:F	r T:(H:F)	HIX
11S2	852	22809	2360	25005	3315	47813	0,069	0,912	0,094	11498	5571	74	0,006	0,013	0,484	154	0,968
11S2	961	24493	2481	25659	3536	50152	0,070	0,955	0,097	12076	6352	77	0,006	0,012	0,526	147	0,969
11S2	865	23809	2519	26007	3460	49816	0,069	0,915	0,097	12009	5697	79	0,007	0,014	0,474	166	0,970
11S2	1009	25404	2668	26564	3784	51968	0,073	0,956	0,100	12441	6381	86	0,007	0,013	0,513	168	0,969
15S2	825	22149	2768	23195	3688	45343	0,081	0,955	0,119	10709	4842	112	0,010	0,023	0,452	247	0,960
15S2	1109	27766	3578	28225	4821	55990	0,086	0,984	0,127	13188	6308	137	0,010	0,022	0,478	287	0,960
15S2	1226	23642	4492	23842	5932	47484	0,125	0,992	0,188	11281	5514	288	0,026	0,052	0,489	590	0,918
19S2	924	29218	2450	30187	3425	59405	0,058	0,968	0,081	13880	6887	65	0,005	0,009	0,496	132	0,981
19S2	839	28066	2299	29239	3198	57304	0,056	0,960	0,079	13319	6463	63	0,005	0,010	0,485	129	0,979
19S2	913	28850	2570	29168	3565	58019	0,061	0,989	0,088	13469	6717	72	0,005	0,011	0,499	144	0,976
19S2	754	22502	1930	24576	2744	47079	0,058	0,916	0,079	11289	5477	53	0,005	0,010	0,485	110	0,979
7S3	989	17774	2509	17742	3728	35516	0,105	1,002	0,141	8258	4552	133	0,016	0,029	0,551	242	0,932
7S3	853	16522	2218	17162	3241	33683	0,096	0,963	0,129	7933	4133	107	0,014	0,026	0,521	206	0,938
7S3	965	21876	2499	23084	3625	44960	0,081	0,948	0,108	10664	5436	102	0,010	0,019	0,510	199	0,953
7S3	962	23857	2758	25904	3851	49762	0,077	0,921	0,106	11908	5722	101	0,008	0,018	0,481	211	0,958
11S3	1052	17468	2477	16886	3727	34353	0,108	1,034	0,147	7945	4529	128	0,016	0,028	0,570	224	0,935
11S3	809	18385	2084	19111	3002	37496	0,080	0,962	0,109	8892	4579	79	0,009	0,017	0,515	154	0,961
11S3	1079	21707	2656	22518	3912	44225	0,088	0,964	0,118	10443	5510	120	0,011	0,022	0,528	227	0,949
15S3	727	14485	1886	14499	2730	28983	0,094	0,999	0,130	6732	3607	96	0,014	0,027	0,536	179	0,941
15S3	1027	19751	2906	21091	4094	40842	0,100	0,936	0,138	9718	4737	158	0,016	0,033	0,487	324	0,937
15S3	1141	15724	3330	15534	4725	31259	0,151	1,012	0,214	7267	4026	237	0,033	0,059	0,554	428	0,889
19S3	704	19050	1770	19756	2549	38807	0,066	0,964	0,090	9133	4588	62	0,007	0,013	0,502	123	0,967
19S3	734	19943	1868	21277	2681	41220	0,065	0,937	0,088	9750	4803	66	0,007	0,014	0,493	133	0,968
19S3	835	22125	2091	23536	3014	45660	0,066	0,940	0,089	10804	5332	75	0,007	0,014	0,494	151	0,967
19S3	1017	23432	2524	23095	3705	46527	0,080	1,015	0,109	10842	5784	99	0,009	0,017	0,533	186	0,960

R Script for the integration of fluorescence intensities in EEM

Inout

```
inout<-function(xvec, yvec, xclass, yclass, nfron)
{
	a <- pi/2
	b <- -0.2121144
	c <- 0.074261
	d <- -0.0187293
	teta <- 0
	result <- (-1)
	#par defaut resultat result=-1
	xref <- xclass[1] - xvec
	#xref coord de reference en x
	yref <- yclass[1] - yvec
	#yref coord de reference en y
	s <- sqrt(xref * xref + yref * yref) + 1e-030
	xref <- xref/s
	yref <- yref/s
	for(j in 2:nfron) {
		x <- xref
		y <- yref
		xref <- xclass[j] - xvec
		yref <- yclass[j] - yvec
		s <- sqrt(xref * xref + yref * yref) + 1e-030
		xref <- xref/s
		yref <- yref/s
		prodv <- x * yref - xref * y + 1e-030
		prods <- x * xref + y * yref
		s <- abs(prodv)
		dteta <- a - sqrt(abs(1 - s)) * (((d * s +
			c) * s + b) * s + a)
		if(prods < 0) {
			dteta <- (pi - dteta)
		}
		teta <- teta + abs(dteta * prodv)/prodv
	}
	if(abs(teta) > pi) {
		result <- 1
	}
	#result=1 si le point est dans le poly
	return(result)}
```

Delimitation of regions and zones in excitation emission matrix (EEM)

```
#Au sein du diagramme "excitation-délimitation" de la fluorescence
# délimite les régions et les zones dans lesquelles les calculs
# de volume d'intensité et d'identification des scripts vont se faire.
# Fait appel à la fonction Inout
source("scripts/inout.R")
# PARAMETRES A MODIFIER EVENTUELLEMENT
# Nombre de fichiers par spectre
nfic<-45
# Nombre de mesures par spectre
```

```
nc<-702
# Etendues en X et Y
yvec<-seq(200.,420.,5.)
xvec<-seq(250.,600.5,0.5)
#
#DIAGRAMME DES REGIONS SUR LESQUELLES SE FERA LE CALCUL DES VOLUMES
#definition des matrices en x et en y
#
nregions<-6
xtriangleg<-matrix(0,ncol=7,nrow=6)
ytriangleg<-matrix(0,ncol=7,nrow=6)
#
#nombre de sommets pour chaque classe
nfrong<-c(5,5,5,5,5,7)
#
#on remplit les lignes des x et des y : coordonnees des nfron points definissant les classes
xtriangleg[1,]<-c(280,330,330,280,280,0,0)
ytriangleg[1,]<-c(230,230,250,250,230,0,0)
xtriangleg[2,]<-c(330,380,380,330,330,0,0)
ytriangleg[2,]<-c(230,230,250,250,230,0,0)
xtriangleg[3,]<-c(380,380,475,435,380,0,0)
ytriangleg[3,]<-c(230,250,250,230,230,0,0)
xtriangleg[4,]<-c(280,280,380,380,280,0,0)
ytriangleg[4,]<-c(250,270,340,250,250,0,0)
xtriangleg[5,]<-c(380,380,575,475,380,0,0)
ytriangleg[5,]<-c(250,300,300,250,250,0,0)
xtriangleg[6,]<-c(380,380,450,600,600,575,380)
ytriangleg[6,]<-c(300,340,400,400,310,300,300)

# fin de creation des 6 Régions
#
#nomination des ZONES
labelregions<-c(1,2,3,4,5,6)
labelregionschar<-c("I","II","III","IV","V","VI")
xlabelregions<-c(305,355,430,330,475,500)
ylabelregions<-c(240,240,240,295,275,350)
#
#ZONES AU SEIN DESQUELLES EST RECHERCHEE L'INTENSITE MAX
#definition des matrices en x et y
xtrianglef<-matrix(0,ncol=6,nrow=7)
ytrianglef<-matrix(0,ncol=6,nrow=7)
#
nzones<-7
#nombre de sommets pour chaque classe
nfronf<-c(5,5,5,5,5,5,5)
#
#on remplit les lignes en x et en y
xtrianglef[1,]<-c(280,280,320,320,280,0)
ytrianglef[1,]<-c(230,250,250,230,230,0)
xtrianglef[2,]<-c(300,320,320,300,300,0)
ytrianglef[2,]<-c(270,270,280,280,270,0)
xtrianglef[3,]<-c(320,350,350,320,320,0)
ytrianglef[3,]<-c(270,270,280,280,270,0)
xtrianglef[4,]<-c(400,500,500,400,400,0)
ytrianglef[4,]<-c(300,300,350,350,300,0)
xtrianglef[5,]<-c(380,475,435,380,380,0)
ytrianglef[5,]<-c(250,250,230,230,250,0)
xtrianglef[6,]<-c(360,420,420,360,360,0)
ytrianglef[6,]<-c(310,310,320,320,310,0)
xtrianglef[7,]<-c(460,460,475,475,460,0)
```

```
ytrianglef[7,]<-c(370,380,380,370,370,0)
#nomination des zones d'intensité
labelzones<-c(1,2,3,4,5,6,7)
labelzoneschar<-c("TY1","TY2","TRY","FL","HL","BE","AL3")
xlabelzones<-c(290,310,335,450,425,400,468)
ylabelzones<-c(240,275,275,325,240,315,375)

# fin de creation des zones d'intensité
## création d'un masque permettant d'exclure des zones mal corrigées
#on remplit les lignes en x et en y
xmasque<-c(380,600,600,430,380)
ymasque<-c(200,310,280,200,200)

regionsref<-matrix(NA,ncol=nfic,nrow=nc)
zonesintref<-matrix(NA,ncol=nfic,nrow=nc)
regionsrefchar<-matrix(NA,ncol=nfic,nrow=nc)
zonesintrefchar<-matrix(NA,ncol=nfic,nrow=nc)

# calcul des zones d'intensité
        for(j in 1:nc)
        {
                for(k in 1:nfic)
                {
                        tempzones<-0
                i<-0
                while(tempzones!=(1)&i<=(nzones-1))
                {
                        i<-i+1

tempzones<-inout(xvec[j],yvec[k]+0.1,xtrianglef[i,],ytrianglef[i,],nfronf[i])
                }
                        if(tempzones==1) zonesintref[j,k]<-labelzones[i]
                if(tempzones==1) zonesintrefchar[j,k]<-labelzoneschar[i]

        }
                        }
# calcul des regions d'intégration
for(j in 1:nc)
        {
                for(k in 1:nfic)
                {
                tempregions<-0
                i<-0
                while(tempregions!=(1)&i<=(nregions-1))
                                {
                                        i<-i+1
                                        tempregions<-
inout(xvec[j],yvec[k]+0.01,xtriangleg[i,],ytriangleg[i,],nfrong[i])
}
                                        if(tempregions==1)regionsref[j,k]<-labelregions[i]
                        if(tempregions==1)regionsrefchar[j,k]<-labelregionschar[i]

                        }
                                }
# recherche des zones correspondant au masque
for(j in 1:nc)
        {
                for(k in 1:nfic)
                {
                tempregions<-0
```

```
                                        tempregions<-inout(xvec[j],yvec[k],xmasque,ymasque,5)

                                        regionsref[j,k]<-ifelse(tempregions==1,NA,regionsref[j,k])
                                        regionsrefchar[j,k]<-
ifelse(tempregions==1,NA,regionsrefchar[j,k])
                                        zonesintref[j,k]<-ifelse(tempregions==1,NA,zonesintref[j,k])
                                        zonesintrefchar[j,k]<-
ifelse(tempregions==1,NA,zonesintrefchar[j,k])

                                }
                                        }
# Effectif des régions et des zones
#
table(regionsref)
table(regionsrefchar)

# Dessin des regions dans un fichier pdf
pdf(file="refregions.pdf",paper="a4r")
image(xvec,yvec,regionsref, ylab="Excitation Wavelength (nm)",
xlab="Emission Wavenlength (nm)",col=rainbow(6),main=paste("Régions de références"))
for(i in 1:6)
{
lines(xtriangleg[i,1:nfrong[i]],ytriangleg[i,1:nfrong[i]])
}
text(xlabelregions,ylabelregions,labelregionschar)
lines(xmasque,ymasque,col=4)
#
table(zonesintref)
table(zonesintrefchar)
#
image(xvec,yvec,zonesintref, ylab="Excitation Wavelength (nm)",
xlab="Emission Wavelength (nm)",col=rainbow(6),main=paste("Zones d'intensité"))
for(i in 1:6)
{
lines(xtriangleg[i,1:nfrong[i]],ytriangleg[i,1:nfrong[i]])
}
for(i in 1:7)
{
lines(xtrianglef[i,1:nfronf[i]],ytrianglef[i,1:nfronf[i]],col=3)
}
text(xlabelzones,ylabelzones,labelzoneschar)
lines(xmasque,ymasque,col=4)

save.image()
dev.off()
print("CALCULS TERMINES
```

Integration of fluorescence intensity volume in each region and zone in EEM

```
# SCRIPT PRINCIPAL
# VERSION du 18 juillet (C. WALTER)
#INITIALISATION PAR L'UTILISATEUR
# METTEZ A JOUR LES NOMS DE FICHIER
# VERSION DU 6 MAI 2008 pour correction UV
# NOM DU BLANC
nomblanc<-"name of blank"
# NOM DU FICHIER SP A ANALYSER
nomfic <-"sample name"
#
# PARAMETRES A MODIFIER EVENTUELLEMENT
```

```
# Nombre de fichiers par spectre
nfic<-45
# Nombre de mesures par spectre
nc<-702
yvec<-seq(200,420,5)
xvec<-seq(250,600.5,0.5)
#
# LES DONNES BRUTES SONT DANS UN REPERTOIRE RAWDATA
# LECTURE DES FICHIERS A ANALYSER
nomfichier<-paste("rawdata/",nomfic,"#",as.character(0),as.character(1),".sp",sep="")
fich<-matrix(scan(nomfichier,skip=54),ncol=2,byrow=T)
dataechant<-matrix(NA,nrow=nc,ncol=nfic)
dimnames(dataechant)<-list(as.character(xvec),as.character(yvec))
dataechant[,1]<-fich[,2]
for (i in 2:9)
{
nomfichier<-paste("rawdata/",nomfic,"#",as.character(0),as.character(i),".sp",sep="")
fich<-matrix(scan(nomfichier,skip=54),ncol=2,byrow=T)
dataechant[,i]<-fich[,2]
}
for (i in 10:45)
{
nomfichier<-paste("rawdata/",nomfic,"#",as.character(i),".sp",sep="")
fich<-matrix(scan(nomfichier,skip=54),ncol=2,byrow=T)
dataechant[,i]<-fich[,2]
}
# 1ère correction des donnees mesurees : mise à 0 des valeurs négatives
datacorrige<-ifelse(dataechant<0,0,dataechant)
# LECTURE DU FICHIER UV
nomfichier<-paste("rawdata/",nomfic,"#","uv",".sp",sep="")
fich<-matrix(scan(nomfichier,skip=86),ncol=2,byrow=T)
datauv<-matrix(NA,nrow=801,ncol=2)
#inversion de l'ordre du fichier UV pour aller de 200 à 600
datauv<-fich[801:1,]
#correction des valeurs négatives pour le fichier UV
datauv[,2]<-ifelse(datauv[,2]<0,0,datauv[,2])
# vecteurs dérivés
datauvemission<-matrix(NA,nrow=702,ncol=2)
datauvemission[1:701,]<-datauv[101:801,]
datauvemission[702,]<-c(600.5,datauvemission[701,2])
datauvexcitation<-datauv[seq(1,441,10),]
# matrice des corrections UV
matrixcorrecuv<-matrix(NA,nrow=702,ncol=45)
for (j in 1:45)
matrixcorrecuv[,j]<-datauvemission[,2]+datauvexcitation[j,2]
#formule commplète de correction
matrixcorrecuv<-10^(+0.5*matrixcorrecuv)
# 2ème correction des données mesurées par le fichier UV
datacorrige2<-datacorrige
for (i in 1:702)
{
        for (j in 1:45) datacorrige2[i,j]<-datacorrige[i,j]*matrixcorrecuv[i,j]
}
# 3ème correction : division par l'intensité de Raman pour être dans les bonnes unités (RU)
# raman correspond ici à la mesure de 25 échantillons d'eau ultra pure (intensité pour une excitation de
350 et une émission de 397)
raman <-25.79
#
datacorrige2<-datacorrige2/raman
#absorbance values at different excitation wavelengths
```

```
a200<-datauv[1,2]
a210<-datauv[21,2]
a220<-datauv[41,2]
a230<-datauv[61,2]
a240<-datauv[81,2]
a254<-datauv[109,2]
a272<-datauv[145,2]
a280<-datauv[161,2]
a340<-datauv[281,2]
a365<-datauv[331,2]
a410<-datauv[421,2]
a465<-datauv[663,2]
a565<-datauv[731,2]
a595<-datauv[791,2]
# LECTURE DU BLANC
nomfichier<-paste("rawdata/",nomblanc,"#",as.character(0),as.character(1),".sp",sep="")
fich<-matrix(scan(nomfichier,skip=54),ncol=2,byrow=T)
datablanc<-matrix(NA,ncol=nfic,nrow=nc)
datablanc[,1]<-fich[,2]
for (i in 2:9)
{
nomfichier<-paste("rawdata/",nomblanc,"#",as.character(0),as.character(i),".sp",sep="")
fich<-matrix(scan(nomfichier,skip=54),ncol=2,byrow=T)
datablanc[,i]<-fich[,2]
}
for (i in 10:45)
{
nomfichier<-paste("rawdata/",nomblanc,"#",as.character(i),".sp",sep="")
fich<-matrix(scan(nomfichier,skip=54),ncol=2,byrow=T)
datablanc[,i]<-fich[,2]
}
# 3ème correction du blanc par Raman
datablanc1<-datablanc/raman
datablanc2<-ifelse(datablanc1<0,0,datablanc1)
#4ème correction des données :  correction par rapport au blanc
datacorrige3<-(datacorrige2-datablanc2)
datacorrige3<-ifelse(datacorrige3<0,0,datacorrige3)
#Exportation dans un repertoire csvcorrectdata au format csv
write.csv2(datacorrige3,paste("csvcorrectdata/",nomfic,"-cor.csv"))
# #ZONES EEM
intmaxzones<-tapply(datacorrige3,zonesintref,"max")
sumregions<-tapply(datacorrige3,regionsref,"sum")
# EXTRACTION SUR LES REGIONS RENSEIGNEES
datacorrigclean<-ifelse(is.na(regionsref),NA,datacorrige3)
datacorrigclean<-ifelse(datacorrigclean<0,0,datacorrigclean)

dataechantclean<-ifelse(is.na(regionsref),NA,datacorrige)
datablancclean<-ifelse(is.na(regionsref),NA,datablanc2)
# HISTOGRAMMES ET COMPARAISON VALEURS BRUTES ET CORRIGEES
pdf(file=paste(nomfic,"-resu.pdf"),paper="a4r",version="1.4")
par(pty="s",mfrow=c(1,2),cex=0.6)
hist(dataechantclean,main=paste("EEM brute de\\",nomfic))
hist(datablanc,main=paste("EEM brute de",nomblanc))
par(pty="s",mfrow=c(1,1))
#plot(datablancclean,dataechantclean,type="n",xlab=paste("EEM brute de",nomfic),
#ylab=paste("EEM brute de",nomblanc),xlim=c(0,1000),ylim=c(0,1000))
#for (i in 1:nfic) points(datablancclean[,i],dataechantclean[,i],col=i)
#corrections UV
hist(matrixcorrecuv,main="Histogramme du paramètre de correction UV")
```

```
plot(datacorrige,datacorrige3,mai="correction UV",xlab="data corrige blanc",ylab="datacorrige blanc+uv")
#abline(0,1)
par(mfrow=c(2,2))
plot(rep(xvec,nfic),dataechantclean,type="n",,ylab="EEM",
xlab="Emission Wavenlength",main=paste("EEM brute de",nomfic),ylim=c(0,1000))
for(i in 1:nfic)lines(xvec,dataechantclean[,i],col=i)
plot(rep(xvec,nfic),datablancclean,type="n",,ylab="EEM ",
xlab="Emission Wavenlength",main=paste("EEM brute de ",nomblanc),ylim=c(0,1000))
for(i in 1:nfic)lines(xvec,dataechantclean[,i],col=i)
plot(rep(xvec,nfic),datacorrigclean,type="n",,ylab="EEM",
xlab="Emission Wavenlength",main=paste("EEM corrigée de ",nomfic),ylim=c(0,1000))
for(i in 1:nfic)lines(xvec,datacorrigclean[,i],col=i)
hist(datacorrigclean,main=paste("EEM corrigée de",nomfic))

# DESSIN DES IMAGES CORRIGEES
par(pty="m",mfrow=c(1,2))
filled.contour(xvec,yvec,datablancclean,zlim=c(0,max(datacorrigclean,na.rm=T)),xlab="Emission
Wavelength (nm)",
ylab="EXcitation Wavenlength",col=rainbow(24),main=paste("EEM du blanc",nomfic))
filled.contour(xvec,yvec,datacorrigclean,zlim=c(0,max(datacorrigclean,na.rm=T)),xlab="Emission
Wavelength (nm)",
ylab="EXcitation Wavenlength",col=rainbow(24),main=paste("EEM corrigée de",nomfic))
par(mfrow=c(1,1))
filled.contour(xvec,yvec,datacorrigclean,zlim=c(0,1200),xlab="Emission Wavelength (nm)",
ylab="EXcitation Wavenlength",col=rainbow(24),main=paste("EEM corrigée de",nomfic))
image(xvec,yvec,datacorrigclean,zlim=c(0,1200),xlab="Emission Wavelength (nm)",
ylab="EXcitation Wavenlength",col=rainbow(24),main=paste("EEM corrigée de",nomfic))
for(i in 1:6)
{
lines(xtriangleg[i,1:nfrong[i]],ytriangleg[i,1:nfrong[i]],col=1)
}
for(i in 1:7)
{
lines(xtrianglef[i,1:nfronf[i]],ytrianglef[i,1:nfronf[i]],col=3)
}
text(xlabelregions,ylabelregions,labelregionschar,col=1)
text(xlabelzones,ylabelzones,labelzoneschar,col=3)
contour(xvec,yvec,datacorrigclean,ylab="Excitation Wavelength (nm)",
xlab="Emission Wavenlength",levels=seq(0,max(datacorrigclean,na.rm=T),50),main=paste("EEM
corrigée de",nomfic))
for(i in 1:6)
{
lines(xtriangleg[i,1:nfrong[i]],ytriangleg[i,1:nfrong[i]],col=2)
}
for(i in 1:7)
{
lines(xtrianglef[i,1:nfronf[i]],ytrianglef[i,1:nfronf[i]],col=3)
}
text(xlabelregions,ylabelregions,labelregionschar,col=2)
text(xlabelzones,ylabelzones,labelzoneschar,col=3)
# calcul de l'indice d'humification HIX
num1hix <-sum(datacorrige3[371:461,12])
num2hix <-sum(datacorrige3[101:191,12])
hix <- num1hix/(num1hix+num2hix)
rm(num1hix,num2hix)
hix2 <-datacorrige3[401,35]/datacorrige3[501,35]
hix3 <-datacorrige3[441,35]/datacorrige3[541,35]
# calcul des statistiques sur les régions et les zones
intmaxzones<-tapply(datacorrige3,zonesintrefchar,"max")
```

```
sumzones<-tapply(datacorrige3,zonesintrefchar,"sum")
sumregions<-tapply(datacorrige3,regionsrefchar,"sum")
sumgeo<-sumregions[3]+sumregions[5]+sumregions[6]
sumbio<-sumregions[1]+sumregions[2]+sumregions[4]
sum53<-sumregions[3]+sumregions[5]
sum56<-sumregions[5]+sumregions[6]
R4sursum56<-sumregions[4]/sum56
R53sur6<-sum53/sumregions[6]
Rbiogeo<-sumbio/sumgeo
# Dans chaque zone, identification des longueurs d'onde d'emission et d'excitation correspondant à une intensité maximale
# ATTENTION changer les valeurs si on change nfic et nc dans l'entete
liste<-match(intmaxzones,datacorrige3)
longexcitmax<-200+floor(liste/702)*5
restetemp<-liste-(floor(liste/702)*702)
longemissionmax<-250+restetemp*0.5
rm(restetemp,liste)
# Dessin des émissions intégrées et des intensités maximales par région et par zones
plot(sumregions,main="EEM intégrée sur les 9 régions de référence",
xlab="Régions",ylab="EEM cumulée",sub=paste("Echantillon : ", nomfic),type="h",col=2)
plot(intmaxzones,main="Intensité maximale au sein des 7 zones de référence",
xlab="Zones de références", ylab="Intensité maximale",sub=paste("Echantillon : ", nomfic)
,type="h",ylim=c(0,1000),col=2)
plot(sumzones,main="EEM intégrée au sein des 7 zones de référence",
xlab="Zones de références", ylab="EEM cumulée",sub=paste("Echantillon : ", nomfic)
,type="h",col=2)
plot(sumzones,intmaxzones,xlab="EEM cumulée par zone",ylab="Intensité maximale",type="n")
text(sumzones,intmaxzones,c("H-L","Al3","F-L","BE","TRY","TY1","TY2"))
plot(longemissionmax,longexcitmax,xlab="Pic d'émission en nm",ylab="Pic d'excitation en nm",type="n")
text(longemissionmax,longexcitmax,c("H-L","Al3","F-L","BE","TRY","TY1","TY2"))
graphics.off()
# EXPORTATION
resutraitstat<-
data.frame(nomfic,date(),t(sumregions),sumgeo,sumbio,sum53,sum56,R4sursum56,R53sur6,Rbiogeo,t(intmaxzones),t(sumzones),t(longemissionmax),t(longexcitmax),a200,a210,a220,a230,a240,a254,a272,a280,a340,a365,a410,a465,a565,a595,hix,hix2,hix3)
write.csv2(resutraitstat,file="resultats-analyse-eem.csv",append=T)
```

Fluorescence tracers of Dissolved Organic Matter in headwater agricultural catchments

Abstract

Dissolved organic matter (DOM) concentrations are increasing in the streams at agricultural headwater catchments in French Brittany, an intensive agricultural region, and Europe during last twenty years. These increasing DOM concentrations are threat to water quality degradation. At small agricultural catchment scale, soil and farm wastes effluents are principle sources of DOM. To propose management actions and to restore stream water quality, three dimensional EEM (Excitation Emission Matrix) was applied to trace DOM issued from farm wastes in the soil and agricultural catchment stream.

Fluorescence tracers were measured on DOM produced from pig slurry, cow manure and composted manures which recycled commonly in cultivated soils. Afterwards, these tracers were analysed in rainfall simulation experiment in the cultivated plots amended with pig slurry and cow manures. The persistence of these fluorescence tracers was evaluated in soil incubation (two months) and in two different experimental dispositives (Champ Noël, 0.9% total carbon and Kerguehennec, 2.5% total carbon) as well as these tracers were compared in mineral vs organic (pig slurry and cow manure) fertilized plots with different recycling time of 14 and 7 years respectively. Finally, the relation between agricultural practices in Valley Bottom Wetlands (VBW) and the presence of these fluorescence tracers in 15 agricultural streams were explored during three storm events. VBW were identified as principle source of DOM in French Brittany catchments. The agricultural practices (crop rotation, quality and quantity of fertilizers, grazing meadows) in the VBW were identified by farm survey.

The fluorescence intensities were integrated in the two regions of EEM spectra (biochemical/geochemical, bio:geo), five regions composed of proteins like, fulvic and humic (I to V), and three zones (tryptophan (TRY), fulvic like (FL) and humic like (HL)). DOM produced from pig and cow demonstrated biochemical fluorescence signatures and discriminated from composted manures which showed geochemical signatures similar to soil DOM. The tracers bio:geo, TRY:FL, TRY:HL, TRY:(HL:FL), TRY trace the DOM issued from farming wastes in simulated runoff two hours after soil spreading. Cow manure DOM was not differentiated from pig wastes DOM with these fluorescence tracers. One year after last recycling, several tracers were found in soil 0.9% C while at the soil with 2.5% C, only TRY persisted. With these results, we are not clear whether the effect is cumulative or it's the influence of last farm wastes spreading. The fluorescence tracers were identified in the headwater catchments impacted by farm wastes recycling. Some catchments demonstrated highly humified DOM which resembled to soil DOM without recycling. Therefore, fluorescence spectroscopy permits to trace the DOM issued from farming wastes. Fluorescence spectroscopy is found a valuable tool for monitoring farming wastes DOM contamination and understanding the biogeochemistry of DOM in soil and water environment.

Key words: dissolved organic matter, farming wastes, agricultural practices, water quality, fluorescence spectroscopy, headwater catchments

Traçage des matières organiques dissoutes par fluorescence dans les bassins versants agricoles

Résumé :

La concentration en carbone organique dissout augmente depuis environ vingt ans dans les rivières de nombreux bassins versants en Bretagne et en Europe. Dans les petits bassins versants agricoles, les principales sources sont les sols et les effluents d'élevage. Afin de proposer des actions pour restaurer la qualité de l'eau, la fluorescence tridimensionnelle EEM (Excitation Emission Matrix) est utilisée pour tracer dans les sols et les cours d'eau la matière organique issue des effluents d'élevage.
Les traceurs de fluorescence sont mesurés sur les MOD issues de lisiers de porc, fumier de bovin et effluents compostés couramment recyclés sur les sols. Ces traceurs sont ensuite recherchés dans les eaux de ruissellement lors d'une simulation de pluie sur parcelle amendée par du fumier de bovin et du lisier de porc. La persistance des traceurs est évaluée dans une incubation de sol (deux mois) et sur deux dispositifs expérimentaux (Champ Noël, 0.9% de carbone total et Kerguehennec, 2.5% de carbone total) comparant des fertilisations minérale et organique (lisier, fumier) respectivement depuis 14 et 7 ans. Enfin, la relation entre les pratiques agricoles dans les zones humides de fond de vallée et la présence de ces traceurs dans les eaux de quinze bassins versants (BV) agricoles est explorée lors de trois crues. Ces zones sont reconnues comme étant les principales zones contributrices en MOD dans les BV bretons. Les pratiques agricoles (rotation, qualité et quantité de fertilisants, paturâge) dans les zones humides potentielles de fonds de vallée sont identifiées par enquête.
La fluorescence est intégrée dans deux régions du spectre (biochimique/géochimique, bio/géo), cinq régions détaillant les composés du type protéine, fulvique ou humique (I à V), et trois zones (Tryptophane (TRY), composés fulviques (FL) et humiques (HL)). La MOD issue des lisiers et fumiers possède une empreinte fluorescente biochimique qui les discrimine des effluents compostés présentant une empreinte plus géochimique similaire aux MOD issues des sols. Les traceurs bio :geo, TRY :FL, TRY :HL, et TRY:(HL:FL), TRY permettent de tracer les MOD issues d'effluents d'élevage dans les eaux de ruissellement quelques heures après l'épandage. Les MOD issues d'effluents bovins ne sont pas discriminées des effluents porcins. Un an après le dernier épandage, plusieurs traceurs des effluents sont retrouvés dans le sol à 0.9% de C, alors que sur le sol à 2.5% de C, seul le TRY persiste. Les résultats ne permettent de conclure sur l'effet cumulatif ou sur l'influence du dernier épandage. Les traceurs sont identifiés dans les BV les plus impactés par le recyclage d'effluents d'élevage. Certains BV ne sont impactés que par des MOD fortement humifiées issues des sols sans recyclage. La fluorescence tridimensionnelle permet donc de tracer des MOD issues des effluents d'élevage.

Mots-clés: matière organique dissoute, effluents d'élevage, pratiques agricoles, qualité des eaux, fluorescence, bassins versant.