Effet de la combinaison de lombrifiltration et du lagunage à macrophytes sur le recyclage des effluents sur le site d'élevage.

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Effet de la combinaison de la lombrifiltration et du lagunage à macrophytes sur le recyclage des effluents sur le site d'élevage

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Avant propos

Cette thèse est le résultat de trois années de travail au sein de l'UMR EcoBio (CNRS - Université de Rennes 1) et de l'UMR SAS (Sol, Agro et hydrosystèmes, Spatialisation ; INRA – Agrocampus Ouest). Elle a été encadrée par Daniel Cluzeau (Université Rennes 1, UMR EcoBio), Philippe Morand (CNRS, UMR EcoBio), Paul Robin (INRA, UMR SAS) et Yinsheng Li (Université Jiao Tong, Shanghai).

Elle a été financée par une bourse du gouvernement Indonésien et de l'ambassade de France en Indonésie sur le sujet « rôle des lombriciens dans le déterminisme des émissions gazeuses d'un lombrifiltre associé à une porcherie ». Elle a contribué aux projets sur le recyclage des effluents d'élevage conduits à Guernévez en collaboration avec les Chambres d'Agriculture de Bretagne (financements Région Bretagne, Département du Finistère, CASDAR, CNRS-ingénierie écologique, ARCUS Languedoc-Roussillon Chine) et au projet de métrologie des émissions gazeuses (financement ADEME).

Ce travail a été suivi par un comité de pilotage auquel ont participé les encadrants, Jean-Marie Paillat (CIRAD), Manuel Blouin (UMR BioEmCo) et Daniel Boujard (Université de Rennes 1).

Je m'intéresse depuis longtemps à l'agriculture biologique car ce mode de production répond à un besoin fondamental de la population, se nourrir, et il fournit des produits favorables à la santé de la population tout en préservant l'environnement et les ressources naturelles.

L'un des facteurs limitants de l'agriculture biologique est la disponibilité en fertilisants organiques. C'est pourquoi j'ai cherché à améliorer mes connaissances sur ce sujet en développant des collaborations internationales, d'abord par une formation de Master de Science du Sol. Puis, pour approfondir mes connaissances, j'ai obtenu une bourse de gouvernement de l'Indonésie et de l'Ambassade de France pour faire une thèse dans ce domaine. Après cette thèse, j'envisage d'appliquer ces connaissances pour améliorer les productions de l'agriculture biologique.

En agriculture, les fertilisants organiques sont fabriqués à partir de produits de l'activité d'agricole : déjections animales, paille, résidus horticoles, etc. Le dispositif pilote sur lequel j'ai travaillé a pour but de conserver les effluents d'un élevage en vue de leur recyclage et de limiter les fuites polluantes. Il se situe à Guernévez (Finistère).

Mon sujet initial a évolué pour s'adapter au contexte des projets en cours. Il a été étendu à l'ensemble du système de recyclage de Guernévez qui inclut des systèmes de lagunage à macrophytes.

Mon sujet définitif a donc porté sur l'intérêt de « combiner la lombrifiltration et le lagunage à macrophytes sur le recyclage des effluents sur le site d'élevage ». En effet, le traitement peut améliorer le recyclage : par exemple la décantation préalable d'un liquide favorise le fonctionnement de la lagune où il est apporté (moins d'accumulation de boues), mais si le traitement est trop poussé il peut défavoriser son fonctionnement biologique, par exemple si l'on enlève trop d'azote et de phosphore les plantes pousseront moins bien. C'est pourquoi mon travail de thèse stricto sensu a porté sur la compréhension d'un système biologique de recyclage, pour en préciser les avantages et les limites. Il s'est inscrit dans un projet plus large incluant la modélisation, la production horticole, la réalisation d'un court-métrage scientifique. Ainsi, mon projet a commencé en Septembre 2007 et il s'est déroulé en 4 étapes :

- analyser l'influence de la saison sur le traitement en utilisant les observations acquises depuis 2006,
- modéliser le recyclage de l'eau et la production des plantes dans le dispositif pilote,
- proposer un système économiquement viable de production de plantes en pots à partir des produits issus des différentes étapes du traitement,
- analyser les possibilités du lombrifiltre pour réduire au maximum les pertes gazeuses d'azote.

J'ai travaillé sur deux dispositifs existant. Le premier s'appelle «station expérimentale», le deuxième système, « prototype ». La station expérimentale est un dispositif de petites dimensions permettant de comparer trois combinaisons de lagunes à macrophytes, chacune étant composée de quatre niveaux successifs. Le prototype est un dispositif plus grand qui assure le recyclage de la chasse d'eau de la porcherie avec un seul traitement à chaque niveau. Il permet l'expérimentation en conditions d'exploitation. L'ensemble de ces dispositifs incluant la porcherie est appelé « Pilote ». Le premier stade de traitement est un tamisage suivi d'une lombrifiltration (l'utilisation de lombricompost pour filtrer des effluents des élevages). Le système de lombrifiltration de Guernévez est associé à un système de lagunage qui comporte quatre lagunes : deux en eau libre alternent avec deux en filtres plantés. Le système a été conçu pour diminuer le niveau des éléments chimiques de l'effluent d'élevage jusqu'à un seuil sans danger pour l'environnement et pour pouvoir nettoyer les déjections à l'intérieur du bâtiment d'élevage. Le système recycle l'eau. L'eau est stockée dans le dernier bassin avant d'être utilisée pour nettoyer les déjections produites dans le bâtiment d'élevage.

La première étape a conduit à un projet d'article sur l'influence de la saison. J'ai analysé le changement de quelques espèces chimiques à chaque niveau sur la période 2006-2008. J'ai trouvé qu'il y avait une influence de la saison sur notre lagunage.

Pour la deuxième étape, j'ai choisi d'utiliser l'environnement Matlab. La modélisation est importante pour dimensionner le système pour d'autres élevages ainsi que pour interpréter les observations et vérifier que notre compréhension des processus est juste. Dans le programme, j'ai défini des paramètres chimiques et des paramètres caractéristiques des plantes. Le programme modifie les résultats de traitement lorsqu'il y a des changements de combinaisons de plantes. Je suis en train d'ajouter au programme des relations pour tenir compte des dernières observations.

J'ai choisi la troisième étape car je crois que le système de lombrifiltration et de lagunages ajoute une valeur économique à l'élevage. Le système que je propose est destiné à l'horticulture. Il utilise tous les matériels qui existent dans la lombrifiltration et les bassins : pour le substrat d'orchidée, j'utilise les plaquettes de bois de la lombrifiltration ; les plantes produites dans les bassins seront compostées pour produire des fertilisants organiques solides ; l'eau des bassins sera utilisée comme fertilisant organique liquide. J'utilise le Poinsettia ou étoile de noël pour comparer le système proposé à celui basé sur un support de culture classique. Pour cette étape, j'ai suivi une formation d'entrepreneurs destinée aux doctorants intéressés par le montage d'entreprises innovantes et j'ai élaboré un protocole destiné à évaluer le potentiel du lombricompost pour la plantation d'orchidée et de Poinsettia en tenant compte du besoin d'eau pendant la plantation. Le comité des Entrepreneuriales de l'Ille Vilaine a soutenu ce projet. Je n'ai pas gagné le prix récompensant le meilleur projet en 2009 mais j'ai ainsi acquis beaucoup de connaissances sur la création d'entreprise.

La quatrième étape concerne les émissions gazeuses car les élevages ont un fort impact sur l'air (ammoniac, gaz à effet de serre) et les nouveaux systèmes ne doivent pas dégrader cet impact même si les productions animales se développent pour améliorer l'alimentation des populations. L'objectif de ce chapitre est de tester l'influence de la population de lombriciens et de la quantité d'azote sur la perte gazeuse d'azote totale et la proportion sous forme d'ammoniac et de protoxyde d'azote. J'ai conçu et réalisé deux expérimentations. Elles ont permis d'accueillir un étudiant dans le cadre d'une collaboration avec l'Université Jiao Tong (Shanghai, Chine). Il a travaillé sur la porosité libre à l'air.

J'ai utilisé les observations de chaque étape pour écrire mes articles scientifiques et communiquer les nouvelles connaissances vers le monde scientifique. J'espère que les autres chercheurs s'en serviront et j'utiliserai leurs critiques, corrections et idées pour améliorer mes prochaines expérimentations. Ce mémoire de thèse permet d'apporter des éléments nouveaux (émissions gazeuses du lombrifiltre et rôle des lombriciens) et de proposer une nouvelle synthèse des travaux effectués à Guernévez par plusieurs équipes coordonnées par Philippe Morand (illustration en annexe 2).

J'ai réalisé un très court film documentaire scientifique destiné à communiquer mes résultats au grand public. Le film parle du principal sujet de ma thèse « la lombrifiltration ». J'ai utilisé un langage simple pour expliquer la vie des vers de terre dans le lombrifiltre. Mon film est une contribution à l'éducation du public sur la possibilité de pollution de notre environnent par les effluents d'élevage, la fonction des vers de terre pour réduire cette pollution et leurs réactions à différentes doses de matières organiques apportées à la lombrifiltration. Mon but est d'informer les personnes sur les problèmes d'environnement, afin qu'elles essayent de réduire la pollution et de préserver l'environnement, pour une meilleure vie maintenant et pour les futures générations. Ce film est aussi un media pour expliquer ma recherche aux scientifiques non spécialistes de mon domaine dans un langage simple (cf. annexe 3).

En septembre 2010, il y a eu une conférence scientifique internationale sur les vers de terre au Mexique. C'était une possibilité de présenter les résultats de ma recherche sur la relation entre les vers de terre, l'apport de matière organique et les émissions gazeuses. J'ai pu aussi présenter mon film et échanger des informations sur la recherche sur les vers de terre avec d'autres chercheurs dans ce domaine et j'ai enrichi mes connaissances sur les développements actuels de ces recherches.

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Résumé

La production animale augmente continuellement à l'échelle mondiale depuis quelques décennies, dans les pays développés d'abord et maintenant dans les pays en développement. Des systèmes industriels ont été développés pour améliorer la productivité des élevages, pour augmenter rapidement la production animale et pour fournir la nourriture consommée par les villes. Ils sont efficaces en termes de biosécurité et d'efficacité de conversion des aliments du bétail mais ils ont des incidences sur l'environnement telles que les émissions d'odeur, les émissions d'ammoniac ou de gaz à effet de serre, ou la pollution de l'eau. La durabilité de ces systèmes dépend de leur capacité à limiter leurs impacts sur la raréfaction des ressources naturelles et à limiter leurs fuites de sorte que l'environnement naturel et la biodiversité puissent être préservés près des élevages. Des systèmes de traitement onéreux ne pouvant pas être employés pour des raisons économiques, l'ingénierie écologique fournit les concepts qui peuvent aider à trouver des solutions plus efficientes économiquement et écologiquement.

Notre travail a commencé avec la mise en route d'un système associant un bâtiment d'élevage de porcs, une séparation de phase liquide/solide de l'effluent du bâtiment, un lombrifiltre et un ensemble de zones humides artificielles. Destiné à augmenter l'efficacité de recyclage de l'eau et à produire des biomasses utilisables pour la nutrition animale, la fertilisation, la production d'énergie, etc., ce système combine la dilution élevée des effluents, permettant la diminution des émissions, à la réutilisation de l'eau et des nutriments. L'eau utilisée pour l'évacuation fréquente des déjections est ainsi recyclée. Les nutriments sont réutilisés sur l'exploitation agricole ou exportés. L'emprise au sol du système est environ 50 fois inférieure à celle requise pour l'épandage des effluents.

L'objectif fondamental de la thèse était d'améliorer la compréhension du système pour en préciser les avantages et les limites. L'objectif finalisé était d'étudier si les connaissances produites permettaient d'améliorer la conception et la gestion du système.

Des méthodes spécifiques ont été développées pour étudier, sous l'angle des processus et sous l'angle systémique, un dispositif dont les dimensions ne permettaient pas une reproduction dans un laboratoire. Elles ont été appliquées aux émissions gazeuses du lombrifiltre et à l'efficacité de traitement des zones humides artificielles.

Nos résultats permettent de définir une « quantité optimale » d'effluent qui maximise la population de vers de terre (preferendum). Au-dessus de ce seuil, les vers de terre meurent en raison de conditions anoxiques. Quand la population de vers de terre est maximale, les émissions d'ammoniac et de gaz à effet de serre sont limitées en regard du flux d'intrant. Par conséquent, l'abondance de vers de terre peut être employée comme bioindicateur de faibles émissions dans les systèmes de transformation d'effluent. L'effet des lombriciens sur les émissions gazeuses est surtout indirect, par leur influence sur la structure de la couche organique, sa porosité, les transferts de matière et sa population microbienne.

La « quantité optimale » transférée entre deux niveaux successifs peut être définie pour la production de végétation des zones humides artificielles. Par rapport à un système ouvert, le recyclage de l'eau induit un changement de la stoechiométrie des nutriments, en raison d'efficacités de traitement différentes de ces nutriments : par exemple, le taux de réduction du potassium est inférieur à celui de l'azote ; cette différence induit une augmentation de concentration en potassium dans l'eau par rapport à l'azote. La concentration en potassium se stabilise lorsque la rétention par tous les compartiments correspond à une diminution de masse équivalente au flux de potassium excrété par les animaux. Cela montre que la stoechiométrie des nutriments devrait changer dans les milieux agricoles et probablement dans les productions où l'efficacité du recyclage est augmentée. L'estimation du bilan de matière du système, montre que les émissions d'ammoniac et de gaz à effet de serre sont réduites par rapport aux flux d'azote, et que les produits organiques (lombricomposts et boues des lagunes) contribuent majoritairement à l'abattement des nutriments.

Des recommandations pour la conception et la gestion des systèmes qui améliorent le recyclage des effluents sont proposées à partir de ces connaissances. Nos résultats ont été et pourront être mobilisés pour des buts socio-économiques.

Abstract

Animal production increased regularly since some decades, in developed countries at first, and now in developing countries. Industrial systems have been developed to increase rapidly the productivity of animal farms and to supply the food consumed by the towns. They are efficient in terms of biosecurity and of feed conversion efficiency but they have severe environmental impacts such as the odor emissions, the ammonia or greenhouse gas emissions, or the water pollution. The sustainability of these systems depends on their ability to limit their impact on resource depletion and to limit their leakages so that the wild environment and the biodiversity can be preserved beside the producing areas. Expensive treatment systems can not be used because of economical reasons. Ecological engineering provides concepts that can help finding solutions more efficient economically and ecologically.

Our work began with the starting up of a new system of animal production that associates a pig house with manure flushing and screening, a vermifilter, lagooning, and constructed wetlands. This system was designed to increases the recycling efficiency of water and to produce biomass for animal feed, fertilization, biogas, etc. The system combines high manure dilution, which allows a decrease in polluting emissions, to the reuse of water and nutrients. Water is reused for excretion flushing. The nutrients are either reused within the farm or exported. The needed surface is around 50 times less than for manure spreading.

The fundamental objective of the present work was to improve the understanding of the system and to define more precisely its advantages and its limits. The applied objective was to study if this new knowledge was useful to improve the design and the management of this system.

Specific methods were developed to study from the process or from a systemic point of view a recycling system that was too large to be reproduced in a laboratory. They were applied to the gaseous emissions of the vermifilter and to the treatment efficiency of the combination of lagoons and constructed wetlands.

The results show that an “optimal transfer” of liquid can be defined that will maximize the earthworm population (preferendum). Above this input the earthworms die because of anoxic conditions. When earthworm population is maximal, the ammonia and the greenhouse gases are minimized as related to the input flux. Therefore, the earthworm abundance can be used as a bioindicator of low energy and low emissions in manure transforming systems. The effect on gaseous emissions is mostly indirect, through the influence of earthworms on the structure of the organic layer, its free air space, transfer of organic particles and its microbial population.

This “optimal transfer” between two successive levels also exists for the vegetation production of lagoons and constructed wetlands. If we compare “recycling” to “open” system, the water recycling will induces a change in the stoechiometry of nutrients, because of the various treatment efficiencies of elements: for example, potassium abatement rate is less than nitrogen abatement rate; this case induces an increase in potassium concentration in the water compared to nitrogen. Potassium concentration reaches a stable level when the retention by all subsystems corresponds to a mass decrease equivalent to the potassium excreted by the animals. This case shows that the stoechiometry of nutrients should change in agricultural systems with increased recycling efficiency. Calculating the mass balance of the system shows that ammonia and greenhouse gas emissions were low, regarding the nitrogen fluxes, and that the organic products (worm casts and sludge from lagoons) were the major contributors to the removal of nutrients.

Recommendations for the design and management of systems that improve manure recycling are proposed, based on this knowledge. Our results were and can be further used for socio-economical purposes.

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Introduction

1. Context

1.1. Global context of animal production

Animal production is important for human nutrition. People also have benefits from the higher fertility in soils fertilized with animal manure. This sector can supply nutrition such as meat, egg and milk. According to (Gilland, 2002) the consumers prefer the diets that contain more animal product. During the process of animal production, there is manure liquid and solid that can be the source of available nitrogen, phosphorus and other nutrients that will increase crop yields.

Every year, the meat consumption in developed or developing country increases (Delgado, 2003) as shown in Table I. Since 1960, several methods of green revolution were used to increase the agricultural production. The increase was particularly important during the last decades because crop production increased for both human nutrition and increased animal production.

Table I. Evolution of meat and milk consumption in developed and developing countries.

	Developing countries					Developed countries				
	1980	1990	2002	2015	2030	1980	1990	2002	2015	2030
Food demand										
Annual per capita meat consumption (kg)	14	18	28	32	37	73	80	78	83	89
Annual per capita milk consumption (kg)	34	38	46	55	66	195	200	202	203	209
Total meat consumption (million tonnes)	47	73	137	184	252	86	100	102	112	121
Total milk consumption (million tonnes)	114	152	222	323	452	228	251	265	273	284

Source: FAO (2006a) and FAO (2006b).

Further increase in crop productions during the next decades is required to supply food for a growing population, feed for increasing animal farms, biomass for increasing needs of bioenergy and green chemistry industries.

However, the system has several negative effects. In India, the negative effects of green revolution is environmental degradation observed in soil, vegetation and water resources (Singh, 2000). It is due to excessive use of mineral fertilizers and pesticides.

The solution of the contradiction between increased needs and negative effects of high agricultural productions is the greening of green revolution. (Tilman, 1998), the greening technology includes the knowledge of ecological processes and feedbacks, disease dynamics, soil processes and microbial ecology. The mix between the intensive agriculture and ecological knowledge can reduce the negatives effect of green revolution if it is used in most of the agricultural areas.

The effluents of animal production can be either solid manure or liquid slurry. When the quantities spread on crops are below plant requirements, both phases can be recycled by the agricultural ecosystem. In this case, animal effluents contribute to fertility. When the quantity is too high, the liquid phase is more dangerous than the solid one because it contains more reactive elements (Dewes, 1999), e.g. more ammonium and less organic nitrogen. These elements can easier change to other form and contaminate surface and groundwater.

The water source is very limited in the world. The use of water must follow this priority. Clean water should be only used for first priorities sectors such as drinking water or consumption needs. The water of effluents can be reused in agriculture.

Manure removal does not need very clean water. If the animal effluent has a high content of water it can not be transported on long distances to areas where fertilizers are needed. The waters which have low concentrations of chemical elements can be used within the farm to transport the nutrients that were released in animal buildings to intensive crop production and organic matter processing where leakages are controlled.

In natural conditions animal excretion is also diluted and does not induce high pollutions.

Therefore, increasing the recycling efficiency of nutrients excreted by animals and of the water used in the farm is the only option to achieve less resource depletion.

1.2. Local context in regions with concentrated animal feeding operations (CAFO)

In animal production, intensive production systems have been developed to increase animal production during green revolution. They improved the conversion ratio of feed to meat and the biosecurity of animal products.

However, negative effects of these systems were observed with several pollutions. During the production process, the quantity of livestock waste is higher than the recycling capability of nature. Ammonia (NH_3), nitrous oxide (NO_2) and methane (CH_4) are released during the process of animal production and manure management. They induce air pollution. Intensive animal production also produces bad odor that can have negative effects on health. When high rates of organic effluents are spread on crop areas, leakages can induce water pollution through contamination by nitrates (NO_3), phosphates (PO_4), organic carbon (COD) and potassium (K^+). Accumulation of copper (Cu), zinc (Zn) and xenobiotics in soils is also pollution.

Agriculture contributes to greenhouse gas emissions worldwide. Four-fifth of agriculture emissions is from livestock sector (Friel et al. 2009). Strategies to reduce negatives effect are proposed in many countries but their application is difficult because of economical competition between countries.

Brittany is the region with the highest animal production in France. Its intensive animal production system is an example of negative effects on the environment. Water consumption by these system is very high, and their wastewater added to mineral fertilizing pollutes the ecosystem (Morand et al. 2009). Pig production is particularly exposed to critics because of the bad odors generated by animal housing and slurry spreading.

“Concentrated animal feeding operations” (CAFO) is the American name for systems that are used for feeding the animal in intensive animal production. This system is effective to increase the animal production. However, CAFO has several negative impacts on the environment; such as air, soil and water pollution because the animal numbers increase without increasing the area of effluent recycling (Constance and Bonanno, 1999). Rule et al. (2005) explained that CAFO in pig building make poor air quality for worker, the community and farm production.

The development of these industrial systems is expected to continue because they allow increasing rapidly the animal production and supplying the food consumed by the developing towns.

In animal farms where water pollution has been observed, treatment facilities were installed to help the reduction of chemical elements in the animal wastewater. There are several systems to reduce the pollution induced by animal farms, including physical and biological systems (Burton and Turner 2003). Ponds or constructed wetland is an alternative way to protect the ground water from CAFO pollution (Sweeten et al., 2003). However, developing treatments to remove the nitrogen is in contradiction with the increasing need for fertilizers to produce the crops.

Therefore, increasing the recycling efficiency of nutrients should be achieved by intensive livestock production units. Such systems should allow high input and high output per unit area without significant leakages (either as gas or as liquid) that could have negative impacts on local environment such as water eutrophication or biodiversity losses. Outputs should be solid products with low water content in order to reduce the cost and the energy used to transport them.

2. Conceptual framework of the study

2.1. General concepts

Biological systems are less intensive than physical and chemical treatments but they are cheaper to install and to adapt to a wide variety of local contexts. The construction costs of some biological system are relative low and they require minimum maintenance. When comparing the construction cost of wastewater treatment plants for towns, the construction of constructed wetlands appears higher than activated sludge treatment plant. In the case of pig slurry, Levasseur (2004) estimates the construction cost of lagooning around 20 euros/m^3 (and running cost around 0.3 euros/m^3), while the construction cost of activated sludge is in the range 5 to 10 euros/m^3 (and running costs 2 to 5 euros/m^3). However, constructed wetlands consume less energy and are easier to manage by farmers. Several physical and chemical treatments have high investment costs (Burton and Turner, 2003)

Ecology knowledge is used to reduce the negative effect of green revolution (Tilman, 1998) but most treatment systems are not designed to increase crop or animal production. Therefore we focused on this category of systems.

Main concepts are used in this study are provided by the knowledge developed to describe the transformations of nutrients in artificial biosystems.

Biological treatments have been used to avoid that chemical elements contained in piggery wastewater transform into compounds that are more dangerous to the environment. Several treatments can be used to keep piggery wastewater in less dangerous forms (Sévrin-Reyssac et al., 1999).

Most biological treatment systems are designed as open systems. Very few literature concern the concept of recycling either the water or the nutrients within the system. When the water and the nutrients are recycled, there is a dependency between the different parts of the system. This dependency can induce an evolution after several months. The dependency is highest when all the water is recycled.

The other principal concept of biological treatments is that each subsystem must decrease the concentration of chemical elements. The combination of treatments will decrease the concentrations of chemical elements (Ferreira et al., 2003).

2.2. Ecological engineering

Transformation processes are important when recycling fresh animal effluents into crop productions. We assume that the recycling efficiency of natural processes is higher when they are less disturbed (Odum, 1971). At the beginning, the animal effluents are too concentrated and too reactive to be directly recycled into crop productions. To improve the recycling efficiency, the animal effluents can be added with low dose, corresponding to natural fluxes of reactive organic matter in soils. If a high dose of organic matter is added to the soil, the animal effluents should be transformed so that the added organic matter is more stable, having similar properties as soil organic matter.

Earthworms have effective digestive system: common knowledge of vermicomposters say that they are supposed to transform each day approximately their weight of organic matter. When organic matter passes the digestive system, the biochemical reaction (the reactions associated to microorganisms) will increase the stability of the organic matter.

In aquatic systems, plants are important factor (Keffala and Ghrabi, 2005). They can absorb mineral forms of nutrients. They can reduce the velocity of liquid flow. This action provokes the sedimentation process of organic particles. The plant root is a habitat for microorganisms. The microorganisms have a major influence on nitrogen transformations through nitrification and denitrification. In freewater lagoons the water is exposed to solar radiation that can contribute to hygienization and increase the rate of some chemical reactions. The experiment of (Vymazal, 2002) with horizontal flow constructed wetland or that of (Brix and Arias, 2005b) with vertical ones showed that ecological systems were efficient for chemical nutrient removal.

Earthworms and plants can be observed easily by farmers. Thus, their use in biological treatment systems is not only to contribute to the treatment, directly or indirectly, but also to help the management, as bioindicators.

2.3. Biological treatment systems

2.3.1. Vermicomposting and vermifiltration

Vermicomposting is a biological transformation of organic wastes. Earthworms can stabilize organic wastes (Sharma et al. 2005). Earthworms are important actors of the processes in this part (Atiyeh et al. 2000). Earthworms and animal manure can help to recycle industrial organic by-products (Garg and Kaushik, 2005).

Vermifilter earthworms need organic matter as their food. The experimental results of Pramanik et al. (2007) demonstrated vermicomposting as an alternate technology for the management of biodegradable organic wastes. Decrease in chemical elements is achieved within the vermifilter. The species that are used for vermifilter are epigeic earthworms such as *Eisenia fetida* and *Eisenia andrei*. These species are often used for a suitable technology for the decomposition of different types of organic wastes (Kaviraj and Sharma 2003, Garg et al. 2006). Vermicompost is also used to filter wastewater and transform the organic matter (Taylor et al., 2003).

Metals in wastewater are absorbed by vermicompost (Urdaneta et al. 2007). Earthworms influence the absorption process. In this case, earthworms work as ecological engineers. In vermifilter, the ecological engineering function of earthworms is used to reduce the pollutions effects of pig slurry. Earthworms make several pores in vermifilter. It promotes air diffusion and the reactions in vermifilter are quite aerobic. It avoids the fermentation that produces polluting gases such as methane and ammonia. The porosity also helps the transfer of liquid and organic matter. Removal of earthworm casts will avoid the accumulation organic matter that could fill the free air space and make the environment toxic.

Transformation process of vermicomposting resulted in significant reduction in C:N ratio and increase in nitrogen phosphorus, potassium, and calcium concentrations (Kaushik and Garg 2004). Gaseous losses can be polluting gases. It should be controlled to avoid pollution transfer when transforming organic matter. Methane (CH4) atmospheric concentration has doubled in the past several hundred years to the present 1.7 ppm which is rising by around 4 ppb/yr. It is 18 around percent of enhanced global greenhouse effect. Nitrous oxide (N2O) atmospheric concentration is approximately 311 ppb and rising by around 0.75 ppb yr (Frederickson et al., 2006).

Vermicomposting could contribute to greenhouse gas emissions (Frederickson and Howell 2003). Nitrous oxide fluxes observed in winter (week 60) were 3.2 ± 0.3 mg m–2 h–1 (unheated beds), 1.8 ± 0.3 mg m–2 h–1 (heated beds). Emissions during summer (week 80) were 20.1 ± 3.0 mg m–2 h–1 (unheated beds), 21.3 ± 2.8 mg m–2 h–1 (heated beds). No relationship between earthworm density and nitrous oxide flux was found for the large-scale beds. However, in a subsequent laboratory experiment, nitrous oxide emissions were positively correlated with earthworm density (R^2 = 0.76)

However, despite these observations, Edwards & Arancon (2008) consider that it is not possible that vermicomposting contributes significantly to global warming, on the basis of the US example. In U.S in 2006, 84 percent of greenhouse gas emissions were carbon dioxide (CO_2), 7.8 percent were methane (CH_4),and 5.2 percent were nitrous oxide (N_2O). The N_2O emissions, 72 percent came from managing agricultural crop residues, 3.9 percent from animal manures and 0.5 percent from all forms of composting, including vermicomposting.

The vermifilter input is diluted pig slurry. It has several functions. First function of pig slurry is organic matter source. If there is not enough pig slurry, the system in vermifilter will not function normally because the feed is not sufficient to meet the needs of the earthworm population. On the contrary, if the input of pig slurry is too high, the excess of fresh organic matter will transform into anoxic compounds and it will induce toxicity for vermifilter earthworm population. In this case, the effect of pig slurry is a source of organic pollutants. Therefore a critical knowledge for the design and management of the vermifilter is to define the optimal quantity of pig slurry as its input. If the quantity of pig slurry is less than earthworm ingestion capability, the earthworm abundance will decrease slowly, organic matter transformation process will work slowly, water movement will increase if earthworm activity induces higher porosity and free air space, and water biotreatment will be stable. On the contrary, if quantity pig slurry is more than earthworm ingestion capability, the earthworm abundance will decrease rapidly, because organic matter transformation will induce anoxic conditions, and there will be earthworm mortality because of the anoxic environment and polluting gases can be emitted such as ammonia (NH_3) or methane (CH_4).

Once the input of vermifilter is defined, critical knowledge concerns the transformation of organic matter. Pig slurry is a source of chemical elements. When crossing the vermifilter the concentration of nutrients change and the chemical nature of nutrients also changes. The same concepts of optimal input and transformations can be applied to the constructed wetlands. The detailed knowledge of all transformation processes occurring in this experiment, including microbial, chemical and physical transformations from molecular to macroscopic scale could not be studied. We focused on the macroscopic changes from liquid phase to solid or gas phase. Solids can be exported and recycled in other agricultural systems. Gases can be released if they are not polluting the environment. Critical knowledge is needed to control the repartition of nutrients between solid, liquid and gas phases.

The life of earthworms in vermifilter depends on pig slurry input. As earthworm population influences the degradation of composted wastes (Castillo, Benito and Iglesias, 2005), we suppose that the knowledge of an optimal input dose is needed for given earthworm population, input and vermifilter chemical and physical characteristics. The experiment that has optimal dose theme must be done because the relation between pig slurry dose and earthworm abundance must be clear. To define this dose, the indoor experiment in a homogeneous environment is preferable in order to limit the number of factors that could induce differences between treatments or between replicates.

2.3.2. Different types of lagooning

Constructed wetland is effective technology for wastewater treatment (Kadlec and Knight, 1996). In tropical region, the experiment of (Kivaisi, 2001) found constructed wetland was potential technology for wastewater treatment. In subtropical region (Kadlec, 2003) found the same result.

Several researchers used constructed wetland for agriculture wastewater treatments (Jordan et al. (2003); Kovacic et al. (2006); Healy, Rodgers and Mulqueen (2007)). Constructed wetlands are often used as alternates to or components of conventional nutrient management practices to reduce or eliminate contaminant and nutrient from animal wastewater (Lansing and Martin, 2006; Dunne et al., 2005; Verhoeven and Meuleman, 1996; Tanner et al. 1995). The experiment of Lavrova and Koumanova (2007) showed that constructed wetland could reduce nutrients of piggery wastewater.

The wastewater will be treated by physical filtration, chemical adsorption on organic particles, and biological transformations by microbial populations and absorption by macrophytes. Although the plants are growing in these constructed wetlands, they are not the first factors that influence the decrease of chemical elements. They help to monitor the processes that are dominant, and to check that the transformations are stable. The treatment functions of a wetland can be optimized depending on the season (Gerke et al., 2001).

For nitrogen specifically, the complexity of nitrogen removal in water systems, including water, plants, sediments, fauna, and microbes with specific seasonal effects and nutrition dependencies, have been extensively reviewed and discussed recently by Birgand et al. (2007).

According to U.S. Environmental Protection Agency (EPA) (1999) and (Vymazal 2007), there are three types of constructed wetlands.

(1) Constructed wetland with free floating plants (FFP)

In this case, basins are covered with floating aquatic plants (Figure 1). It has open water areas. The free floating plants can be water hyacinth (*Eichhornia crassipes*), duckweed (*Lemna* spp., *Spirodela* spp., *Wolffia* spp.), water fern (*Azolla caroliniana* and *Salvinia rotundifolia*) or water lettuce (*Pistia stratiotes*). Also common are rooted plants growing in a floating form, including pennywort (*Hydrocotlyle* spp.), water lily (*Nymphaea* spp.), frog's bit (*Limnobium spongia*), spatterdock (*Nuphar* spp.), and pondweed (*Potemogeton* spp.).

In the open water source system like FFP, volatilization works effective as removal elements. This system is lacking soil process. Plant uptake is the major removal mechanism. Ammonification is effective in this system. The removal level by denitrification is medium.

The major mechanism of P removal is plant uptake. The microbial uptake is low. The adsoption and soil accretion has very small influence on P removal (Vymazal 2007).

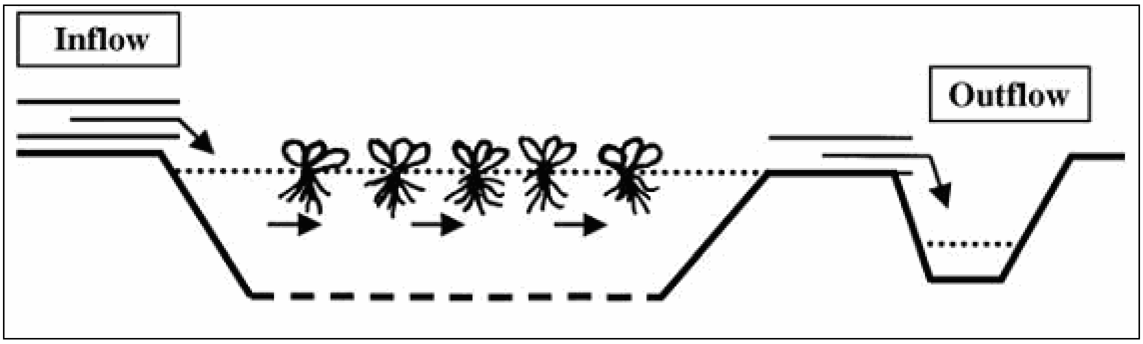


Figure 1. Free floating plants (FFP) constructed wetland (Vymazal 2007)

(2) Free water surface (FWS)

Constructed wetlands are designed using a combination of open-water areas and emergent vegetation. These wetlands are constructed wetlands that provide wastewater treatment through flocculation and sedimentation during the flow of wastewater through stands of aquatic plants growing in shallow water. In some FWS wetlands, there are also open areas where aerobic bio-oxidation complements the physical removal processes (Figure 2). FWS systems resemble natural wetlands in function and appearance. FWS systems have also been termed “surface flow systems.”

Denitrification is the major process of nitrogen removal in this system. Volatilization and nitrification are effective in this system. Plant uptake does not have an important role in nitrogen removal. The soil processes are very limited (Vymazal 2007).

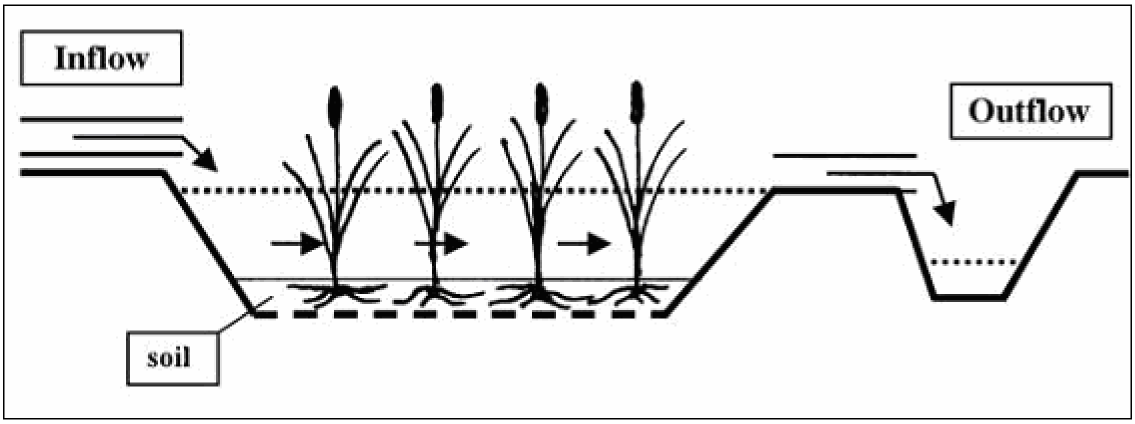


Figure 2. Constructed wetland with free water surface (Vymazal 2007)

(3) Constructed wetland with subsurface flow (SF)

This type of systems provide wastewater treatment within a filter media. Water is not directly exposed to the atmosphere but may be slightly influenced by the roots of surface vegetation. Subsurface flow (SF) wetlands systems also have been termed rock reed filters, submerged filters, root zone method, reed bed treatment systems, and microbial rock plant filters. Gravel beds rather than hydric soils are the support media for wetland plants; as a result, the systems are not truly wetlands.

If the flow of liquid is horizontal, the system is called Horizontal subsurface flow (HSF, Figure 3). If the liquid flows from up to bottom, the system is called Vertical subsurface flow (VSF, Figure 4).

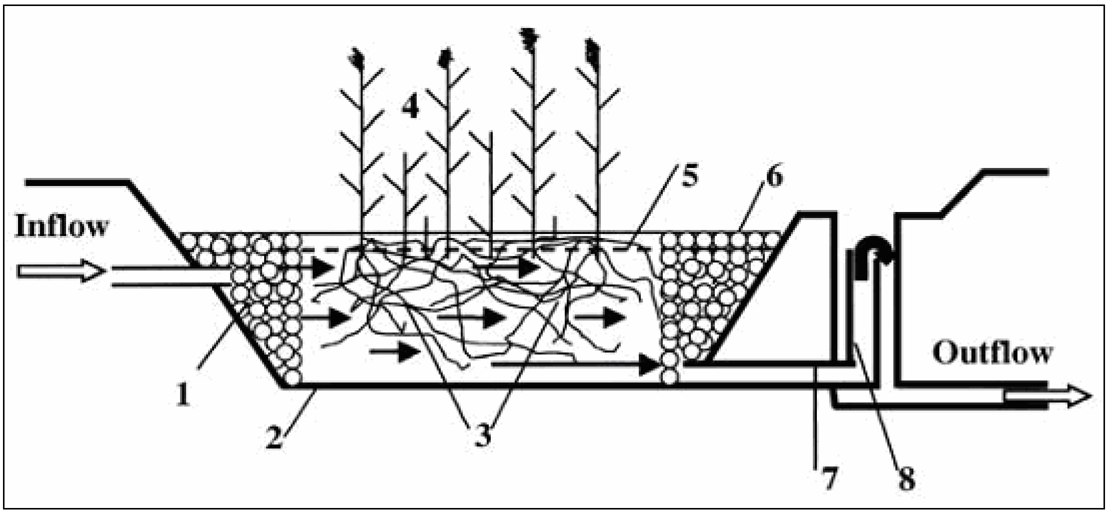


Figure 3. Schematic representation of a constructed wetland with horizontal sub-surface flow. 1, distribution zone filled with large stones; 2,impermeable liner; 3, filtration medium (gravel, crushed rock); 4, vegetation; 5, water level in the bed; 6, collection zone filled with large stones; 7, collection drainage pipe; 8, outlet structure for maintaining of water level in the bed. The arrows indicate only a general flow pattern (Vymazal, 2007).

Denitrification is the major removal process in the horizontal subsurface flow (HSF). Volatilization is a minor process. Nitrification is in very low level. The influence of plant uptake is small compared to other processes (Vymazal 2007).

Phosphorus is removed primarily by ligand exchange reactions, where phosphate displaces water or hydroxyls from the surface of Fe and Al hydrous oxides. Plant uptake is not a major removal process in cold regions. This mechanism may play more significant role in nutrient removal in tropical and subtropical regions (Vymazal 2007).

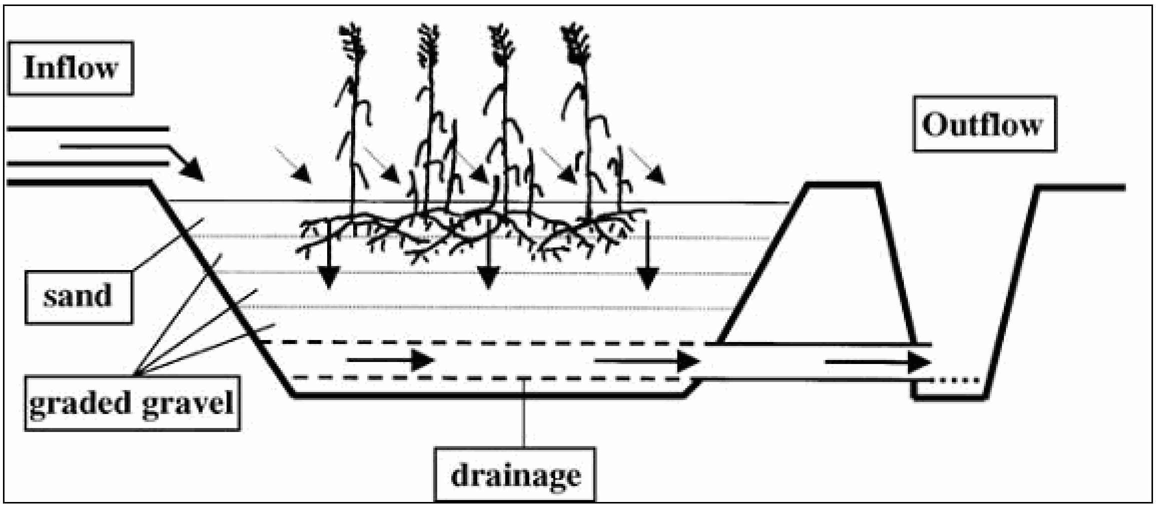


Figure 4. Constructed wetland with vertical subsurface flow (Vymazal 2007)

Nitrification is a major removal process in this system. But denitrification is low because of the high oxygenation. Volatilization does not influence much the removal processes. Plant uptake also influences less the nitrogen removal processes (Vymazal 2007)

Phosphorus removal in vertical flow constructed wetlands is very limited (Brix and Arias, 2005b). Vymazal (2007) informed adsorption is a major factor of removal process. Soil accretion does not influence the removal process. The influence of plant and microbe uptake on the removal process is small .

2.3.3. Combination of systems

Every system has strengths and weaknesses. Single-stage constructed wetlands cannot achieve high removal of total nitrogen due to their inability to provide both aerobic and anaerobic conditions at the same time. Vertical flow constructed wetlands remove successfully ammonia-N but very limited denitrification takes place in these systems because microsites with anoxic conditions occupy a very limited volume. On the other hand, horizontal-flow constructed wetlands provide good conditions for denitrification but the ability of this system to nitrify ammonia is very limited.

The treatment of wastewater requires several functions such as removal of various pollutants, sedimentation, hygienization. Therefore, various types of constructed wetlands may be combined with each other in order to exploit the specific advantages of the individual systems (Vymazal, 2007).

Integrated wetlands could reduce chemicals elements (Keffala and Ghrabi 2005). Integrated wetlands is recommended for use in domestic wastewater which should result in high treatment performance, especially on P removal (Park 2009). In hybrid systems (also sometimes called combined systems) the advantages of the Horizontal Flow (HF) and Vertical Flow (VF) systems can be combined to complement processes in each system (Vymazal 2005).

3. Experimental system

The experiments took place in the experimental farm of Guernévez located in Saint-Goazec (Finistère, France) working on pig rearing, housing, and manure management (Figure 5).

Figure 5. Aerial view of the experimental farm of Guernévez (more details on http://www.bretagne.synagri.com/ca1/synagri.nsf/TECHDOCPARCLEF/00000233?OpenDocument&P1=00000233&P2=&P3=&P4=PAGE&SOURCE=I)

Guernévez purification system applies the ecological principles of the combination of various animal and plant species for the transformation of nutrients transferred by the water. Every step of Guernévez purification system uses the principal of bio-recycling (Figure 6).

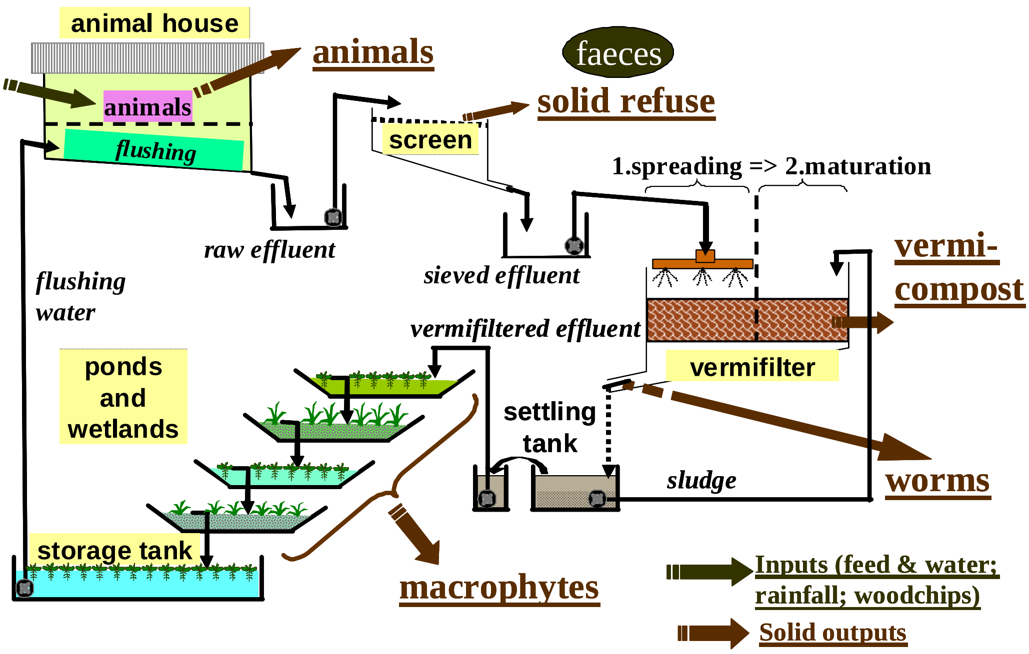


Figure 6. General organization of the system set up in 2007 (cf. Appendix 2)

The Guernévez purification system transforms the wastewater (liquid) into the other phases (solid, liquid and gaseous) that are better adapted to other uses. Solid and liquid phase are good output because they can be used for other processes. Gaseous outputs should not be polluting.

In this experiment, piggery wastewater is transferred to successive subsystems. Wastewater will be transformed by different treatments. There are physical, chemical and biological treatments. It was not possible to get a detailed knowledge of all treatments but we looked for the knowledge that was relevant to discuss the effects of the combination of different treatments.

Figure 7. Construction of the system; view from the piggery.

The piggery building of this pilot system has two floors. In main floor, the pigs are reared. The piggery uses slatted floor. The below floor has the function to collect the pig effluent. The pig effluents fall from the main floor to the below one (Figure 8).

Figure 8. Kipping tanks used in the piggery for effluent flushing

Below the animal building, the recycled water stored in the final lagoon is used to transport the pig slurry. Flushing is used to evacuate the manure. The wastewater that arrives in the last part of the treatment facility will be used as an input in the first part of the system, the piggery. This process will repeat 4 to 6 times per day. It was constructed in 2007, a picture is shown on Figure 7 Some modifications to the initial design were achieved between 2007 and 2008 (Morand et al, 2011). The detailed system that was used during our experiments is presented on Figure 12.

Its first aim is that the water can avoid the manures rest a long time within the building and dilute the concentration of chemical elements of the manure. This is to limit the emission of bad odors, ammonia or methane by the pi manure. Its second aim is that the nutrients are used to produce valuable products even if they are diluted in the circulating water. The dilution has aims for decrease the wastewater concentration for next step (sieve) or the concentration input of sieve will be less than piggery ones. In this part, animal urine and feces are treated by physical removal and chemical dilution.

Effluent will be pumped from first collector tank to sieve (Figure 9). In this phase, the solid and liquid parts are separated. The solid part (particle size above 0.1 mm) will be composted and the liquid one will be flowed to collector tank.

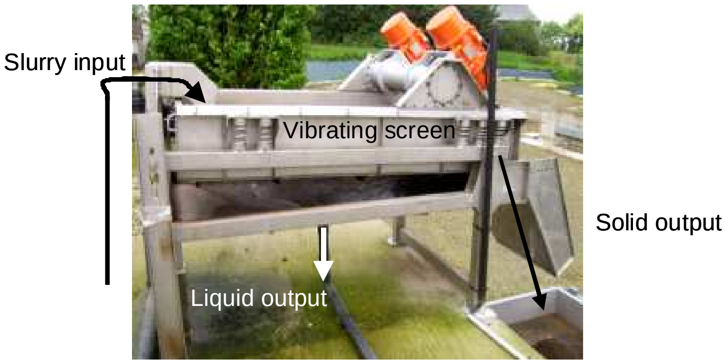


Figure 9. Sieve

Sieve is used to separate the solid from the liquid phase. The wastewater will be treated by physical treatment that separates the raw particles (>0.1 mm) from the liquid. For optimal output, solid phase will be composted. The liquid phase is pumped to the vermifilter.

In second collector tank, the liquid part of pig wastewater is stored as source before feeding the vermifilter. The wastewater is pumped from second collector tank to vermifilter. The pig wastewater is sprayed on surface of vermifilter (Figure 10).

Figure 10. Vermifilter (width: 4m, length: 10m, height: 0,6m)

The media of the vermifilter is woodchips. After each flushing and sieving, the pig slurry is sprayed to the vermifilter surface. The liquid that passes the vermifilter is filtered by the vermicompost. The earthworms *Eisenia fetida* and *Eisenia andrei* will help filtration process. In this part, wastewater will be treated by biological transformations, chemical adsorption of ammonia, and physical removal of fresh organic particles. After vermifilter, pig slurry will be transferred to constructed wetlands.

After vermifilter, the pig wastewater flows by gravity to a settling tank of 5 m^3. The sludge accumulating in settling tank is pumped to a second vermifilter (around 100 L/day) that is less porous. The resulting vermicompost is exported from the site as solid manure. Then the liquid is pumped to a combination of constructed wetland (Figure 11). There are four level constructed wetlands (P1 to P4) and a storage lagoon (P5) with a variable level and with goldfishes to have a visual control that the water quality is acceptable. The media of first and third level constructed wetland is water (floating macrophytes) and the second and fourth are gravel (horizontal subsurface flow).

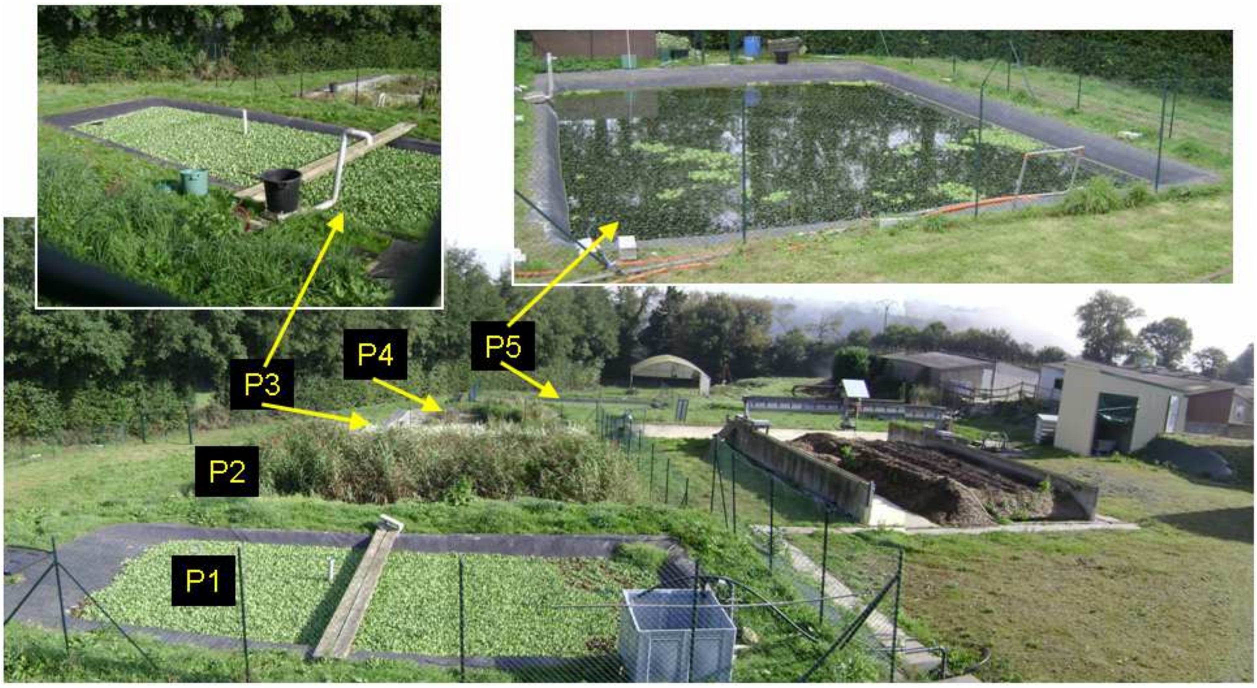


Figure 11. Combination of floating macrophyte and horizontal subsurface flow constructed wetlands

The constructed wetlands are installed at decreasing topographic levels. Because of these different heights, the liquid flow does not need additional energy. This system needs less energy to transport the liquid and the nutrients between subsystems. The first and third levels of constructed wetlands are free water lagoons with floating macrophytes. The second and fourth levels of constructed wetlands have gravels as media and are subsurface wetlands with emerged macrophytes.

The liquid flows by gravity from P1 to P5. The size of the different basins is given in Table II.

Table II. Dimensions of the basins used for the combination of constructed wetlands (adapted from Oudart, 2009)

basin	length (m)	width (m)	maximum height (m)	maximum volume (m^3)	harvesting area (m^2)	material
P1	11,8	4,0	1,5	25	38	water
P2	12,2	8,4	0,75	50	102	gravel 6/10mm
P3	12,2	4,4	0,75	25	44	water
P4	11,6	15	0,6	50	174	gravel 6/10mm
P5	16,8	10,8	2,1	250	181	water

In the final phase, the pig wastewater is collected in storage constructed wetland (P5) and used as flushing water to evacuate the pig effluents which are collected below piggery building (Figure 6).

If the wood chips don't be used anymore as vermifilter, they can be used as compost of agriculture plantation. The biomass of plant will be composted then they can be used as compost. The azolla biomass in third level could be used as animal feed.

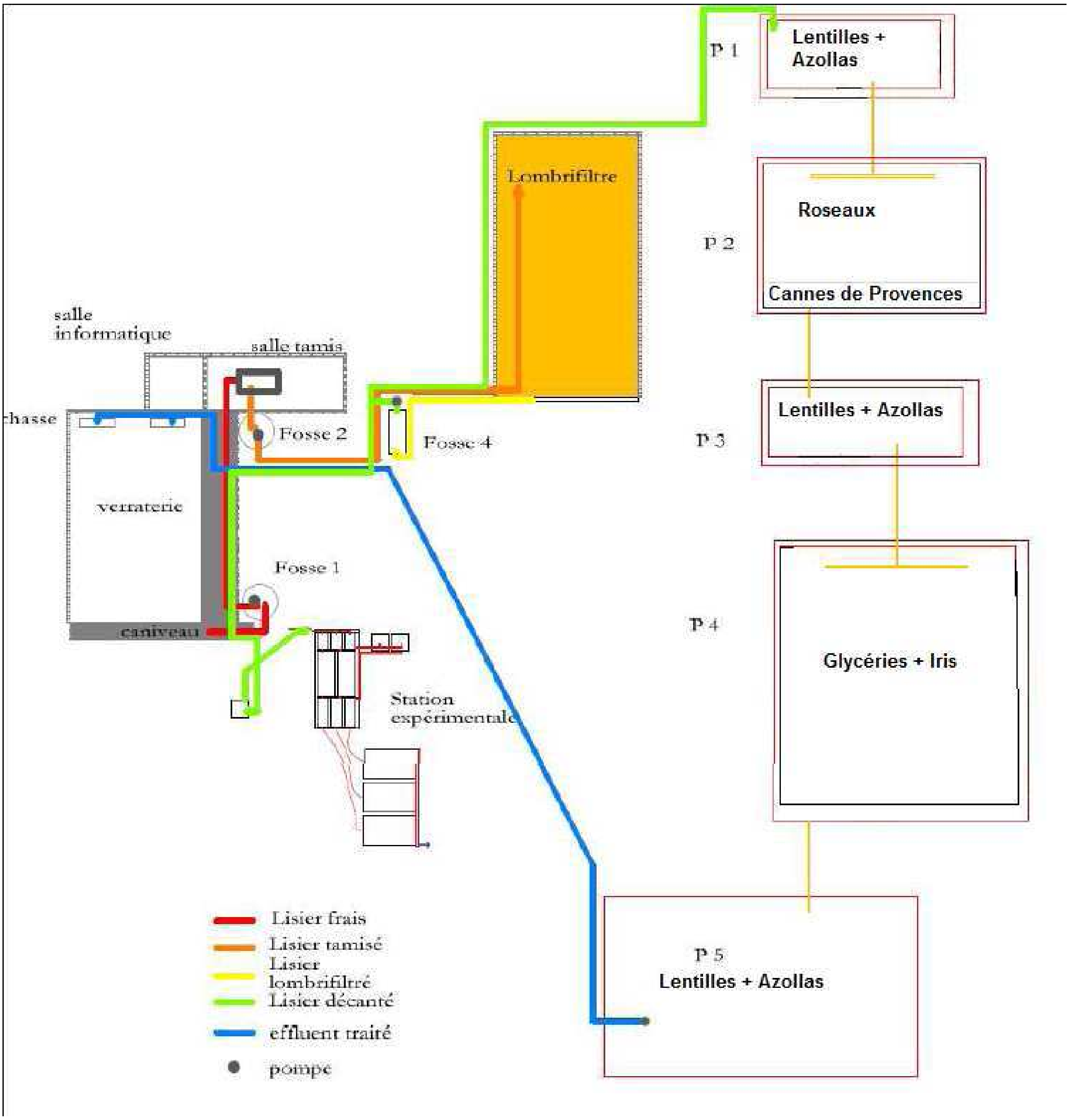


Figure 12. Map of the system (from Fiévet, 2008; the system was progressively modified during successive trainings in Guernévez, cf. Appendix 2)

4. Objectives and Method

The objective of the present work is to identify, and add new when necessary, the knowledge that is critical for the design and the management of the subsystems:

- is it possible to design a biological system that will achieve together water recycling and exportation of excreted nutrients as plants and organic matters?
- how can be defined an optimal input at each level, when different biological subsystems are combined successively, the quantity of water transferred between subsystems being the same?
- for this design, is it possible to use knowledge that has been deduced from studies in open systems, without recycling?

Therefore, this study addresses two levels: the processes at subsystem level, the interactions between subsystems at system level. These two levels will be addressed in two different parts.

At first, we will focus on the vermifilter subsystem because the organic load is highest and the area is limited. The transformations of organic effluent are most intensive. High losses of carbon and nitrogen have been already observed. We had a particular attention to the gaseous emissions because the development of animal production should not induce an increase in emissions of polluting gases. Therefore, the first chapter looks for the concept of “optimal input” in the case of vermifiltration with pig slurry and the second chapter analyzes the specific effect of earthworms on gaseous emissions during vermifiltration.

Experiment of earthworm abundance and gaseous emission is the next step. The indoor experiment is used. Earthworm activity can recover a portion of the more labile nutrients and promote favorable physical and chemical conditions (Mitchell 1994). The solid and liquid phases of experimental media and the earthworm abundance must be examined as the factors that can explain differences in gaseous emissions.

Then we will focus on the macrophyte lagooning and analyze the consequences of the interactions between the different subsystems, vermifilter and constructed wetlands, on the nutrient concentrations in the water, and on the harvested plants. Therefore, the third chapter analyzes the spatial interactions between the different subsystems, while the fourth chapter analyzes the temporal interactions when the seasons influence the plant growth.

Pig slurry has many chemical elements. During experiment, the value efficiency is used to identify the decrease of chemical elements. The experiment of different plant varieties is done in order to know their effect on the evolution of chemical concentrations. The evolution of efficiency during different seasons must be verified because variations in temperature can influence the biochemical reaction in lagoon.

Part 1: Vermifiltration process: optimal input and gaseous emissions

Chapter 1: Optimal input of pig fresh liquid manure during vermifiltration

1. Résumé du chapitre 1 : Intrant optimal de lisier frais d'un élevage de porcs pour la lombrifiltration

Les vers de terre mélangent et transforment l'azote et le carbone de la matière organique fraîche sans consommer d'énergie additionnelle. L'objectif de ce chapitre 1 est de vérifier s'il y a un intrant optimal de lisier qui peut maximiser la population des vers de terre, et donc de l'effet des vers de terre sur les transformations de la matière organique fraîche.

L'expérience a employé 17 mésocosmes d'environ 50 L, constitués à partir d'un lombrifiltre recevant le lisier d'un bâtiment de porcs. Huit doses de lisier ont été ajoutées aux mésocosmes, pendant un mois, avec deux répétitions. Un mésocosm témoin a reçu seulement le liquide filtré et réutilisé pour l'arrosage. L'abondance de vers de terre a été mesurée trois fois et l'évolution de la porosité libre à l'air a été estimée. Les abondances de vers de terre ont été analysées avec l'analyse de groupement.

Les résultats de l'analyse statistique indiquent que la population est réduite quand l'intrant est très bas ou très haut. Par conséquent, nous proposons que l'abondance de vers de terre puisse être employée comme bioindicateur de l'intrant optimal de lisier frais sur le lombrifiltre, indiquant que les transformations sont maximisées par unité de surface et que le système est géré de façon stationnaire. On en conclut qu'un intrant optimal peut être défini pour caractériser le « preferendum » d'un lombrifiltre.

Si cette conclusion est appliquée aux systèmes agricoles avec une productivité élevée, elle implique que le concept du « preferendum » devrait inclure des variables de flux, telles que des intrants d'eau ou d'éléments nutritifs, et non seulement des variables d'état, telles que la température ou la composition de l'environnement. Le chapitre 2 qui suit dans ce rapport analysera si le fonctionnement au voisinage de ce « preferendum » augmentera ou pas les émissions polluantes telles que l'ammoniac ou les gaz à effet de serre habituellement émis par les effluents d'élevage dans des élevages intensifs.

2. Abstract

Earthworms mix and transform nitrogen and carbon from fresh organic matter without consuming additional energy. The objective of this chapter 1 is to check if there is an optimal input of liquid manure that can maximize the population of earthworms, and therefore the effect of earthworms on the transformations of fresh organic matter.

The experiment used 17 mesocosms of around 50 L, made from a vermifilter treating the liquid manure of a swine house. Eight levels of slurry were added to the mesocosms, during one month, in two replicates. There was one control where only the percolated liquid was reused for spreading. Earthworm abundance was measured three times and the evolution of free air space was estimated. The abundances of earthworms were analyzed with cluster analysis.

The results of the statistical analysis indicate that the population is less when the input is either very low or very high. Therefore, we suggest that earthworm abundance can be used as a bioindicator of the optimal input of fresh liquid manure to the vermifilter, indicating that the transformations are maximized per unit area and that the system is managed in a steady-state way. It is concluded that an optimal input can be defined to characterize the "preferendum" of a vermifilter.

If this conclusion is applied to agricultural systems with a high productivity, it implies that the concept of "preferendum" should include flux-variables, such as water and nutrient inputs, and not only state-variables, such as temperature or composition of the environment. The following chapter 2 of this report will analyze if working close to this "preferendum" will increase or not the polluting emissions such as ammonia or greenhouse gases usually emitted by animal manure in concentrated animal feeding operations.

Keywords:

Vermifiltration, free air space, liquid manure

3. Introduction

Concentrated animal production consumes resources such as energy, crops, or fertilizers, and its effluents pollute the environment in ways such as by producing greenhouse gases, water eutrophication, or soil contamination (FAO, 2006a). As animal production is expected to increase (FAO, 2006b), there is an urgent need to increase resource efficiency and decrease the polluting impact. Both can be achieved through the evolution of systems that increase the recycling rate of water, energy, and nutrients, at local or global scales, and that allow producers to certify reduced polluting leakages and increased efficiencies.

A system of animal production has been designed that increases the recycling efficiency of water and animal feed (Morand et al., 2011). The system associates a pig house with manure flushing, a vermifilter, lagooning, and constructed wetlands, in order to combine water and nutrient reuse to high manure dilution. Nutrients are exported either as organic matter or as plants. Water is reused after abatement of pathogens and micropollutants. The vermifilter contributes to a major part of carbon and nitrogen abatement in the liquid. It produces casts that can be exported for use as vermicompost.

The concept of *preferendum* is used in ecological studies to describe the optimal environment of organisms or populations (Fry, 1947), i.e. the range of environmental variables that is preferred for growth and reproduction. As cited by Hermoso et al. (2009), it has been introduced for fish by Shelford (1911) in the "law of tolerance" used in geographical ecology. A cross search in Web of Science using the only word "preferendum" shows that this concept has been used to study wild environments but not to optimize agricultural or animal production. A cross search using "preferendum" and "earthworm" revealed no citations. The concept has been used by Edwards & Bohlen (1996) to indicate the optimal temperature ranges for various earthworm species and first reference cited on this subject is Grant (1955).

In this chapter, we evaluate if a larger meaning of this concept can be used: not only state-variables characterizing the environment, but also flux-variables characterizing the exchanges through the limits of the managed system. As a matter of fact, agricultural systems that were developed to increase production (the so-called “green revolution”) are characterized by increased inputs (fertilizers, energy, pesticides) and increased outputs (kg yield per hectare, kg meat per kg feed).

In the case of vermifiltration, there are both high inputs and high outputs of water and organic matter each day. As the system can rapidly change depending on these fluxes, the concept of “optimal input” should be added to the description of the preferendum of vermifilters. When the system receives a high input of organic matter, the transformations of the substrate depend on the abundance of earthworms (Ndegwa et al., 2000; Clarke et al., 2007). The effect of earthworms on the transformations is both direct and indirect. The direct effect is for example the transformations that occur to the organic matter ingested by the earthworms. The indirect effect is for example the transformations by aerobic microbes that can develop in the added organic matter because the earthworms maintain a free air space in it.

When the input of fresh organic matter is too high, the system becomes anoxic because the free air space is filled with water and particulate organic matter, which reduces oxygen diffusion inside the media. In the specific case of animal manure, the availability of carbon (C) and of nitrogen (N) is high and the C:N ratio is low. In the case of vermifiltration, the abundance of water input, compared to vermicomposting, can also induce specific requirements. From these theoretical considerations, confirmed by practical observations during the development of the system (Morand et al., 2009, 2011), it was expected that a too low dose would not be enough to feed the earthworms and maintain the moisture of the vermifilter. It would induce a decrease in the earthworm abundance. On the contrary, a too high input dose would induce anoxic conditions and earthworm mortality. Thus, an optimal dose should exist, which allows the maximal population of earthworms.

The objectives of this work were: (i) to analyze if a maximum in the abundance of earthworms can be defined when various doses of animal wastewater are applied to a vermifilter; (ii) to analyze if the free air space decreases when the input dose and the duration of experiment increase.

4. Hypothesis

There is an optimal input of fresh liquid manure where the earthworm abundance is highest.

The evolution of the population according to the amount of fresh liquid manure is sufficiently fast to show some evolution after 30 days, therefore we focused on a short term experiment.

The relationship between the input of fresh liquid manure and the abundance of earthworms is supposed to be a bell-shaped curve (Figure 13). The top of the curve corresponds to the optimal input of the vermifilter. It is assumed that the input of the running vermifilter (14 L per day observed in the large-scale vermifilter) is close to the optimal input because it allowed a continuous operation of the vermifilter from 2008 to 2009.

The earthworm population in low or high pig slurry dose is lower than in optimal dose. The cause of lower population is different for low or high dose. The low population in the low dose is caused by the insufficient food. In contrary, low population in the high dose is caused by the toxicity of the environment.

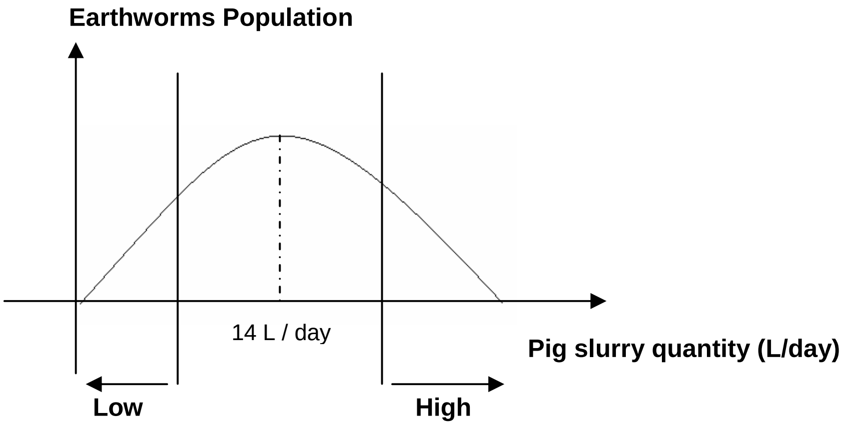


Figure 13. Hypothesis of variation of the population of earthworms according to the input of fresh liquid manure

The earthworms are supposed to be mainly located close to the surface because Eisenia fetida and Eisenia andrei are supposed to be the main species. They are epigeic earthworms and live in soil surface (Bouché, 1972). When sampling the surface of the mesocosm, the estimate of total population of the mesocosm should be close to the estimate based on an average sample of all mesocosm. However, large scale vermifilter is outdoor and it is an open system. Migration of of fauna between vermifilter and its environment is possible. Thus there is a possibility that several non epigeic species live in the vermifilter and are in the mesocosms and not located close to the surface.

5. Material and methods

We use the term “vermifilter” for the system that is spread (substrate and its container) and “vermicompost” or “vermifilter material” the substrate that is sampled and analyzed.

We use the expressions “pig slurry” or “fresh liquid manure”, or the terms “liquid” or “wastewater” to designate the liquid used to spread the vermifilters because it is more convenient. In fact it is not exactly slurry that designates the liquid resulting from excretion and conservation of the effluent during several weeks.

5.1. Experimental site

The research was conducted at the Piggery Experimental Farm of Guernévez, Saint Goazec, France from October 2009 to November 2009. In this site, a vermifilter 48 m^2 in area and 0.5 m in height is in use since 2007. Most earthworms are *Eisenia andrei* (Bouché) and *Eisenia fetida* (Savigny). It was designed according to the conclusions of Li et al. (2008). It recycles the wastewater of a piggery with 30 sows with 4 to 6 flushing per day (800 L water per flushing). Wood chips are added twice a year, and the vermicompost is progressively removed. The wastewater goes through a screen, a vermifilter, a settling tank, and four levels of lagooning and constructed wetlands, until it reaches a storage basin that accumulates rainwater in winter to compensate for summer evaporation. The water is pumped back from the storage basin to flush the piggery. The composition of the liquids at the various levels is given in Morand et al. (2009).

Both the vermifilter and the liquid of this system result from its continuous operation during two years. It could not be reproduced in a laboratory using new materials but it was considered to be sufficiently stable to ensure reproducibility of the experiment: it was considered as a “reference material”. Therefore, the experiment had to use the organisms, the solids, and the liquids of the large-scale vermifilter.

Mesocosms of 53 L vermifilter were each sprinkled with different doses of fresh slurry. There were 2 repetitions of each treatment. The mesocosms were placed within a not insulated building so as to avoid rain inputs but to have temperature variations similar to outside climate. The initial material for filling the mesocosms was taken from the surface of the existing large-scale vermifilter. It was thoroughly mixed before filling all mesocosms simultaneously (Figure 14).

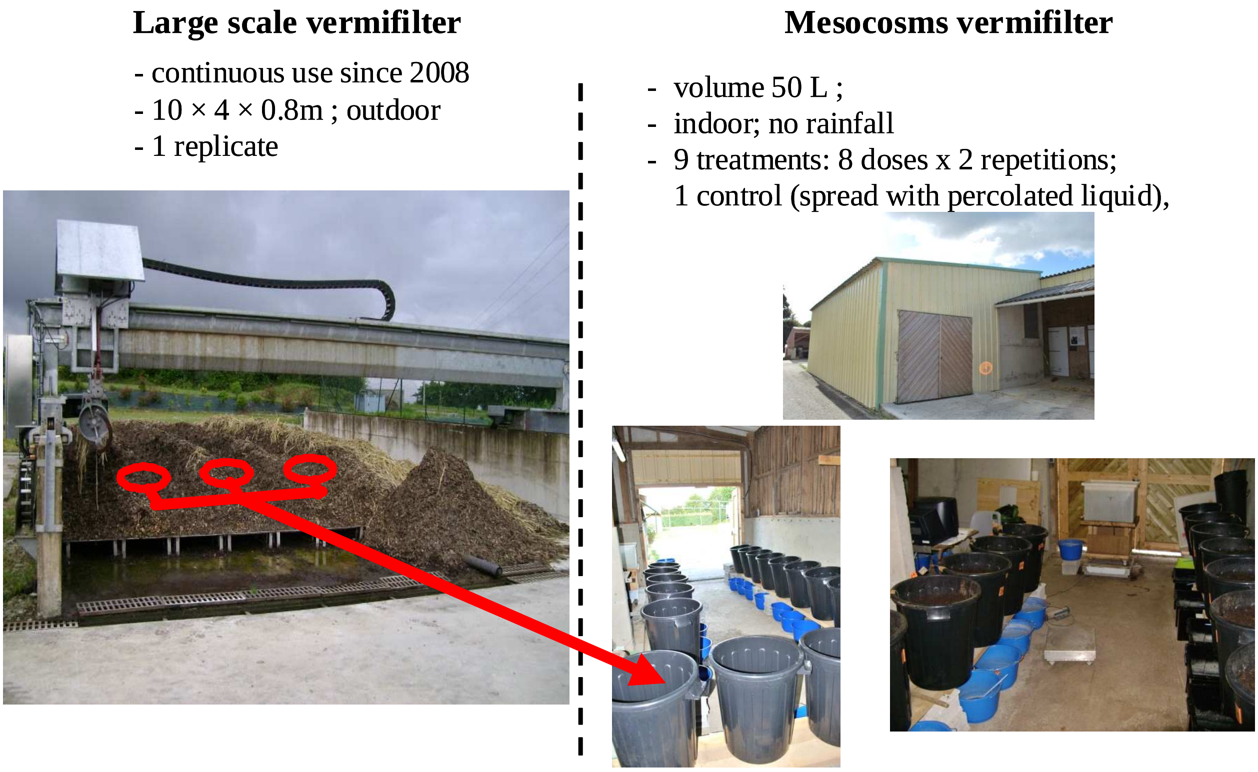


Figure 14. Large scale vermifilter and mesocosm experiment in a not insulated building; both received the same liquid added each day

The experiment took around 4 weeks. The shape of the container containing the mesocosms was cylindrical with 42,4 cm in height and 0.52 m upper diameter (0.21 m^2). The vermifilter material was placed on a grid to allow easy drainage of the water. The percolated liquid was collected in a second container, for daily sampling and weighing (Figure 15). A previous experiment showed that a similar evolution of the population of earthworms was observed with this type of mesocosms compared to the large size vermifilter.

The wastewater was taken once a day from the liquid input of the running vermifilter. The time of sampling was variable, depending on the time of spreading.

The 17 mesocosms were divided into 8 groups and one control. Each group included 2 replicates with the same input. 30.0±0.01 kg of vermicompost was placed into the mesocosms and occupied 53.7±0.7 L. The 8 groups received respectively 1, 2, 3, 5, 7, 9, 11, 14 liters of swine effluent twice per day (morning and afternoon), except the days of sampling and earthworm counting, from September 12^{ve} until October 5th. A previous experiment showed that adding the liquid twice a day manually was acceptable in terms of practical operation during several weeks, and in terms of representativity of the normal vermifilter spread automatically from 4 to 6 times a day.

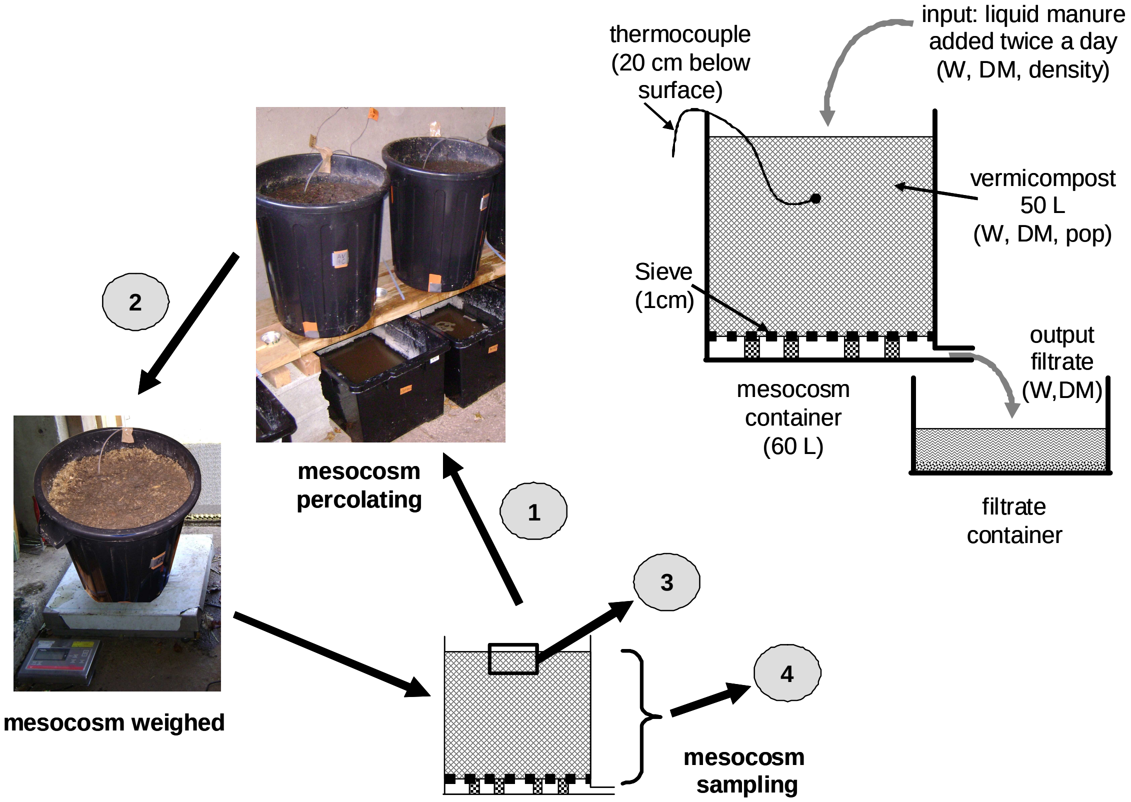


Figure 15. Diagram and photographs illustrating the experimental procedure for the first experiment. W: weight; DM: dry matter; pop: population. (1) mesocosm percolating between sampling operations; (2) weighing before sampling; (3) sampling the surface of the mesocosm; (4) gentle mixing and sampling the mesocosm.

5.2. Pig slurry application

During the first week of the experiment, the slurry was not continuously mixed during application. Therefore, the first treatments (lowest dose) received less concentrated slurry and the last treatments (highest dose) received the slurry with the most organic particles. To avoid a too high difference between the two repetitions of the same treatment, the vermicompost of the two repetitions was mixed the day of sampling. However, this operation of mixing the repetitions made difficult to analyze the evolution of population during the whole experiment, i.e. to confirm the trend observed between the two first sampling dates. Therefore, the mesocosms were not mixed during the experiment with gaseous emissions described in the next chapter of this report.

Pig slurry was applied to mesocosms using a pitcher, each of which was weighed to ensure correct application rates (Figure 16).

During several days in the last week of experiment, pig slurry was taken at 10 o'clock or after the morning pig feeding time. According to the schedule defined on the farm, pigs were fed approximately at 8 o'clock. The pig slurry during these days was more concentrated because the flushing collected the excretions of the night and the first feeding period. This change was decided because no mortality was observed with the high dose after the first weeks, whereas on the large scale vermifilter, the high dose was known to induce earthworm mortality after 14 days.

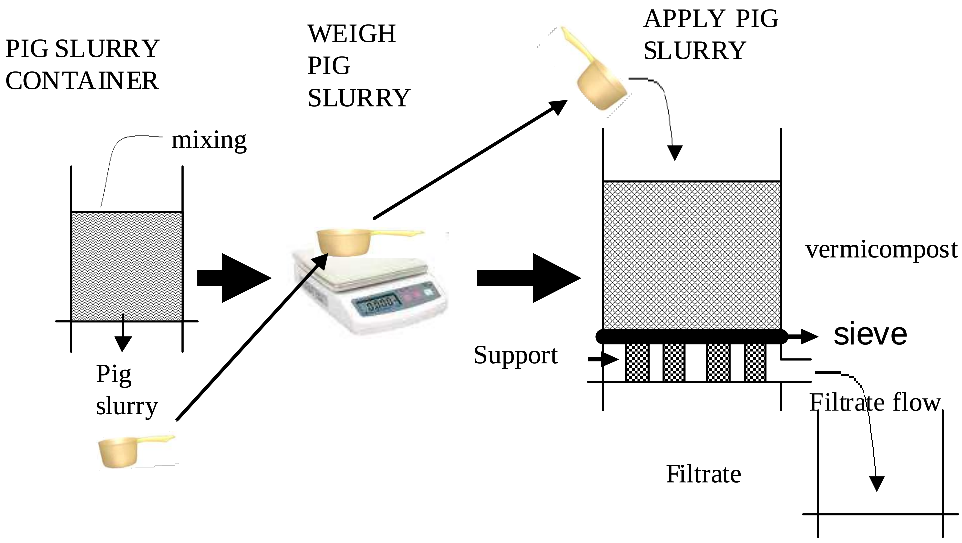


Figure 16. Addition of the liquid to the mesocosms. Temperature, density, mass of input and output, dry matter of input and output, were measured each day.

Table III. informs on the chemical composition for every dose. The pH of pig slurry was 8,2 ± 0,56. The increase in pig slurry quantity induced an increase in all chemical nutrients.

Dose L/mesocosm/day	**DM** g/m²/day	**DCO** g/m²/day	**SS** g/m²/day	**NTK** g/m²/day
2	38	34	15	3
4	76	69	30	5
6	114	103	46	8
10	190	171	76	13
14	267	240	107	18
18	343	309	137	23
22	419	377	168	28
28	533	480	213	35

Table III. Chemical input for each dose

5.3. Sampling

5.3.1. Solid (vermicompost)

For each treatment, a sample of the vermifilter media was taken on days 18th September, 30th September and 7th October. Samples were taken after mixing the vermicompost in each mesocosm. In the running vermifilter, the vermicompost was also mixed every week. Thus it is assumed that the sampling and mixing operation did not alter the representatives of the experiment. Samples were also taken at the surface of the mesocosm (0-10 cm).

The sampling procedure is shown in Figure 17. On sampling days, there were not pig slurry applications. Each container was weighted. The surface solid samples were taken. The tray (11 cm x 10cm x 6 cm) was used for surface sampling.

The vermicompost was taken out from container. The vermicompost was gently mixed to preserve the earthworms. Vermicompost was divided into two parts. The operation (mix-divide) has been done three times.

One-half part of vermicompost was returned to the container and the other part was mixed. This operation (mix-divide) was repeated for each container until the quantity of material in a sample (about 2 l) was obtained. Three trays (11 cm x 10 cm x 6 cm) were used for mix samplings.

The part of vermicompost that was not used as mix solid sampling would be returned to container. After the sampling, the new quantity of vermicompost was weighed to have the initial mass of the next period, the container was progressively filled, the thermocouple was installed horizontally around 20 cm below the surface (e.g. 21±2 cm on 11th September 2009), finally the surface was slightly compacted to ensure homogenous infiltration of the wastewater.

The sample was divided among 3 trays (approximately 400 mL per tray), and each tray was weighed. One tray was conserved in a deep freezer (-18℃) for further analysis. One tray was used for earthworm counting. One tray was used for dry matter analysis, and then its contents was ground and analyzed for C and N by INRA (Institut National de la Recherche Agronomique) in Rennes.

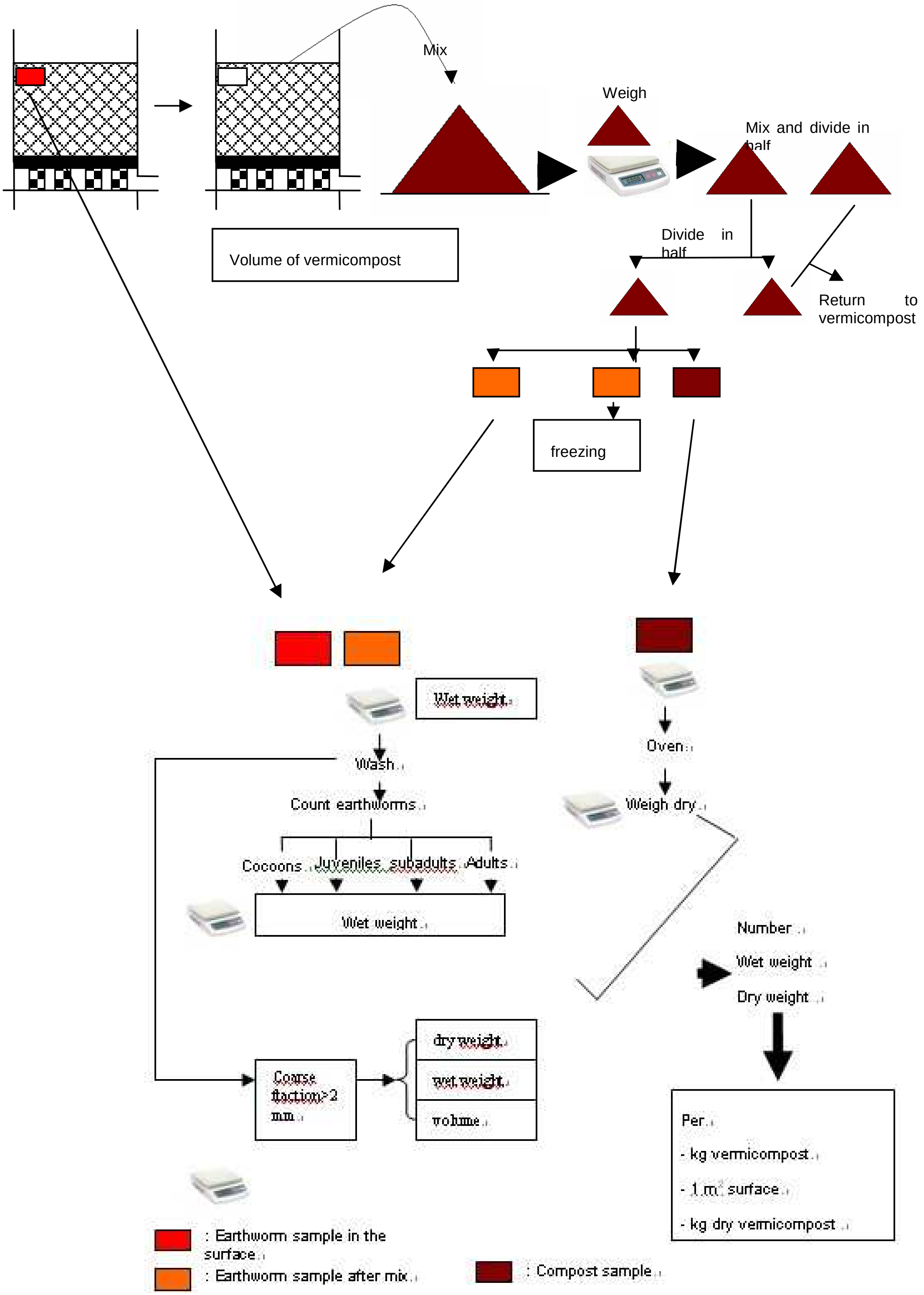


Figure 17. Mesocosm sampling for earthworm counting and dry matter analysis

5.3.2. Liquid

Liquid samples were taken for each time where input liquid was taken at the running vermifilter. The dry matter was measured for initial and final liquid to check the homogeneity of the input for all mesocosms. One liquid sample was taken from the filtrate received in each second container. Each day, input and filtrate samples were either frozen, for further chemical analysis, or dried for dry matter measurement.

5.3.3. Earthworms

The vermicompost in the remaining tray was washed in sieves of decreasing mesh size (10mm, 2mm, and 0.5mm). The earthworms were taken manually in each sieve with a tweezers and put in different cups containing tap water. After having washed the entire sample, the earthworms were counted (abundance measurement) and put on paper towel to remove the water before weighing (biomass measurement).

After having counted the earthworms, the volume of the coarse fraction (> 2 mm) was measured by immersion in water and its dry matter was measured with oven drying at 60℃.

The population abundance and biomass were then extrapolated to the whole mesocosm in two ways:

- for the average sample, it was extrapolated on the basis of the wet weight of the sample and the net weight of vermicompost in the mesocosm,
- for the surface sample, it was extrapolated on the basis of the area of the sample and the surface area of the mesocosm.

5.4. Analytical methods

5.4.1. Statistical analysis

Cluster analysis was performed using SAS® software, with or without hierarchy (procedures named “fastclus” or “cluster method”) to analyze the groups of treatments at the different dates (18^{th} September, 30^{th} September or 7^{th} October), for either the surface sample or the average sample, for either the total biomass or the total population. The software calculates the best association between the treatments (clusters) and it calculates a criterion of separation between the clusters (cluster criterion) which makes it possible to choose the best number of clusters to be considered. If there are at least three clusters, an optimum can be defined as the last cluster; its population is maximum, while the other ones are less optimal, and the population being least in the first cluster.

Treatments are divided into three clusters (Figure 18). The first cluster is the group of treatments that have less earthworm population. The second cluster is the group of treatments that have middle earthworm population. The third cluster is the group of treatments that have maximum earthworm population. Cluster three should be clearly separated from cluster one. Clusters one and three should contain less treatments.

Using this method the treatments with the maximal or minimal population should be better identified.

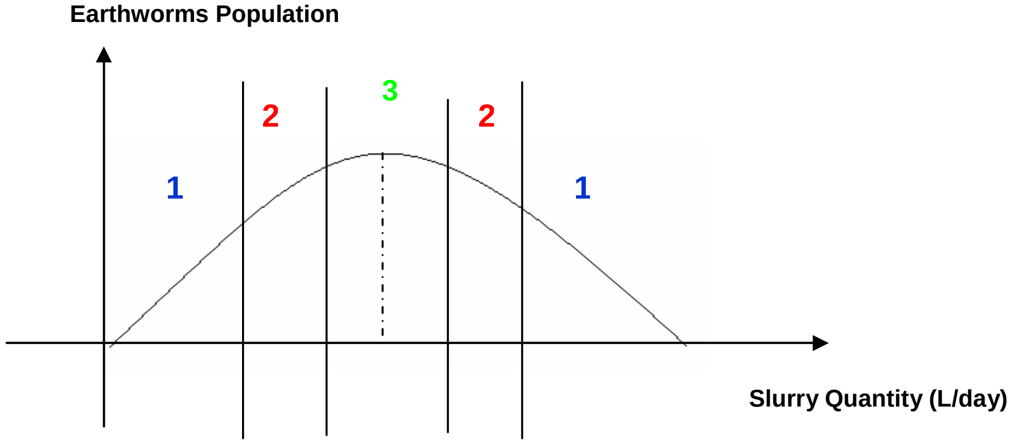


Figure 18. Cluster Area; 1: Cluster of minimal population (strong amount, weak amount); 2: Cluster of intermediate population (separation); 3: Cluster of maximum population (Optimal amount)

The cluster analysis was chosen in order to anticipate the unpredictable result that were presented in curve of relation between earthworms population and slurry quantity (Figure 13). There were possibilities that form of curve did not follow the hypothesis. When the form of curve were not same as the hypothesis ones, it would be difficult to determine the position of slurry quantity.

The first result experiment would be used for the second experiment. Three doses with different characters must be found. The regression curve of relation between earthworms population and slurry quantity (Figure 13) would be less satisfied than cluster analysis because it couldn't divide the slurry quantities to the groups that are different character. Cluster analysis could divide exactly all pig slurry dose to group that has similar character.

5.4.2. Mass balance

The mean of mass balances was calculated for each of the 8 treatments and for each period. It used the measurements of total weight and dry matter for the solids and liquids, and the carbon and nitrogen analysis for the vermicompost.

5.4.3. Calculation of the porosity

Porosity and free air space were calculated from the measurements of weight, dry matter, and volume described above (Dai, 2009).

Hydraulic conductivity was not measured because it was considered not relevant. As a matter of fact, the value can be very heterogenous within te vermifilter when there is clogging at the surface. When the media is homogenous, there is a large range of acceptable values (i.e. the added quantity of liquid can drain within a few minutes or during several hours after input) provided there is enough oxygen diffusion for the earthworm needs, and the daily input has drained after 24h.

Similarly, the moisture of the media could be rather constant but induced various oxygen availability depending on the macroporosity provided by the coarse materials, and the oxygen consumption by the media after liquid input.

The calculation of free air space used assumptions concerning density of organic matter and density of water, and that the volume of air inside the coarse fraction does not contribute to the free air space that is efficient for oxygen diffusion inside the vermicompost. It was based on following equations:

Volume Free air space = Total Volume inside container - Volume Coarse - Volume Fine - Volume of Water in Fine

Total Volume inside container = Volume of vermicompost above sieve.

Volume Coarse = Volume of Coarse Fraction / wet weight of sample * net weight of vermicompost

Volume Fine = (**Mass of Fine Particles** / wet weight of sample * net weight of vermicompost)/ Density Organic Matter

Volume of Water in Fine= Total Mass of Water / Density of water – Wet weight of coarse fraction * water content of coarse fraction / Density of water

Mass of Fine Particles = wet weight of sample * dry matter content – dry weight of coarse fraction

Total Mass of Water = Net weight of container * water content

Density Organic Matter = 1.7kg/L

Density of Water = 1.0kg/L

6. Results

6.1. Abundance of population and biomass

There was no clear optimum of the input dose from the population observations at any date, as shown by Figure 19. Similar results were observed with the biomass.

As the population of the control (no slurry input, only recycled water to maintain the moisture) did not change, it can be assumed that the nutrients present in the vermifilter are enough to feed the earthworm population during at least one month.

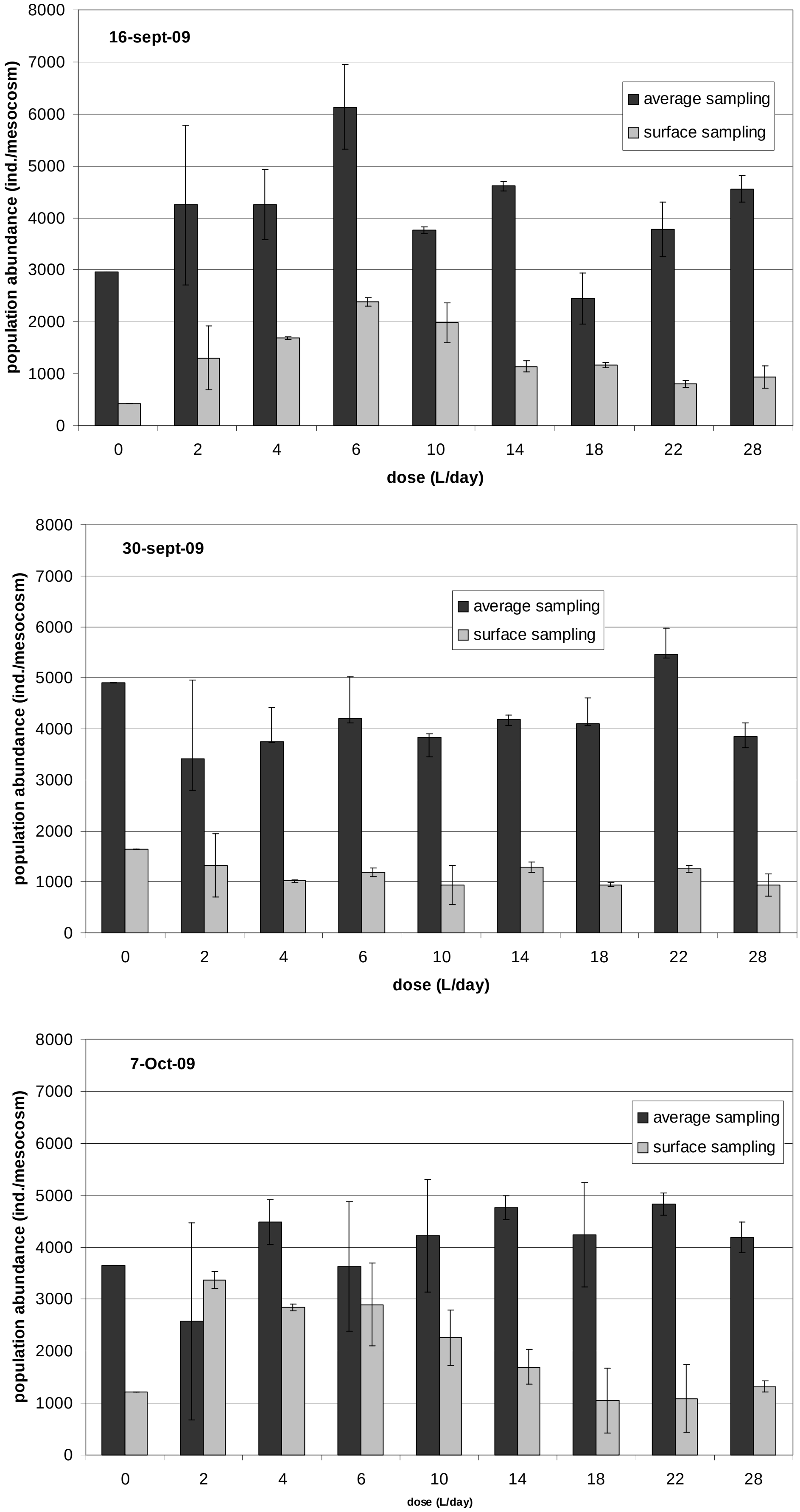


Figure 19. Evolution of population abundance when input dose varies from 2L/day to 28 L/day. One graph for each date, bars indicate the maximum and minimum values of both repetitions.

6.2. Cluster analysis

Results of cluster analysis are given in Table IV. The detailed results are given in Appendix 1. Cluster 1 corresponds to the group with the lowest values of abundance or biomass, while cluster 3 corresponds to the group with the highest values. Therefore, optimal input should correspond to cluster 3 and lower or higher input dose should be found in cluster 1 and 2.

Table IV. Results of cluster analysis for the different population characteristics (average or surface sampling, abundance or biomass of population; three sampling dates); all details of data and statistical analysis are in Appendix 1.

variable	sampling date	cluster 1	cluster 2	cluster 3
average population abundance	16-Sept.-2009	18	2, 4, 10, 14, 22, 28	6
average population abundance	30-Sept.-2009	2, 6	4, 10, 14, 18, 28	22
average population abundance	7-Oct.-2009	2	4, 14, 22, 28	6, 10, 18
average biomass	16-Sept.-2009	10, 18	2, 4, 14, 22, 28	6
average biomass	30-Sept.-2009	2, 6, 14	4, 10, 18, 28	22
average biomass	7-Oct.-2009	2, 4	14, 22, 28	6, 10, 18
surface population abundance	16-Sept.-2009	2, 22, 28	4, 14, 18	6, 10
surface population abundance	30-Sept.-2009	2	4, 10, 18	6, 14, 22, 28
surface population abundance	7-Oct.-2009	2, 4, 6	10	14, 18, 22, 28
surface biomass	16-Sept.-2009	2, 22, 28	4, 14, 18	6, 10
surface biomass	30-Sept.-2009	2, 4, 10	6, 18, 28	14, 22
surface biomass	7-Oct.-2009	14, 28	18, 22	2, 4, 6, 10

As expected from graphical results presented above, statistical analysis gives heterogeneous clustering depending on the considered variable. However, it appeared that input dose 6 L/day was found in a majority of cluster 3, while input dose 2 L/day was found in a majority of cluster 1. Many results of input dose 28 L/day are in cluster 1.

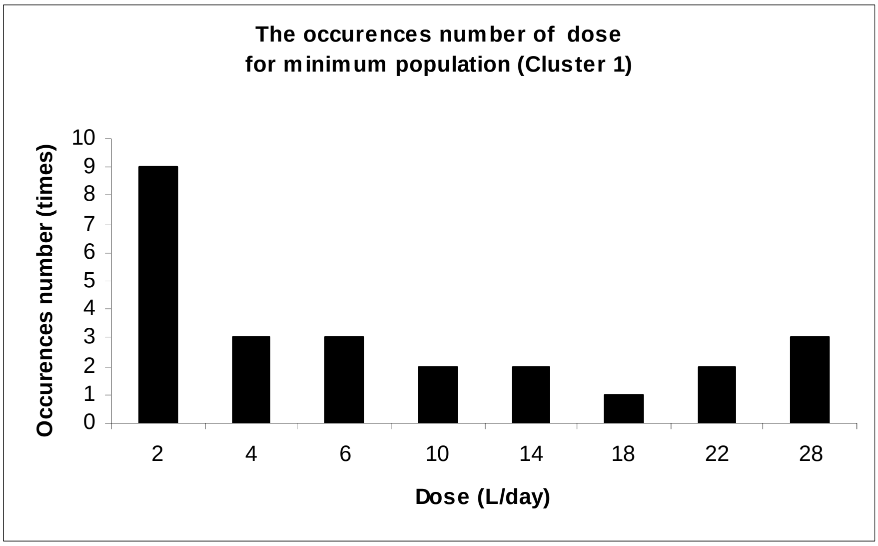


Figure 20. The occurrence number of dose for minimum population (Cluster 1)

According to Figure 20, in the low dose condition (less than 14 L/day), most of result of dose 2 L/day were often found in cluster 1. The treatment 2 L/days made the earthworms parameters in mesocosm were often in less level. In high dose condition (less than 14 L/day), most of result of dose 28 L/day were often found in cluster 1. The treatment 28 L/days made the earthworms parameters in mesocosm were often in less level too. Although the results of two doses were same, the causes of these cases were different. Therefore, these two doses were considered as minimum and maximum doses in the next experiment.

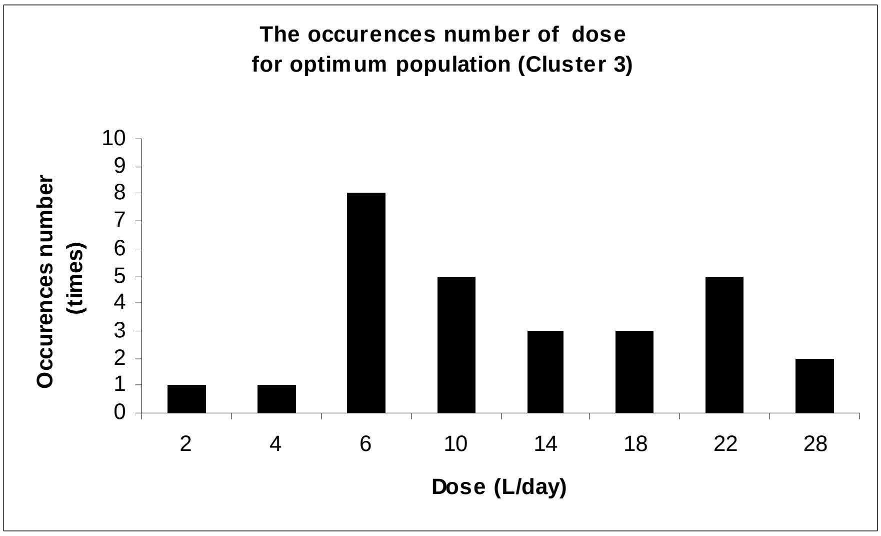


Figure 21. The occurrence number of dose for optimum population (Cluster 3)

The experiment results of dose 6 L/day were often found in cluster 3 (Figure 21). The input treatment of 6 L/day made the most earthworms parameters of mesocosm were often found in maximal level. Therefore it was chosen as medium input for next experiment.

This result experiment will be used in the next experiment. According to hypothesis (Figure 13), three doses are needed for the next experiment. First dose is the dose with both lower earthworm population and lower input. The second dose is the dose with maximum earthworm population and optimal input. And the third dose is the dose with lower earthworm population and higher pig slurry input.

Therefore, it can be concluded from these results that, despite the probable limit due to representatively of the liquid used, there is an optimal input corresponding to 6 L/day that will allow a maximum of the earthworm population. The dose 2 L/day is chosen as the lowest dose because most of its results were in lower condition (population and biomass). The dose 28 L/day is chosen because three results were in lower condition (population and biomass).

6.3. Evolution of free air space

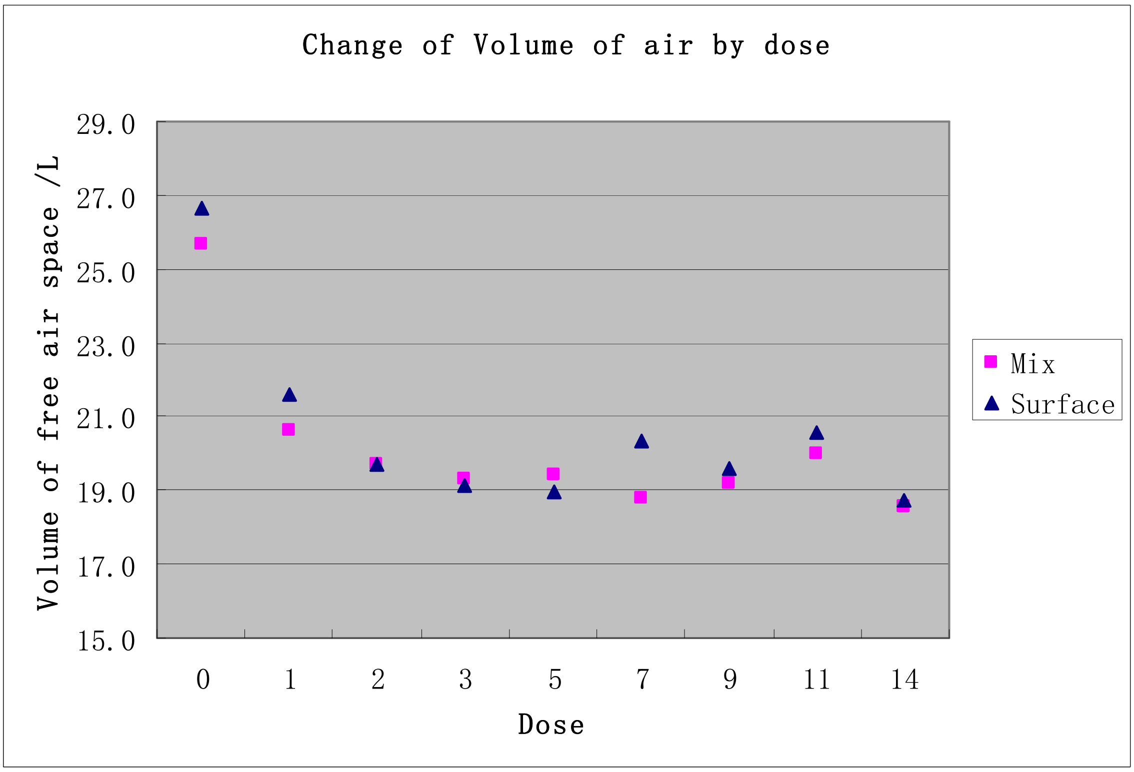


Figure 22. Change of Volume of air by dose on the basis of either the average samples or the "surface" samples on 7th Oct. 2009 ; dose is expressed in L/spreading, i.e. half of the daily dose.

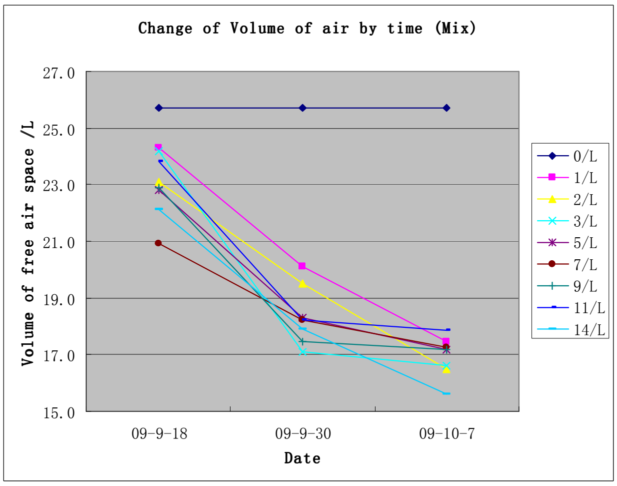
Results of free air space evolution presented in Figure 22 show that the porosity decreased as the dose increased. The maximal volume of free air space in average samples was 20.6L/mesocosm when the dose was 2 L/day. The minimal volume of free air space in surface or average samples was 18.6L/mesocosm when the dose was 28 L/day.

The volume of free air space in mesocosms spread with slurry was always smaller than the control spread with recycled water. It can be explained by the "washing effect" of the water that removes the earthworm casts, while it does not add new organic particles to the mesocosms in the case of the control.

Average samples show similar results than surface samples. It shows that the organic particles added with the slurry did not accumulated at the surface, i.e. they were removed either by the earthworms or by the water. This observation is in contradiction with practical observations on the large-scale vermifilter that show a clear accumulation of fine particles at the surface, so that high input dose can induce clogging. It confirms the hypothesis that the liquid used for this experiment was poorer in organic particles than the average liquid spread on the vermifilter.

Evolution of free air space with time is shown in Figure 23. It decreased in all treatments but the control. Most of the decrease occurred during the first period. During the second period, the decrease was more heterogeneous with the surface samples than with the average samples.

These results show that a monthly period is enough to detect a change in the free air space of mesocosms when the input dose is modified, but not enough to show if the new state is stable or not.



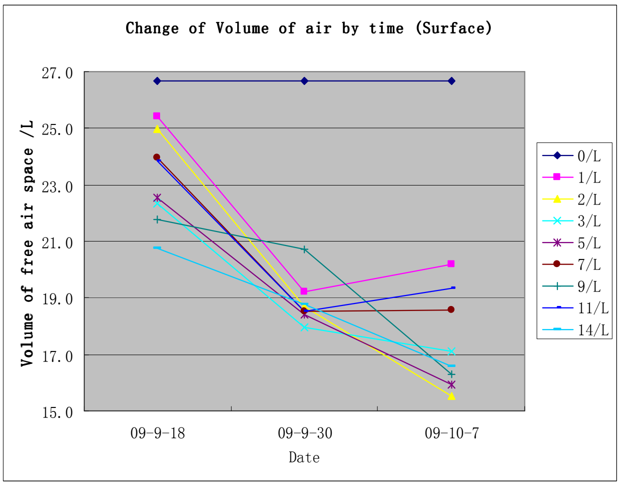


Figure 23. Change of Volume of air by time calculated on the basis of either the average samples or the "surface" samples

6.4. Evolution of C and N contents

Results of nitrogen and carbon content of the mesocosm at the end of the experiment are given in Figure 24 and Figure 25. The evolution is not clear, i.e. the total N input did not induce a significant change in the N stock of the mesocosms. The hypothesis of a small increase in N content can however be proposed from Figure 24. The negligible difference between average and surface samples, even for the highest doses, confirms the hypothesis that the input liquid was not rich enough to represent the average input of the large-scale vermifilter.

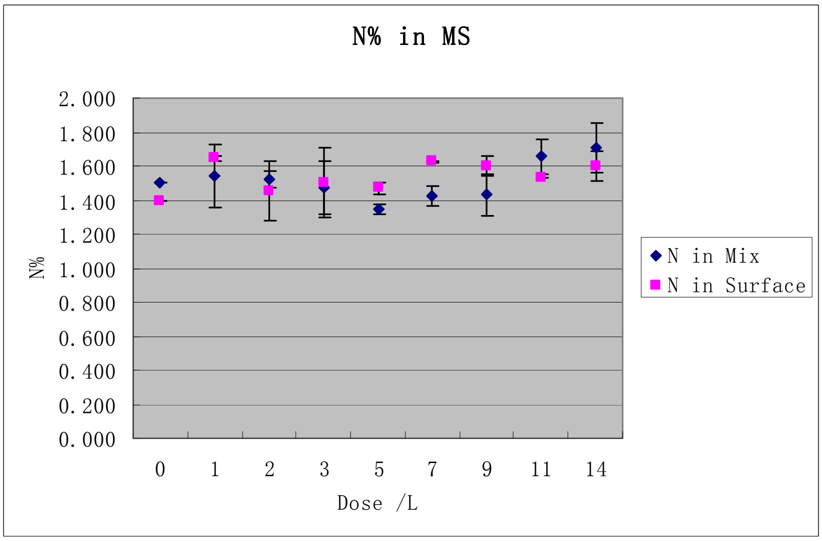


Figure 24. Change of N with dose (sampled in 7th Oct. 2009)

The change in carbon content was less than the change in nitrogen content. It can be interpreted as the effect of a major role of the woodchips used as support material within the vermifilter.

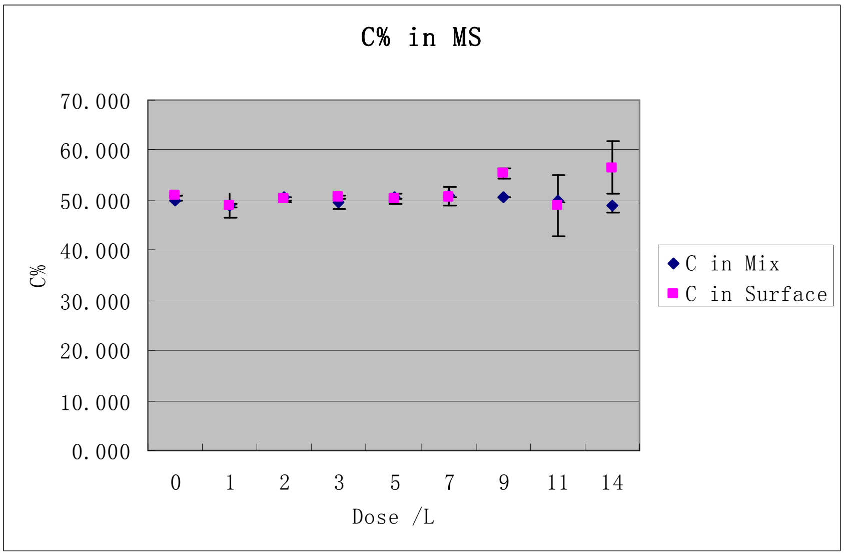


Figure 25. Change of C with dose (sampled in 7th Oct. 2009)

7. Discussion of hypothesis

7.1. Existence of an optimum

The theoretical hypothesis of the existence of an optimal input that should maximize the earthworm population was not clearly demonstrated for population and biomass measurements during a one month experiment.

This unclear result can be due to the liquid used for the experiment. Practical observations with the large-scale vermifilter have shown that too high input doses induce surface clogging and earthworm mortality after one week. Experimental results with population, free air space, nitrogen content did not show clear difference between average and surface samples whatever the input dose. Therefore, it is concluded that he liquid spread on the vermifilter is not homogenous during the day and more attention should be paid to its variability so that further experiments use a liquid that is either more representative or at least richer in organic particles (increase of hydraulic and organic loads with increased input dose).

However the statistical analysis on abundance and biomass at the three sampling dates clearly showed that the maximum values were obtained with the dose 6 L/day while minimum values were obtained with the doses 2 L/day and 28 L/day.

Therefore, it can be concluded from this experiment that there is not enough knowledge to propose a response curve of population to input dose but, for further experiments using various input doses, the three doses 2, 6, 28 L/day can be used, while more attention should be paid to the liquid taken from the large-scale vermifilter.

7.2. Speed of evolution of the earthworm population

The hypothesis that the evolution of either abundance or biomass of the earthworm population in a vermifilter can be modified by input dose within one month was not clearly demonstrated by this experiment.

In the first period experiment, the mortality that should have been observed at high doses was not observed, probably because the organic load was not high enough to induce surface clogging and anoxic conditions within the vermifilter.

Some days before the end of experiment, the pig slurry was more concentrated. This change induced the expected mortality. On dose 28 L, there were several cases of earthworms mortality (Figure 26).

Because the number of days with the more concentrated input was less than one week, the measurements of earthworm abundance did not show a clear decrease. However, there were several earthworms that went out of vermifilter on treatment 28 L. With this high dose, there was only a little earthworm mortality. The clogging was not clear with the high dose treatment: when adding the liquid at the surface, the duration of infiltration was not clearly higher than for other treatments. The organic matter accumulation in surface of high dose treatments was clearly observed but toxicity of earthworm environment was only located close to the surface. Until the end of experiment, most of earthworms in high dose treatment could adapt the environment condition by moving to non-toxic places.

The expected population decrease, when no feed was distributed to the vermifilter, was not observed in the control. It shows that the stock of nutrients is probably sufficient for the earthworm population for a period of one month provided the other environmental conditions remain stable (temperature between 15 and 20℃ and moisture around 80%).

Figure 26. Mortality observed on treatment 28 L.

7.3. Distribution of the earthworm population in the vermifilter

The comparison of population abundance estimated from surface or average sampling showed that the hypothesis that most of the earthworm population was located close to the surface is false in the case of our vermifilter.

As a matter of fact surface sampling underestimates the total population. It can be explained by the fact that our vermifilter is characterized by a high free air space induced by the raw material used as organic substrate (woodchips). This induces a repartition within almost all the height of the vermifilter of the organic input and sufficient oxygen availability. As convenient environment is provided in all the vermifilter, the earthworms explore all the volume.

Therefore, it is recommended in further experiments to associate both sampling methods as far as possible, and to prefer average sampling if both methods can not be achieved simultaneously.

7.4. Clogging

During first period of experiment, there was not clogging in high dose. The qualities of pig slurry application were not same between the treatments. In the lower dose treatments were less concentrate than the higher ones.

Several days before the end of experiment, the method of pig slurry application have been changed. Pig slurry was taken in same period. During the pig slurry application, pig slurry in container was mixed. The qualities of pig slurry were same for all application doses.

This new method made the significant change. The clogging was found in several high doses treatments. The phenomena of earthworm mortality were found in high doses too.

8. Conclusions

Including flux-variables in the concept of “preferendum” is relevant in order to optimize highly productive agricultural systems. The existence of an optimal input to the vermifilter was demonstrated from a statistical point of view. However, it was not obviously detected from a clear decrease in earthworm population at either too low or too high input doses. It can be concluded that hydraulic load was not a limiting factor for the vermifilter material used in this experiment, and that organic load was not too high because the liquid was more diluted than the average liquid of the large-scale vermifilter.

Therefore, this type of vermifilter can accept a maximum hydraulic load that is above or equal 28 $L.day^{-1}.mesocosm^{-1}$ where each mesocosm has an area of 0.21 m^2, a volume around 55 L and a free air space around 20 L. The organic load of pig fresh slurry to achieve the maximum population is above or equal 120 g dry $matter.day^{-1}.mesocosm^{-1}$ where each mesocosms had a population around 500 g earthworms within around 30 kg of wet vermicompost.

The decrease in the population abundance or biomass due to a decrease in the nutrients stored in the vermifilter occurs slowly than the decrease due to mortality when anoxic conditions appear. In vermifilters, the quality of pig slurry application influenced the earthworm mortality. In highly productive systems, the stock of nutrients is usually high. Therefore, experiment duration should be of several months to study an effect of a decrease in nutrient stock on earthworm growth and reproduction.

The comparison of surface and average sampling showed that the earthworms are not only located close to the surface. If the conditions are convenient regarding oxygen and nutrient availability, as well as moisture and temperature, the earthworms will move within the vermifilter and average sampling is necessary to have a representative sampling. “Preferendum” is not linked to a position close to the surface. It is recommended in further experiments to associate both sampling methods as far as possible.

9. Knowledge application to design and management

For design purposes, the daily hydraulic load corresponds to the maximum number of flushing the excretion per day, and the organic load to the association of the maximum excretion flux and the average earthworm population.

For management needs, the hydraulic load can be reduced without changing the organic load by reducing the number of flushing per day. The organic load can be reduced by decreasing the ratio of animal number and vermifilter area. In the large-scale vermifilter we observed bigger earthworms in regions richer in organic particles and more cocoons in dryer areas. Our pragmatic conclusion was that the optimal input for growth is different from that for reproduction. Therefore, the vermifilter is now divided in rows around 0.7 m width: one half of the rows is spread with the wastewater 5 days a week, the other half is spread 2 days a week. This management allows to maintain heterogeneity in the vermifilter with richer regions for adult earthworms and regions poorer in organic matter for cocoons and juvenile earthworms.

Chapter 2: Effect of input dose and earthworm presence on gaseous emissions during vermifiltration

1. *Résumé du chapitre 2 : effet de la dose et de la présence des lombriciens sur les émissions gazeuses durant la lombrifiltration*

La lombrifiltration est employée pour traiter les eaux usées. Comme les effluents d’élevages sont riches en azote et carbone disponibles, le traitement des effluents d’élevages peut être une source d'ammoniac (NH_3), de protoxyde d'azote (N_2O) et de méthane (CH_4). Les vers de terre ont l'avantage de mélanger et de transformer l'azote et le carbone sans consommer d'énergie additionnelle. Cependant cette technologie ne doit pas générer des émissions gazeuses polluantes accrues.

Le chapitre précédent de ce rapport (chapter 1) a montré que le « preferendum » du lombrifiltre peut être défini en utilisant le concept d’« intrant optimal » de lisier frais de porc.

L'objectif de ce chapitre (chapter 2) est d'analyser le rôle spécifique des vers de terre sur des émissions de NH_3, de N_2O, de CH_4 et de CO_2 pendant la lombrifiltration de lisier frais. On se demande en particulier si les vers de terre augmenteront les émissions de gaz polluant ou pas quand le système est géré de manière à maximiser la population de vers de terre et les transformations induites de l'effluent d'élevage frais.

L'expérience a utilisé dix-huit mésocosmes d'environ 50 L, faits à partir d'un lombrifiltre transformant le lisier d'un bâtiment de porcs. Trois doses de lisier ont été ajoutées aux mésocosmes, avec ou sans des vers de terre, pendant un mois, avec trois répétitions. Les émissions gazeuses et l’abondance des vers de terre ont été mesurées cinq et trois fois respectivement.

On observe une diminution des émissions d'ammoniac et de protoxyde d'azote ainsi qu’un puit de méthane dans les traitements avec des vers de terre. Pour ce qui concerne le N_2O, les résultats suggèrent qu'un seuil d'intrant d'azote existe : au-dessous du seuil, les vers de terre augmentent les émissions de protoxyde d'azote du fait d’une teneur et une disponibilité accrus en azote dans les turricules de vers de terre comparées à l'environnement ; au-dessus du seuil, les vers de terre diminuent beaucoup les émissions du fait du mélange accru de la matière organique apportée avec le substrat. Nous proposons que l'abondance de vers de terre puisse être employée comme bioindicateur d'une faible consommation d'énergie, et d’une faible émission de gaz à effet de serre et d'ammoniac dans les systèmes qui associent le lisier frais au recyclage de l'eau.

La conclusion de ce chapitre est que, quand le lombrifiltre est géré en maximisant la population de vers de terre avec le flux d'effluent d'élevage, il réduit l'émission de gaz polluant par rapport à l’émission par l'effluent d'élevage s'il avait été conservé à la ferme.

Si cette conclusion est appliquée aux systèmes agricoles avec une productivité élevée, elle implique qu'un recyclage continu des éléments devrait être assuré en combinant divers sous-systèmes de plantes et d'animaux, que l'utilisation des

coproduits frais d'un sous-système par un autre sous-système réduisent au minimum les fuites vers l'environnement naturel. Le chapitre suivant de ce rapport (chapter 3) analysera dans quelle mesure l'équilibre est changé quand le transfert entre les sous-systèmes est basé sur l'eau et les nutriments recyclés : que devient la composition du liquide quand elle dépend en partie de l'eau et des nutriments apportés par un sous-système, et en partie de l'eau et des nutriments qui viennent de l’amont et n'ont pas été utilisés par le sous-système ?

2. Abstract

Vermifiltration is used to treat wastewater. As wastewater of animal farms is rich in available nitrogen and carbon, treatment of wastewater can be a source of ammonia (NH_3), nitrous oxide (N_2O) and methane (CH_4). Earthworms have the advantage of mixing and transforming nitrogen and carbon without consuming additional energy. However this technology should not increase gaseous emissions.

The previous chapter 1 of this report showed that the “preferendum” of the vermifilter can be defined using an “optimal input” of pig fresh manure.

The objective of this chapter 2 is to analyze the specific role of earthworms on emissions of NH_3, N_2O, CH_4 and CO_2 during vermifiltration of animal wastewater. A particular question is whether the earthworms will increase polluting gas emissions or not when the system is managed in order to maximize the earthworm population and the induced transformations of fresh animal manure.

The experiment used eighteen mesocosms of around 50 L, made from a vermifilter treating the liquid manure of a swine house. Three levels of slurry were added to the mesocosms, with or without earthworms, during one month, in triplicate. Earthworm abundance and gas emissions were measured three and five times respectively.

There was a decrease in emissions of ammonia and nitrous oxide and a sink of methane in treatments with earthworms. Concerning N_2O, the results suggest that a threshold of nitrogen input exists: below the threshold, earthworms increase a little the nitrous oxide emissions due to increased nitrogen content and availability in the earthworm casts compared to the environment; above the threshold, earthworms decrease a lot the emissions due to increase mixing of the added organic matter. We suggest that earthworm abundance can be used as a bioindicator of low energy input, and low greenhouse gas and ammonia output in systems using fresh slurry with water recycling.

The conclusion of this chapter is that when the vermifilter is managed in order to maximize the earthworm population with a flux of fresh animal manure, it reduces the emission of polluting gas that would have been emitted by the animal manure if it would have been conserved on the farm.

If this conclusion is applied to agricultural systems with a high productivity, it implies that a continuous recycling of elements should be ensured by combining various plant and animal sub-systems, that use the fresh byproducts of another subsystem and that minimize the leakages to the wild environment. The following chapter 3 of this report will analyze to what extent the equilibrium is changed when the transfer between subsystems is based on recycled water and nutrients: does the composition of the liquid transferred downstream change when it depends partly on water and nutrients added by

the subsystem, and partly on water and nutrients that come from upstream and were not used by the subsystem?

Keywords:

Vermifiltration, gaseous emissions, NH_3, N_2O, CH_4, CO_2

3. Introduction

Concentrated animal production consumes resources such as energy, crops, or fertilizers, and its effluents pollute the environment in ways such as by producing greenhouse gases, water eutrophication, or soil contamination (FAO, 2006a). As animal production is expected to increase (FAO, 2006b), there is an urgent need to increase resource efficiency and decrease the polluting impact. Both can be achieved through the evolution of systems that increase the recycling rate of water, energy, and nutrients, at local or global scales, and that allow producers to certify reduced polluting leakages and increased efficiencies.

A system of animal production has been designed that increases the recycling efficiency of water and animal feed (Morand et al., 2011). The system associates a pig house with manure flushing, a vermifilter, lagooning, and constructed wetlands, in order to combine water and nutrient reuse to high manure dilution. Nutrients are exported either as organic matter or as plants. Water is reused after abatement of pathogens and micropollutants. The vermifilter contributes to a major part of carbon and nitrogen abatement in the liquid. It produces casts that can be exported for use as vermicompost.

The previous chapter (chapter 1) of this report showed that the “preferendum” of the vermifilter can be defined using an “optimal input” of pig fresh manure. This “optimal input” will ensure a maximum abundance of earthworm population. Several advantages are expected from functioning close to this preferendum such as: easier management using the earthworm abundance as a visible indicator characterizing a steady-state functioning, maximal fluxes of fresh manure transformation, maximal production of earthworms and vermicompost, accelerated adaptation of earthworms to evolutions of the farm, the climate, the pathogens, etc. However, the transformation of organic matter could also induce increased emissions of ammonia or greenhouse gases.

Until now, the specific effect of the earthworms and dosage of fresh manure on the gaseous emissions has not been observed and can not be forecast from the existing literature. Previous observations on a vermifilter fed with pig manure showed negligible nitrous oxide emissions (Li et al., 2008) while a lot of literature suggests that earthworms enhance nitrous oxide emissions through gut-associated denitrification (e.g. Drake and Horn, 2006; Wüst et al., 2009). However, the rise in nitrogen–poor environments can be simply explained by higher nitrogen content in the gut due to biological nitrogen fixation (Striganova et al., 1993; Umarov et al., 2008). Methane is usually emitted by liquid manure systems, e.g. in most pig farms, while enhanced methane consumption in the presence of earthworms has been already observed (Kammann et al., 2009). Beside the direct production or consumption of greenhouse gases or ammonia, earthworms have an indirect influence on the net emission of the organic substrate through the microbial community (Binet et al., 1998; Aira et al., 2007) and through their physical impact on the porosity and its connectivity (Lee and Foster 1991, Boyle, Curry and Farrell 1997). When the system receives a high input of organic matter, the transformations of the substrate depend on the abundance of earthworms (Ndegwa et al., 2000; Clarke et al., 2007). When the input of fresh organic matter is too high, the system becomes anoxic that induces earthworm mortality.

Therefore, the objectives of this work were: (i) to analyze the specific effect of the earthworms (i.e. what happens when they are removed) on the evolution of the vermifilter and on the emissions of NH_3, N_2O, CH_4, and CO_2, with various doses of animal wastewater; (ii) to discuss if observed effects can be directly related to the earthworm abundance or if they are indirectly due to modifications of the organic substrate by earthworms; (iii) to evaluate if earthworm abundance can be considered as a bioindicator in systems using vermifiltration to improve manure recycling.

4. Hypothesis

The three doses are around the optimal input. The low dose should not induce a significant decrease in earthworm population before the end of experiment (four weeks). On the contrary, the high dose should induce visible mortality of earthworms and significant decrease of population. This second experiment should confirm more clearly the results deduced from statistical analysis in first experiment.

Gaseous emissions could not be measured continuously neither on the mesocosms nor with the large vermifilter. Therefore, this experiment is based on two assumptions :

- emissions of mesocosms were representative of the large vermifilter;
- emissions between observation days did not take values highly different from observations.

These assumptions are supported by the use of the same material and liquid as the large vermifilter, and by the rather steady-state conditions within the various mesocosm as shown by temperature and humidity measurements.

The expected effect of earthworms on CO_2 emissions is an increase because of the respiration of earthworms and because of an increase in microbial respiration due to oxygen diffusion improved by the higher free air space and connected porosity in the treatments with earthworms.

The expected effect of earthworms on CH_4 emissions is a decrease because earthworm casts are a sink of CH_4 (Héry et al., 2008; Kammann et al., 2009; Moon et al., 2010).

The expected effect of earthworms on NH_3 emissions is a decrease because the fresh organic matter added at the surface should be removed and transformed by earthworms (Bohlen & Edwards, 1995; Binet et al., 1998), thus avoiding ammonium increase due to mineralization of the fresh organic matter.

The expected effect of earthworms on N_2O emissions is an increase for at least three reasons:

- theoretical considerations show that earthworm gut is favorable to N_2O emissions (Drake & Horn, 2006);

- large scale observations with vermicomposting showed an increase (Frederickson & Howell, 2003);
- high input in organic nitrogen combined with higher oxygen diffusion should induce higher N_2O emissions.

As earthworms are considered as "ecosystem engineers", the observed effects on gaseous emissions should be more due to indirect effects, through modifications of the environment induced by earthworm activity, than to direct effects of the earthworm metabolism.

5. Material and methods

5.1. Experimental site

The research was conducted at the Piggery Experimental Farm of Guernévez, Saint Goazec, France from October 2009 to November 2009. In this site, a vermifilter 48 m^2 in area and 0.5 m in height is in use since 2007. Most earthworms are *Eisenia andrei* (Bouché) and *Eisenia fetida* (Savigny). It was designed according to the conclusions of Li et al. (2008). It recycles the wastewater of a piggery with 30 sows with 4 to 6 flushing per day (800 L water per flushing). Wood chips are added twice a year, and the vermicompost is progressively removed. The wastewater goes through a screen, a vermifilter, a settling tank, and four levels of lagooning and constructed wetlands, until it reaches a storage basin that accumulates rainwater in winter to compensate for summer evaporation. The water is pumped back from the storage basin to flush the piggery. The composition of the liquids at the various levels is given in Morand et al. (2009).

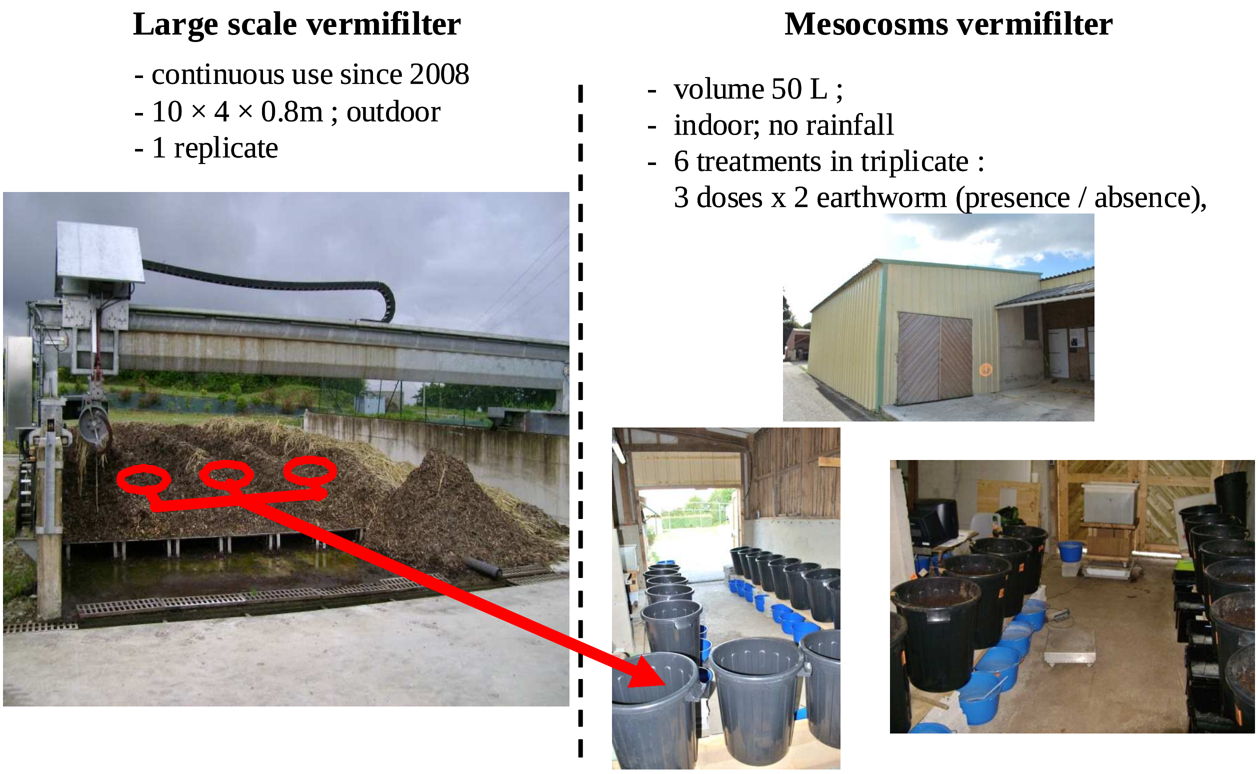


Figure 27. Large scale vermifilter and not insulated building for mesocosm experiment; both received the same liquid added each day

Eighteen mesocosms of 50 L vermifilter were sprinkled with different doses of fresh slurry. Three different doses of slurry were chosen from the experiment described in the previous chapter. Half of the mesocosms had an abundant population of earthworms and half had a negligible population. There were three repetitions of each treatment. The mesocosms were placed within a not insulated building so as to avoid rain inputs and have similar temperature variations as outside. The initial material for filling each mesocosm was taken from the surface of the existing running vermifilter for both experiments. Sawdust was added to increase carbon availability and therefore nitrogen organization (Figure 27).

The experiment took around 4 weeks. The shape of the mesocosms was cylindrical with 0.5 m in height and 0.52 m in diameter (0.21 m^2). The vermifilter material was placed on a grid to allow easy drainage of the water towards a tank for daily sampling and weighing (Figure 28).

The material with or without earthworms was prepared from the material collected from the surface of the large-scale vermifilter. The material was spread on a flat surface and illuminated. Within some minutes, the earthworms went down to avoid the light. Then, the surface was taken to constitute the material “without earthworms” (in fact some earthworms and the cocoons remained in the material) and the bottom layer of material was taken to constitute the material “with earthworm”. As a result of this procedure, the initial population abundance of the mesocosms “with earthworms” was higher than the population of the large-scale vermifilter.

In the rest of the text, the treatments with earthworms are designed by AV and the treatments without by SV.

From both types of materials, with or without earthworms, the same mass of vermifilter material was weighed (21.5 kg; initial water content before mixing with sawdust was 77.3±0.6 % and 76.3±0.5 % for vermifilter material with or without earthworms respectively). A mixture of dry sawdust and woodchips was added to the vermifilter material to ensure an initial free air space and promote nitrogen organization during the experiment, even in the treatment with high slurry inputs. The sawdust was first moistened, in order to limit the effect of dry sawdust on the earthworms. The liquid used came from the tank after the vermifilter. The sawdust was put into water, and then drained. The resulting water content before mixing with the vermifilter materials was 69.3±0.5 %. The same mass of moist sawdust (13.5 kg corresponding to around half of the volume of vermifilter material) was mixed with the material of each mesocosm. A sample of 1 kg was taken after mixing for initial analysis, so that all repetitions used the same weight of 34.12 kg material. The next day, the material was installed in the mesocosms. After 12 to 36 hours, the material weighed between 33.30 and 33.94 kg. The mass loss since the day before, probably mostly as CO_2 and H_2O, was 0.44±0.124 kg for the treatments prepared with earthworms and 0.61±0.101 kg for the treatments prepared with the substrate without earthworms. The material was added in two phases with a slight compaction after adding half of the mass and at the end. The volume was adjusted to the same height in all mesocosm to ensure a similar density of the material.

The wastewater was taken once a day from the liquid input of the running vermifilter. As the liquid was too diluted in the first experiment, the time of sampling was fixed in this one, after the first soup distribution in the morning in order to increase the organic load and reduce the variability of the input liquid.

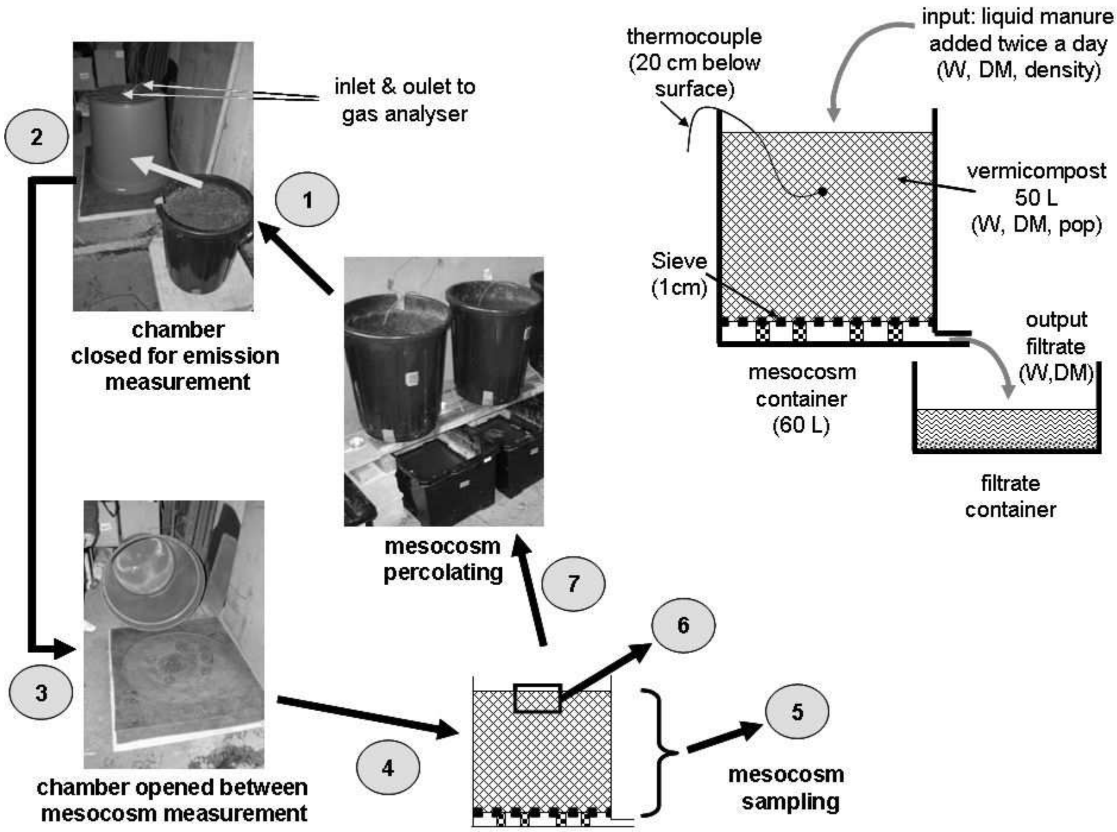


Figure 28. Diagram and photographs illustrating the experimental procedure for the second experiment. W: weight; DM: dry matter; pop: population. (1) weighting the mesocosm before gas measurement; (2) gas emission calculated from concentration increase in static chamber; (3) mesocosm returned to percolating or sampling stage; (4) weighting the mesocosm before sampling; (5) gentle mixing and sampling the mesocosm; (6) sampling the surface of the mesocosm; (7) controlling the weight between sampling and percolating stage.

5.2. Containers

One repetition requires two containers. The first container contains the vermicompost, a screen, and supports to improve liquid flow. The pig slurry is added to the surface of the vermifilter material in the container. At the bottom of container, a hole allows excess liquid to drain. The second container sits below the first one to collect this liquid for sampling.

5.3. Pig slurry application

The wastewater was used to sprinkle the mesocosm morning and afternoon. The mesocosms were not sprinkled on the days of gas measurement, solid sampling, or earthworm counting. The total of sprinkling days was 12.

During the experiment, the three levels of wastewater input were 1, 3 and 14 L, twice a day, giving 2, 6, and 28 L/day as resulted from the experiment described in the previous chapter. For each level, there was one treatment with earthworms and one treatment without. Each treatment had three repetitions.

The dose is indicated with a number: 2, 6, or 28 depending on the daily volume of liquid that was added to the mesocosms. In the following text, the different treatments are designated by: 2AV, 2SV, 6AV, 6SV, 28AV, 28SV.

In the treatment without earthworms and 28 L/day, clogging was rapidly observed. During the first period of 2 weeks, between two vermicompost samplings, the quantity of added slurry was therefore reduced to the maximum amount that could filter through the system. This induced a difference of liquid input between the treatments with and without earthworms, above 15%. Therefore, during the second period of two weeks, the surface (0-5 cm) was pierced to allow liquid infiltration and to have the same liquid input in treatments with or without earthworms.

Pig slurry was applied to mesocosms by using a pitcher on days: 1, 2, 6, 7, 8, 9, 10, 15, 16, 19, 23, and 24 of November. Some days, the mesocosm were not spread because other measurements were ongoing: gas emissions, weighing and sampling, earthworm counting. The quantities of liquid were adjusted on a weight basis, after a measurement of the slurry density, to ensure identical application rates to all mesocosms receiving the same amount. The weights of input and output liquids were recorded every day.

Slurry was applied twice a day (morning and afternoon) to avoid introducing too much water at the same time. The pig slurry used comes from the prototype after screening. It was mixed continuously to avoid particle settling when taking the quantity for each mesocosm.

Filtrate that collects in the lower container was weighed the day after spreading. The filtrate was sampled before adding new liquid. One sample was used for dry matter measurement and another one was frozen for further chemical analysis.

Other details of the procedure of slurry application and filtrate characterization were similar to those described in chapter 1 of the present report.

5.4. Sampling

5.4.1. Solid (compost)

For each treatment, a sample of the vermifilter media was taken on days 29th October, 12th and 25th November. Samples were taken at the surface of the mesocosm (0-10 cm). Other samples were taken after mixing the vermicompost in each mesocosm. In the running vermifilter, the vermicompost was also mixed every week. We assume that mixing the vermicompost did not reduce the representativeness of the experiment.

The sampling procedure was similar to the sampling procedure described in chapter 1 of the present report.

On sampling days, all containers with and without vermicompost were weighted. Then the vermicompost was mixed and divided into two parts. One-half was returned to the container and the other part was mixed. This operation (mix-divide) was repeated for each container until the quantity of material in a sample (about 2 l) was obtained.

The sample was divided among 3 trays (approximately 400 mL per tray), and each tray was weighed.

One tray was conserved in a deep freezer (-18℃) for further chemical analysis, another one was used for dry matter analysis; the third one was used for earthworm counting.

5.4.2. Liquid

Samples were taken for each time where input liquid was taken at the running vermifilter. Two samples were taken: at the beginning and at the end of the spreading period, resulting in four samples each day of spreading, to check the homogeneity of the input for all mesocosms. One liquid sample was taken from the filtrate received in each second container. Each day, input and filtrate samples were either frozen, for further chemical analysis, or dried for dry matter measurement.

5.5. Measurements

5.5.1. Temperature

Temperature was measured continuously with thermocouples (type K, Thermoelectric ; coated with Teflon tubes 2x4mm) connected to a Campbell datalogger (21X with AM416 multiplexer). One thermocouple was planted in each mesocosm at 12 cm below the surface. Air temperature was measured at two levels, and slurry temperature was also measured with thermocouples. The datalogger took a measurement every 10 seconds and the 10 minute averages were recorded.

5.5.2. Water and dry matter input and output

Samples for dry matter analysis were oven-dried (60 ℃ for at least 24 hours or more until complete drying shown by stable weight). Dry matter content was calculated from the wet and dry weights after subtracting the weight of the container.

The water or dry matter input were calculated from the mass of input liquid measured the day of spreading and the average of the dry matter analysis processed on all samples of input liquid of the same day.

The water or dry matter output were calculated from the mass of filtrate measured the day after spreading and the dry matter analysis of its sample.

The net inputs of wastewater, water or dry matter were calculated from the difference between inputs and outputs as calculated above.

All daily values were summed for the two periods between sampling dates for earthworm counting or vermifilter material analysis.

5.5.3. Earthworms

The vermicompost of one sampling tray was washed in sieves of decreasing mesh size and the earthworms were removed. The volume of the coarse fraction (> 2 mm) was measured in water and its dry matter was measured as previously described. Hand sorting was used to count earthworm abundance in both surface and average samples as described in previous chapter 1. The population was divided into four classes: cocoons, juveniles (small), sub-adults (large, without clitellum) and adults (large, with clitellum). Each class was characterized by number (abundance) and fresh weight (biomass).

The surface sample was used to determine the abundance per square meter close to the surface. This value was then extrapolated, on an area basis, to the mesocosm. The earthworms are epigeic, therefore, they are supposed to be located close to the surface, and many literature references use surface counting. The abundance of the mesocosm on area basis was calculated using following calculation:

$$\textbf{abundance}_{\text{mesocosm, surface}} = [\ \textbf{abundance}_{\text{surface sample}} / \textbf{area}_{\text{sample}}\ ] * \textbf{area}_{\text{mesocosm}}$$

where **abundance** $_{\text{mesocosm, surface}}$ is the total number of individuals per mesocosm (juvenile + subadults + adults), **abundance**$_{\text{surface sample}}$ is the abundance counted in each sample taken at the surface, **area**$_{\text{sample}}$ is the area of the sampling box (14 x 11 cm box giving an area of 0.015 m²), **area**$_{\text{mesocosm}}$ is the area of vermifilter (52 cm diameter giving an area of 0.21 m²).

The average sample was used to determine the abundance per kilogram wet weight of vermicompost. This value was then extrapolated, on a weight basis, to the mesocosm using following calculation:

$$\textbf{abundance}_{\text{mesocosm, weight}} = [\ \textbf{abundance}_{\text{average sample}} / \textbf{weight}_{\text{sample}}\ ] * \textbf{weight}_{\text{mesocosm}}$$

where **abundance** $_{\text{mesocosm, weight}}$ is the total number of individuals per mesocosm (juvenile + subadults + adults), **abundance**$_{\text{average sample}}$ is the abundance counted in each sample taken after mixing the mesocosm, **weight**$_{\text{sample}}$ is the net weight of the wet sample before counting, **weight**$_{\text{mesocosm}}$ is the net weight of wet vermifilter material measured before the sampling operations.

5.5.4. Gaseous emissions

The approach used is that of a static chamber (Figure 29) because the emission kinetics of different gases can differ (for example, relatively strong emission for CO_2, relatively weak emission for N_2O). In addition, static rooms allow one to control the period over which one calculates emissions. Thus, accumulation over short periods can be used for some gases and longer periods can be used for other gases.

Measurements of gaseous emissions were performed each week, the first time before sampling and the second time, the day before mesocosm sampling: 31st October, 5th, 11th, 18th, and 25th November. Each mesocosm was placed in a hermetically-closed chamber of known volume connected to a gas analyzer. A chamber with a large volume was chosen so that volume variations due to heterogeneous free air space inside the mesocosms or volume variations of the mesocosms could be considered negligible.

Gaseous concentrations were measured continuously during the day of measurement and measurements were recorded in the same file. Concentrations in outside air were first measured for at least 15 minutes until concentrations were low and stable. Then, the mesocosms were put entirely inside the measurement chamber, the chamber was closed and the inside concentration measurements lasted between 30 minutes and 2 hours. Then, the chamber was opened, the mesocosm removed, the absence of water condensation was checked because it can dissolve ammonia, and the chamber was opened until low and stable concentrations were reached again.

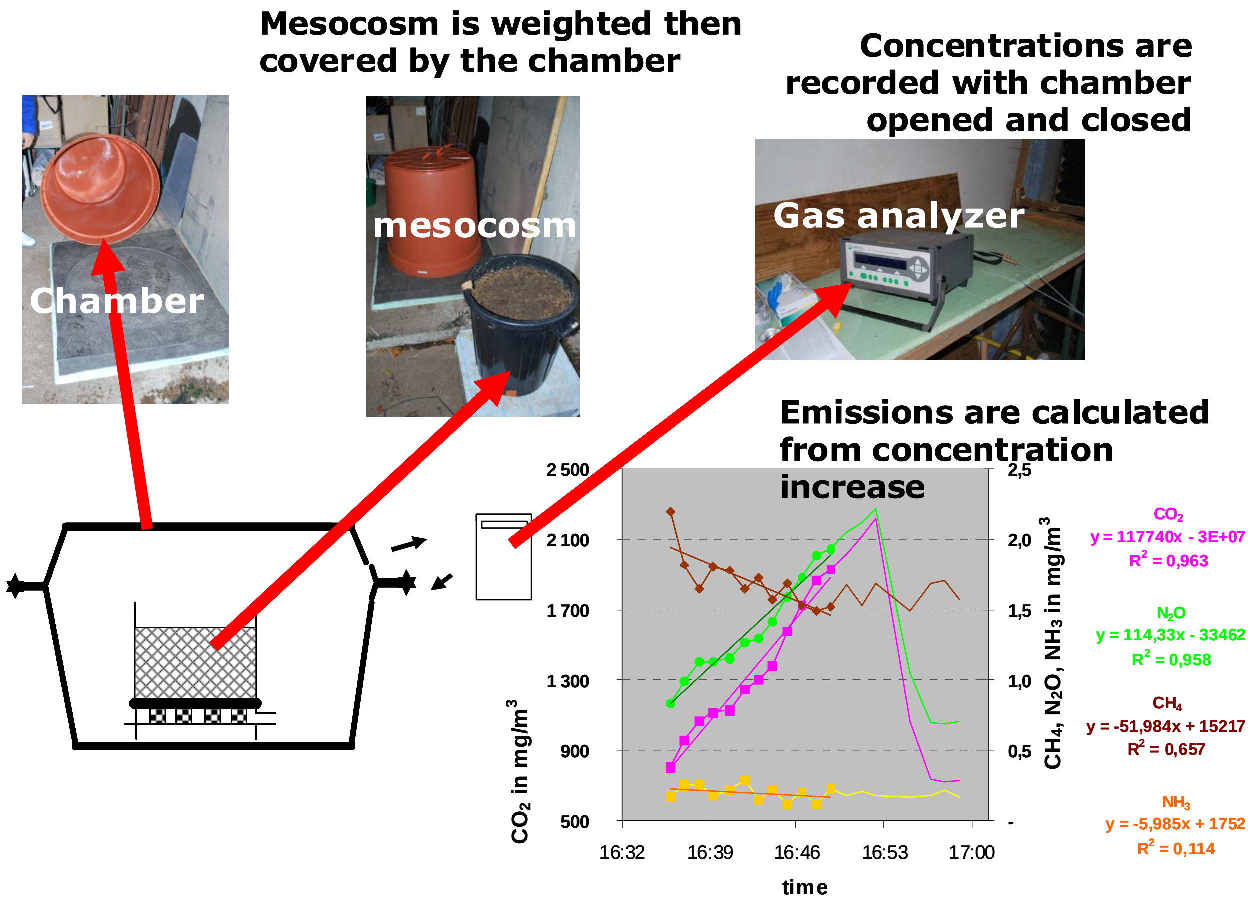


Figure 29. Procedure for measuring gaseous emissions

From the measurements, the raw data were first controlled for outside concentrations. The outside concentrations of CO_2, N_2O, and NH_3 did not change over the days of measurement. The outside concentrations of CH_4 and H_2O could change. The emission was considered not detectable when the concentration variation inside the close chamber was less than the concentration variation between the initial and final outside concentrations (i.e. the concentration did not change when the chamber was closed but it changed when the chamber was opened in order to change to the next mesocosm).

Then, a sample of points was chosen that presented the highest correlation coefficient of gas concentration with time. It was chosen in the excel sheet by plotting the regression line and the correlation coefficient for all gases on the same graph, for increasing periods. Concerning H_2O, the concentration increase could be slower after some minutes. Therefore, the chosen period was sometimes shorter for H_2O compared to the other gases (CO_2, N_2O, CH_4, and NH_3). For these four last gases, two gases were chosen to choose the period: CO_2 and N_2O, because they always changed with time. The number of measurements used to calculate the slope was over 15 values. As the change was not clear for NH_3 and CH_4 in some cases, the lowest acceptable correlation coefficient was 0.3. This rather low value was chosen because for NH_3 and CH_4, the outside concentrations were close to the detection level of the gas analyzer. The correlation coefficient could not be high because of the dispersion of the values.

When the correlation coefficient was above 0.3, it was always possible to see a clear trend in the concentrations, despite the variations between two successive values. When the correlation coefficient was below this threshold, the emission was considered to be less than the detection level, i.e. close to zero.

The raw concentrations were given in mg gas/m3 air. Therefore, the emission was only related to the air volume inside the chamber and the slope of concentration evolution with time:

emission = **slope** * **volume** / 1000

where "**emission**" is given in "g gas. day^{-1} . $mesocosm^{-1}$", "**slope**" is given in "mg gas . day^{-1}", "**volume**" is given in m^3 air (0.248 m^3 deduced from 300 L of empty chamber and 52 L of mesocosm), 1000 is for the conversion of mg gas, given by the gas analyzer, into g gas. Then "**emission**" of gas was converted into each element: C for CO_2 and CH_4, N for N_2O and NH_3 using the ratio of the molar masses, for example:

emission C-CO_2 = **emission CO_2** / (12+16*2) * 12

emission N-N_2O = **emission N_2O** / (14*2+16) * (14*2)

The results are presented for three dates: the first date corresponds to the measurements before beginning the spreading, i.e. as all treatments with earthworm were similar as well as all treatments without earthworms; the second date corresponds to the average of the measurements done on 5th and 11th November; the last date corresponds to the average of the measurements done on 18th and 25th November.

5.6. Data processing

Calculations, figures, Student T-tests, and tables were prepared using Excel software. Results related to fluxes are given for the two successive periods of 2 weeks: before and after the middle sampling. Results related to states are given for three dates: beginning, after 2 weeks, and after four weeks.

The results were analyzed either in term of differences between two periods for the same treatment, or in terms of differences between treatments with or without earthworms (or between different doses) for the same date. Temperatures are given in Celsius degrees (℃) while temperature differences are given in Kelvin (K). "dw" denotes "dry weight" and it is same as "dry matter". "ww" is used for "wet weight".

6. Results

6.1. Liquid budget

6.1.1. Liquid input

Accumulation of organic particles caused clogging in treatment 28 l/day without earthworm (28 SV). The treatment 28 SV received a similar mass of wastewater during both periods of 15 days. Both of them were 134 ± 3 kg. For the other treatments, the mass of input was heavier during the first period of 15 days than during the second period of 15 days.

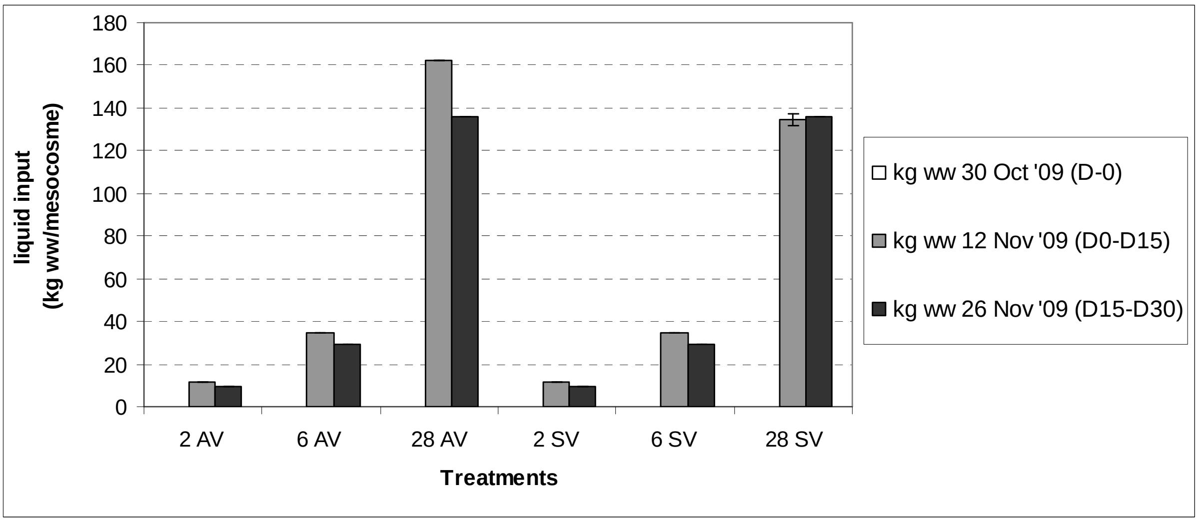


Figure 30. Liquid inputs of each treatment

6.1.2. Liquid output

The mass filtrate of both dose 2 and 6 l/day in treatments with earthworms and without earthworms were not different. Mass filtrate of dose 2 l/day were 7-9 kg and of dose 6 l/day were 26-31 kg both treatment with and without earthworms. In the dose 28 l/day, there was different mass filtrate between treatment with earthworms and without earthworm. The mass filtrate was lower in treatment 28 SV.

We found liquid could not flow well in treatment 28 SV. There were clogs in surface. The mass filtrates of dose 28 l /day with earthworm were heavier than treatment without earthworm until 15^{th} day. The mass filtrates of treatment with earthworm were 155 and 132 kg for each period. It was only 125 and 130 kg in treatment 28 SV. From 15^{th} to 30^{th} day, the mass filtrates between treatments with and without earthworms were more similar than during the first period because the clogged surface was pierced to allow liquid infiltration.

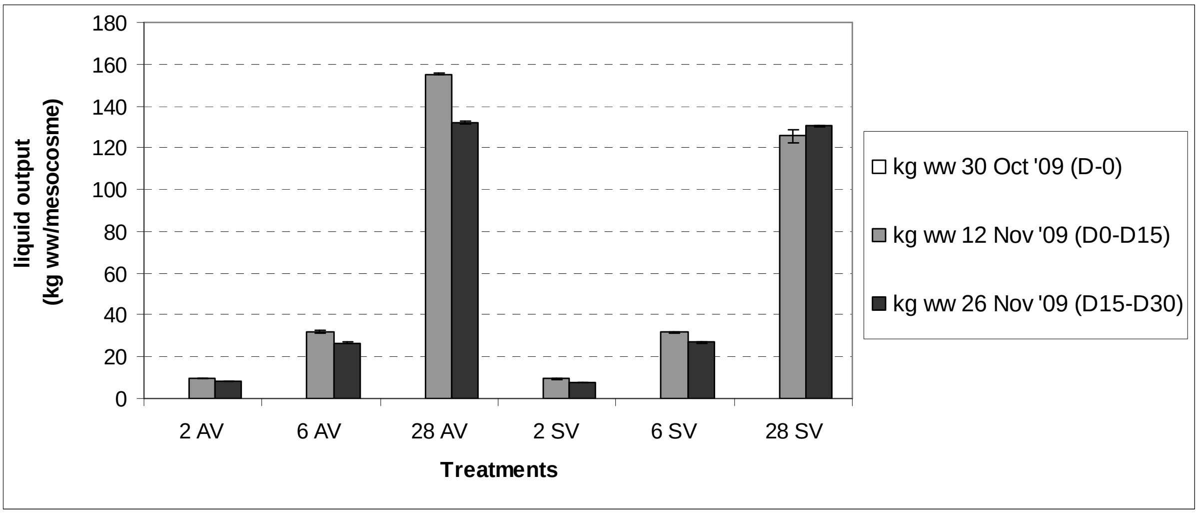


Figure 31. Liquid outputs of each treatment

6.1.3. Net liquid input of mesocosm

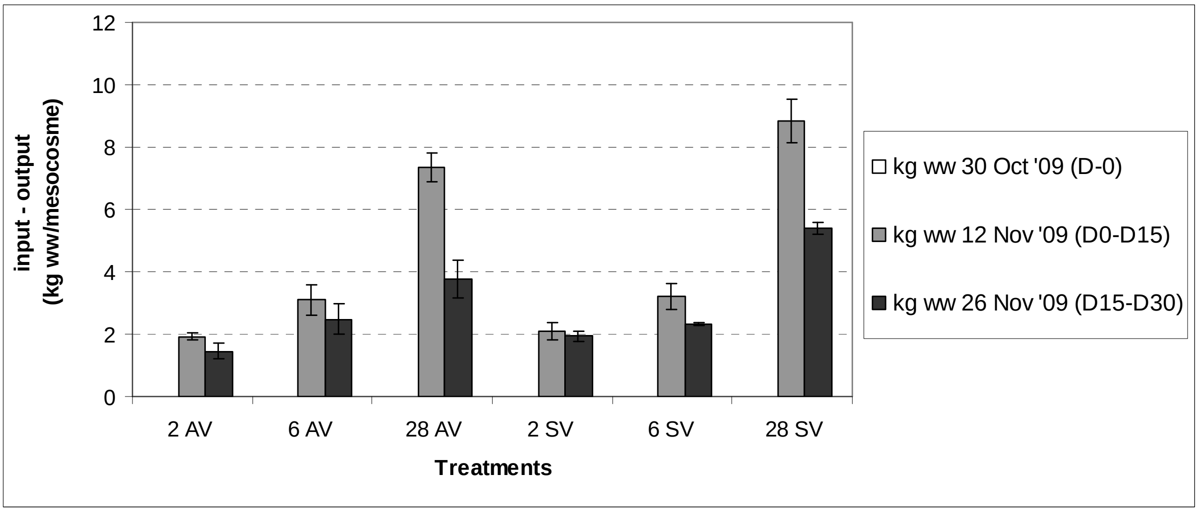


Figure 32. Net liquid input of mesocosms

The net mass input resulting from the difference between input mass and filtrate mass showed that treatment 28 SV accumulated between 5-9 kg, that is heavier than 28 AV that accumulated only 4-7 kg. There were no difference in net mass input between treatments with or without earthworm in doses 2 and 6 l/day. The net mass input of those treatment varied between 1-3 kg. The net mass input increased following the increase of dose.

6.1.4. Water input

The water input during the first 15 days was 11-161 kg. It was heavier than during the second 15 days period for all treatments that were 9-135 kg, except for treatment 28 SV. On that treatment, input during the first 15 days was 134 kg and was almost same as during the second period of 15 days that was 135 kg. This reduction was due to a lower input because of clogging.

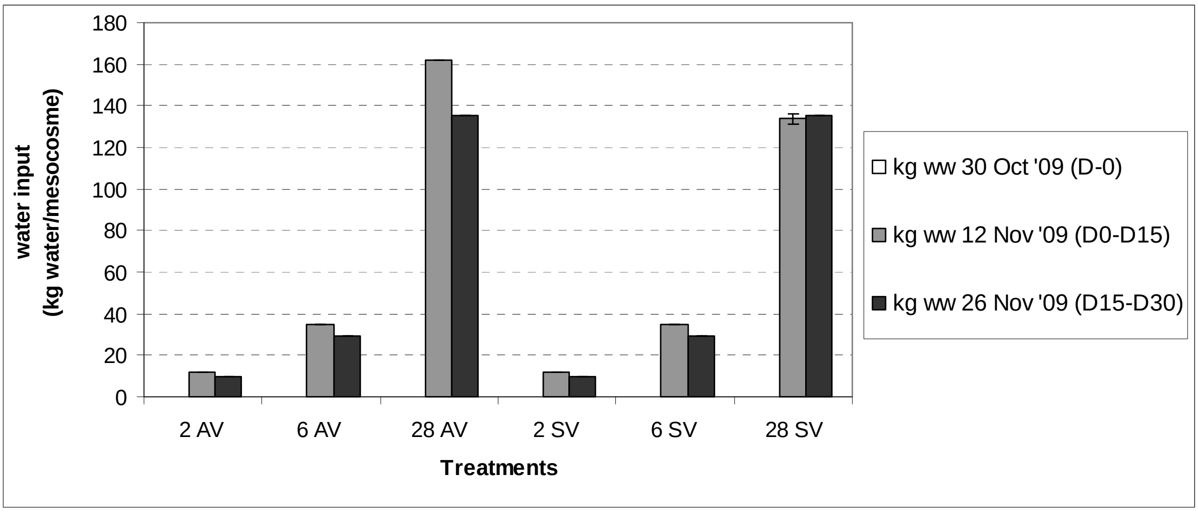


Figure 33. Water input

6.1.5. Water output

During the first period of 15 days, there was almost no difference of water output between treatments with or without earthworms for dose 2 l/day and 6 l/day. The mass of water output of treatments with or without earthworms was in the range 10 - 32 kg. A difference was clearly observed in dose 28 l/day during this first period because of a very different input: the mass of water output for treatment with earthworms was 154 kg and it was only 125.17 kg for treatment without earthworm.

During the second period of 15 days, the difference of water output between treatments with or without earthworms was not clear for doses 6 and 28 l/day: both mass of water output were around 26.5 kg for dose 6 l/day and 130 kg for dose 28 l/day. During this period, the mass of water output of dose 2 l/day was clearly higher for treatment with earthworm that had 8.28 ± 0.26 kg of water output compared to treatment without earthworms, which had 7.79 ± 0.17 kg of water output.

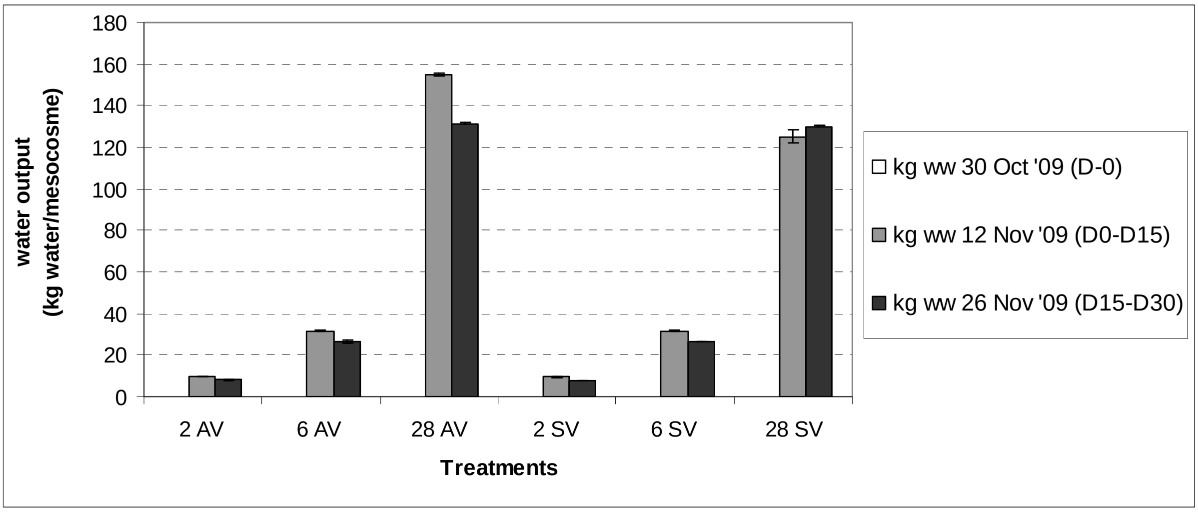


Figure 34. Water output

For the dose of 28 l/day contrasted phenomena were observed. In treatment with earthworms the mass of water output during the first period was 154 kg and it was more than the mass of water output during the second period where it was only 131 kg. On the contrary, the mass of water output in treatment without earthworms during the first period was 125 kg that was less than during the second period where it was 129.97 kg despite similar inputs during both periods, around 135 kg.

6.1.6. Net water input in mesocosms

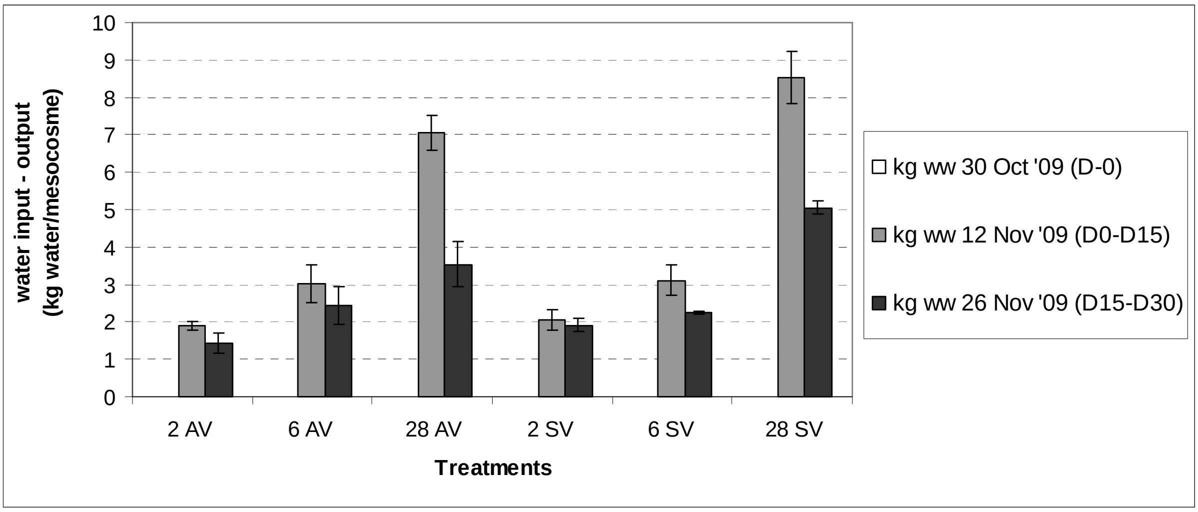


Figure 35. Net water input in mesocosms

During the first period, the net water input of all treatments without earthworms was heavier than treatments with earthworms, respectively in the range 2.1 - 8.5 kg and 1.9 - 7.1 kg. However, the differences between treatments with or without earthworms were not significant because of the variations in filtrate mass between replicates.

During the second period, the same phenomena as during the first period was observed for doses 2 l/day and 6 l/day: the net water input of treatments without earthworm was heavier than treatments with earthworms but the difference was not significant because of variability between replicated. However, the net water input of dose 28 l/day on treatment without earthworms was clearly heavier than for treatment with earthworm, respectively 5.1 kg and 3.5 kg.

6.1.7. Dry matter content of the liquids

Table V. Dry matter content of the liquids

period	30 Oct '09 - 12 Nov '09		12 Nov '09 - 26 Nov '09	
dose	average (g dw/kg ww)	standard deviation	average (g dw/kg ww)	standard deviation
2 AV	2.90	0.10	4.04	0.11
6 AV	2.90	0.07	4.20	0.14
28 AV	3.06	0.09	3.89	0.03
2 SV	2.14	0.03	2.28	0.11
6 SV	2.35	0.05	2.91	0.01
28 SV	2.59	0.05	3.05	0.02
inputs	4.72	1.95	5.42	2.54

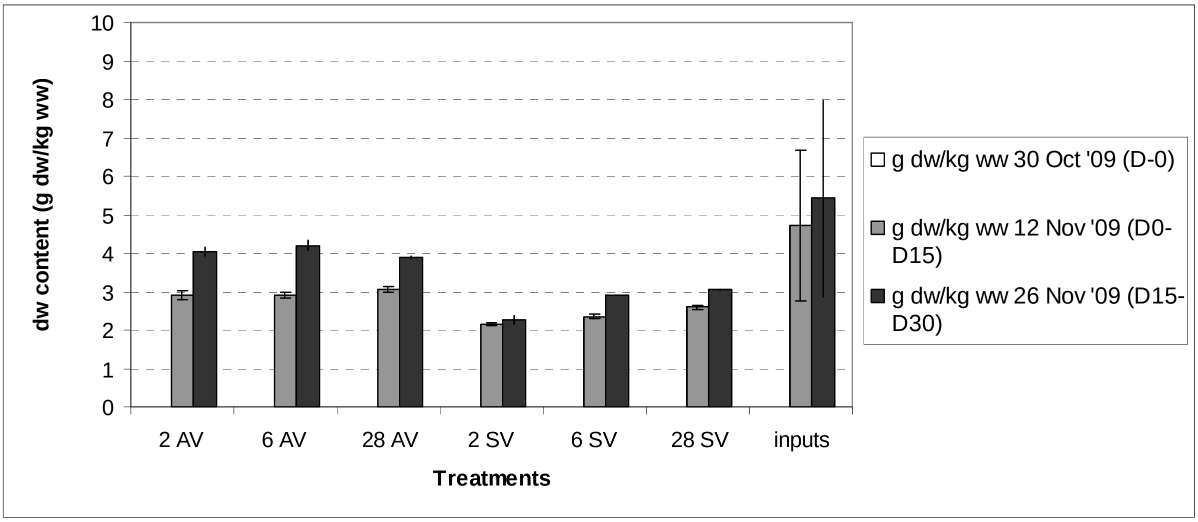


Figure 36. Dry matter content of the liquids

The dry matter content of the input liquid was higher than the dry matter content of the filtrate, respectively in the range 4.7 - 5.4 g dw/kg ww and 2.1 - 4.2 g dw/kg ww. The dry matter content of the filtrate of treatments with earthworms was higher than for treatments without earthworms, respectively in the range 2.9 - 4.2 g dw/kg ww and 2.1 - 3.1 g dw/kg ww. The differences between doses were not significant for treatment with earthworms. For treatments without earthworms, the dry matter content of the filtrate increased following the dose increasing.

The dry matter content of the filtrate in treatments with earthworms was higher during the second period than during the first period, respectively 3.9 – 4.2 g dw/kg ww and 2.9 – 3.1 g dw/kg ww. A similar tendency was observed for the treatments without earthworms but the difference between the second and the first periods was smaller, respectively 2.3 -3.1 g dw/kg ww and 2.1 – 2.6 g dw/kg ww.

6.1.8. Dry matter input of mesocosms

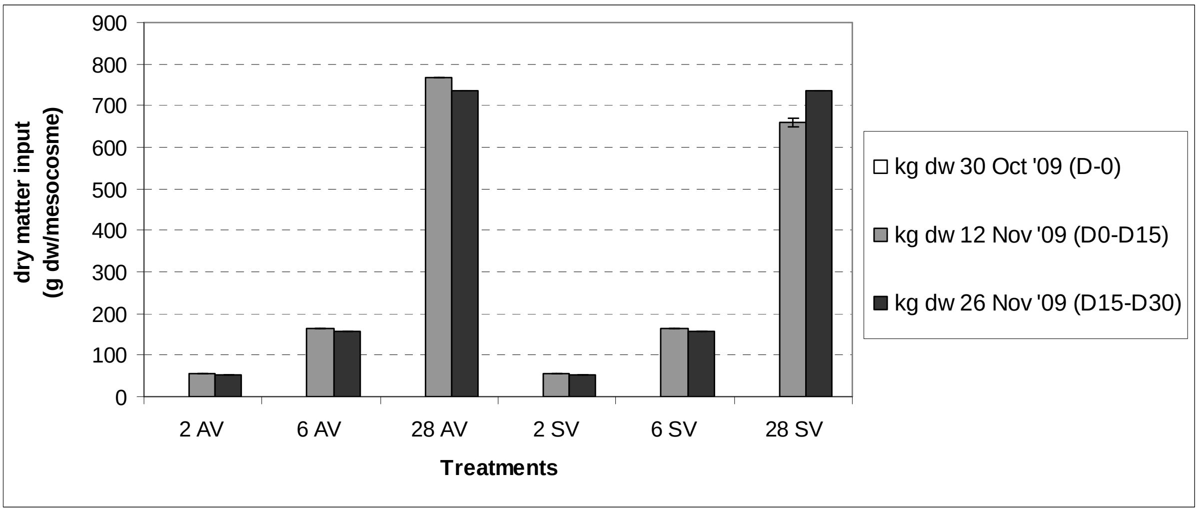


Figure 37. Dry matter input of mesocosms

Mass of dry matter input were similar during first and second period of treatments with or without earthworms, respectively 52 - 55 g dw for dose 2 l/day and 157 - 165 g dw for dose 6 l/day.

Dose 28 l/day had different phenomena. In treatment with earthworms, the mass of dry matter input was 768 g dw during the first period and it was heavier than during the second period where it was 735 g dw. In treatment without earthworms, the mass of dry matter input was less during the first period than during the second as a result of less fresh manure input, respectively 658 g dw and 735 g dw.

6.1.9. Dry matter output of mesocosms

The mass of dry matter in filtrate was clearly heavier in treatments with earthworms than in treatments without earthworms. The difference between treatments with or without earthworms increased with the dose.

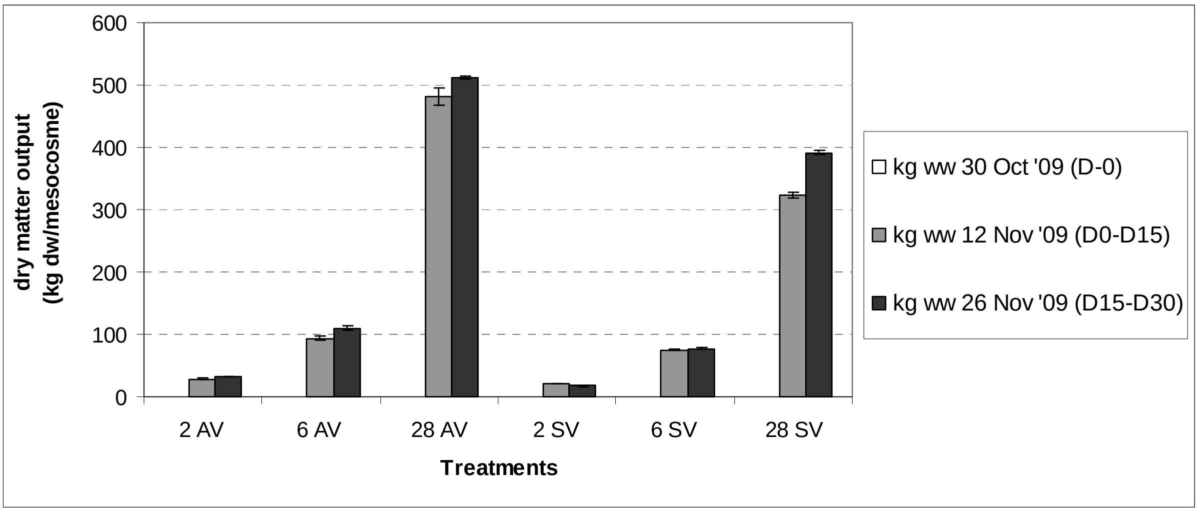


Figure 38. Dry matter output of mesocosms

6.1.10. Net dry matter inputs of mesocosms

The net input of dry matter increased with the input dose. It was smaller in treatments with earthworms than in treatments without earthworms for all doses. There was a clear decrease in the net input of dry matter between the first and the second period for the treatments with earthworms but not for the treatments without earthworms. In dose 2 l/day, the net dry matter input was between 20 and 27 g for treatment with earthworm and between 34 and 35 g for treatment without earthworms. In dose 6 l/day, the net dry matter input was between 47 and 71 g for treatment with earthworm and between 80 and 90 g for treatment without earthworms. In dose 28 l/day, the net dry matter input was between 223 and 287 g for treatment with earthworms and were around 340 g for treatment without earthworms.

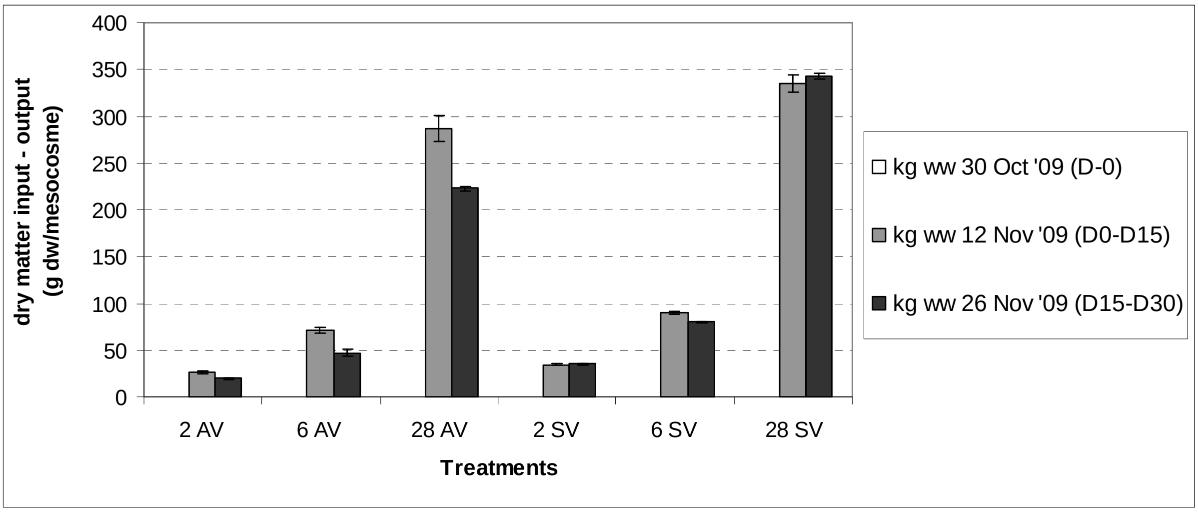


Figure 39. The dry matter balance between input and output liquid

6.1.11. Conclusions concerning the liquid input

Liquid inputs were very homogeneous between the repetitions except during the first period, where it was limited in treatment 28 SV to the maximum infiltrated amount because of clogging. On the basis of former measurement of concentrations (Morand et al, 2009), the nitrogen input varied between 0.6 and 8.4 g N mesocosm^{-1} day^{-1}. Liquid outputs were also very homogeneous, and slightly lower than inputs due to water accumulation inside the mesocosms, and water evaporation. The differences between two different doses were significant ($P<0.05$), whether earthworms were present or not. The differences between treatments with or without earthworms, for the doses 2 and 6 l/day, were not clear for the liquid input and for the filtrate. There was only a high difference for dose 28 l/day, since the beginning of the experiment where clogging was rapidly observed on treatment without earthworms. The net input of liquid in treatment 28 SV was in the range 5-8 kg while it was only in the range 3-7 kg in treatments with earthworms (28 AV), i.e. water accumulation was significantly higher in treatment 28 SV ($P<0.05$).

The dry matter content was always higher in the input than in the output, showing filtering efficiency whether earthworms were present or not. The temporal variability was much higher for the input than for the output. There was an increase in dry matter content of the filtrate between the first and the second period ($P<0.01$). This increase was higher in treatments with earthworms. The dry matter content of filtrate was higher in the treatments with earthworms than for the treatments without earthworms (respectively from 2.9 to 4.2 g dw/kg ww for all treatments with earthworms and from 2.1 to 3.1 g dw/kg ww for treatments without earthworms; $P<0.001$).

As a consequence of the differences in liquid budget and dry matter content in the liquids, the treatments without earthworms accumulated more dry matter and liquid than the treatments with earthworms. Surface accumulation of organic particles in treatment without earthworms explained the clogging.

6.2. Mesocosm weights

6.2.1. Water content of the vermifilters

Table VI. Water content of the vermifilters

sampling date	29 Oct '09		12 Nov '09		25 Nov '09	
dose	water content (%)	standard deviation	water content (%)	standard deviation	water content (%)	standard deviation
2 AV	74.6%	0.3%	75.1%	0.8%	75.2%	0.3%
6 AV	74.6%	0.3%	75.5%	0.4%	75.6%	0.5%
28 AV	74.6%	0.3%	75.8%	0.6%	76.1%	0.4%
2 SV	73.7%	0.7%	74.0%	0.5%	74.7%	0.9%
6 SV	73.7%	0.7%	74.3%	0.2%	75.5%	0.3%
28 SV	73.7%	0.7%	75.4%	0.5%	76.8%	0.2%

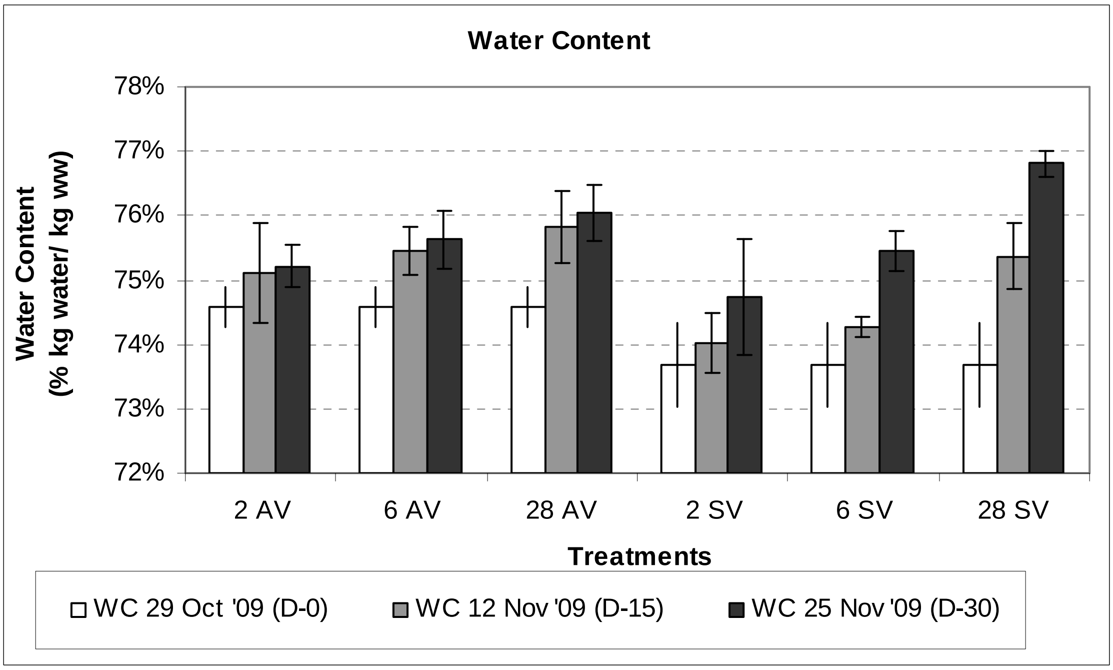


Figure 40. Water content of the vermifilters

Compost humidity increased during the experiment for all treatments. The differences between treatments were usually close to the variability within the replicates of one treatment. However clear trends could be observed.

Initial water content of treatments with earthworms was higher than treatments without earthworms are small following dose. This difference remains during the experiment except for dose 28 where final humidity was higher for 28 SV than for 28 AV, respectively 76.8 % and 76.1 %.

The increase in water content between two sampling dates was higher when the dose was higher: humidity of dose 2 l/day increased from 73.6 to 75.2 %; humidity of dose 6 l/day increased from 73.7 to 75.6 %; and humidity of dose 28 l/day increased from 73.7 to 76.8 %.

For a given dose, the increase in water content between two sampling dates was higher for the treatments without earthworms: the humidity of 2 AV increased from 74.5 to 75.2 % and from 73.6 to 74.7 % for 2 SV; humidity increased from 74.6 to 75.6 % for treatment 6 AV and from 73.7 to 75.5 % for treatment 6 SV.

Variations of dry matter content were opposite to variations of water content.

6.2.2. Wet weight of the mesocosms

Table VII. Wet weight of the mesocosms

day of measurement	31 Oct '09		12 Nov '09		25 Nov '09		
dose	wet weight (kg)	standard deviation	wet weight (kg)	standard deviation	wet weight (kg)	standard deviation	mass increase (kg)
2 AV	33.70	0.26	33.80	0.31	33.94	0.45	0.24
6 AV	33.52	0.04	34.73	0.22	35.47	0.23	1.95
28 AV	33.45	0.03	36.96	0.12	38.73	0.03	5.28
2 SV	33.37	0.08	34.00	0.45	34.87	0.48	1.50
6 SV	33.42	0.11	34.99	0.35	36.41	0.59	2.99
28 SV	33.32	0.14	38.57	0.85	41.85	0.75	8.53

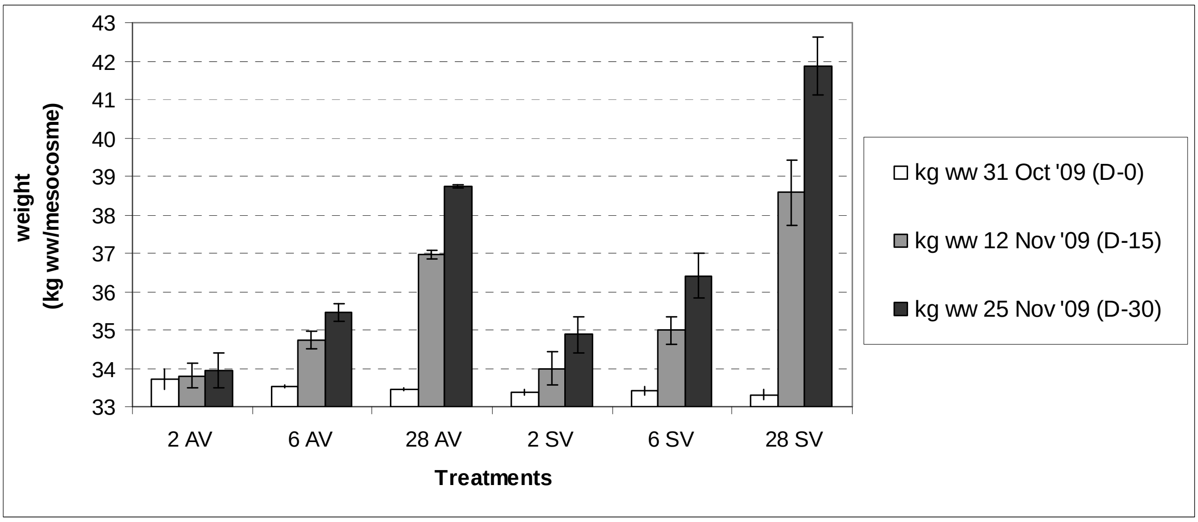


Figure 41. Wet weight of the mesocosms

The wet weight of vermicompost increased following the dose in both treatments with or without earthworms. The increase was higher in treatments without earthworms than in treatments with earthworms for the three doses. It can also be observed that the variability between replicates within one treatment also increased with the dose and was higher in treatments without earthworms compared to treatments with earthworms. This increased variability was due to the transformations inside each vermifilter and not to differences in either the initial state or the input of fresh manure.

Wet weight in treatment 2 AV increased non significantly. Its mass was 33.7±0.26 kg at the beginning of the experiment, and it was 33.9±0.45 kg at the end of the experiment. On the contrary, treatment 2 SV increased in wet weight: from 33.4±0.1 kg at the beginning of the experiment, to 34.9±0.5 kg at the end experiment.

The increase of wet weight on dose 6 l/day was higher than on dose 2 l/day. In treatment with earthworms, the wet weight increased significantly from 33.5 to 35.5 kg. In treatment without earthworms, the increase of wet weight was higher: wet weight changed from 33.4 to 36.4 kg.

In dose 28 l/day, the increase of wet weight was highest. The wet weight in treatment with earthworms increased from 33.5 to 38.7 kg. At the beginning of the experiment wet weight of treatment 28SV was 33.3 kg and, at the end of experiment, wet weight changed to 41.9 kg.

6.2.3. Mass of water in the mesocosm

The evolution of the mass of water in the mesocosms show the same trends as the wet weight of the mesocosms: increase in water with the dose and in treatments without earthworms compared to treatments with earthworms. Treatments with earthworms gained less water less than treatments without earthworms.

However there were two small differences with the results of wet weight:

- for treatment 2 AV, an increase in the mass of water was observed while it was not observed with wet weight;
- Standard deviation did not show clear trends with increasing dose or with earthworm presence as it was the case for wet weight.

Table VIII. Mass of water in the mesocosms

day of estimate	31 Oct '09		12 Nov '09		25 Nov '09		
dose	water weight (kg)	standard deviation	water weight (kg)	standard deviation	water weight (kg)	standard deviation	water increase (kg)
2 AV	25.13	0.22	25.39	0.13	25.53	0.23	0.39
6 AV	25.00	0.09	26.20	0.27	26.82	0.33	1.82
28 AV	24.95	0.11	28.03	0.29	29.45	0.16	4.50
2 SV	24.59	0.16	25.17	0.27	26.06	0.23	1.47
6 SV	24.63	0.22	25.99	0.24	27.47	0.39	2.85
28 SV	24.55	0.11	29.07	0.52	32.15	0.51	7.60

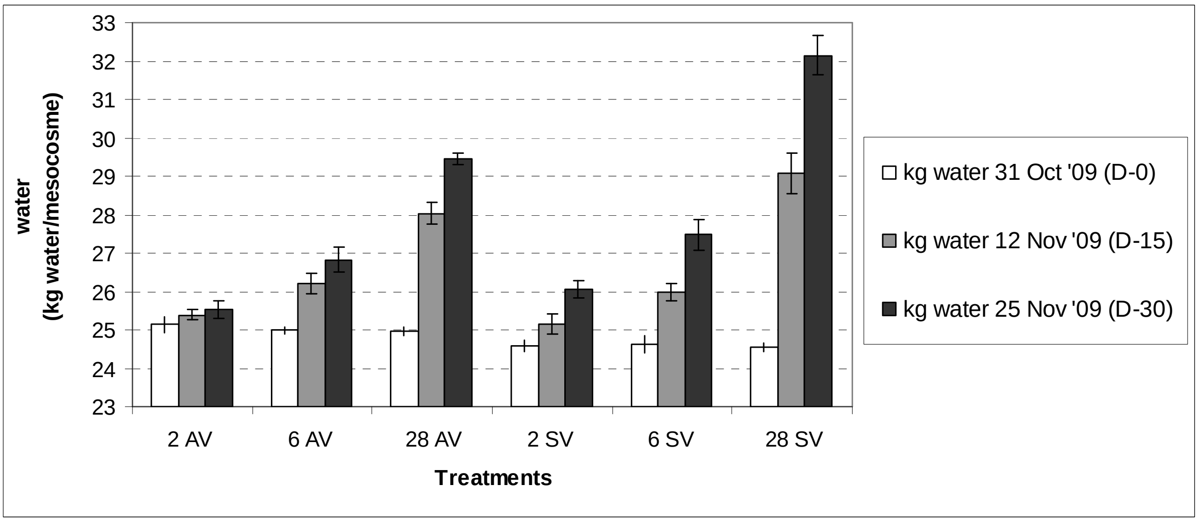


Figure 42. Mass of water in the mesocosms

6.2.4. Dry matter of mesocosm

Table IX. Dry matter of mesocosm

day of estimate	31 Oct '09		12 Nov '09		25 Nov '09		
dose	dry matter weight (kg)	standard deviation	dry matter weight (kg)	standard deviation	dry matter weight (kg)	standard deviation	dry matter increase (kg)
2 AV	8.57	0.12	8.41	0.34	8.41	0.22	-0.16
6 AV	8.52	0.11	8.52	0.10	8.64	0.11	0.12
28 AV	8.50	0.10	8.93	0.19	9.28	0.17	0.78
2 SV	8.78	0.24	8.83	0.25	8.81	0.41	0.03
6 SV	8.79	0.23	9.01	0.13	8.94	0.23	0.15
28 SV	8.77	0.26	9.50	0.38	9.71	0.25	0.94

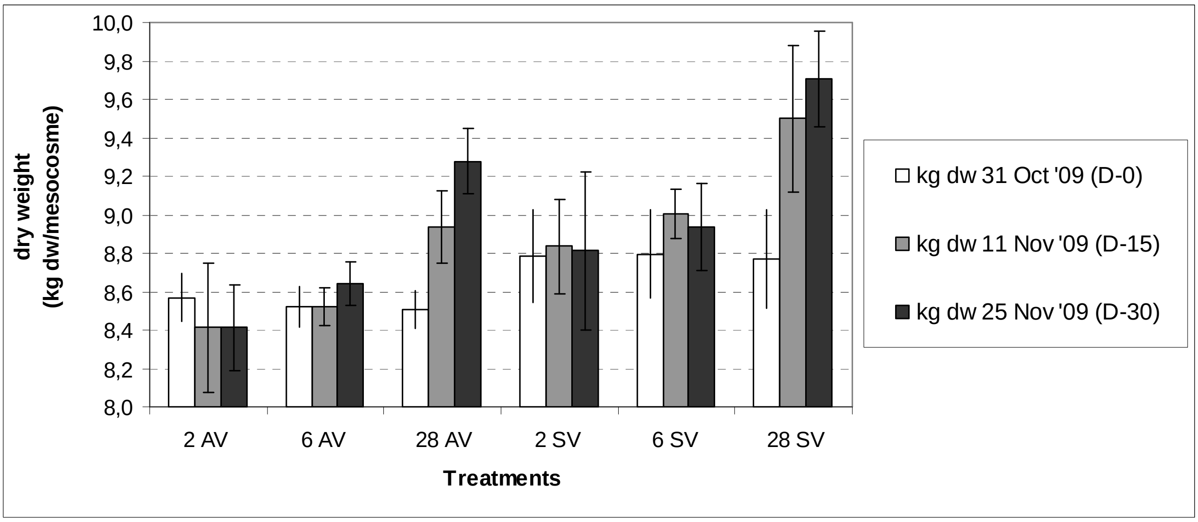


Figure 43. Mesocosm dry matter

Dry matter of treatment 2 AV decreased insignificantly from 8.6 kg to 8.4 kg. Dry matter mass of the other mesocosms increased from the beginning to the end of the experiment. The increase was higher for higher dose and in the absence of earthworms.

The increase was not significant for treatments 6 AV, 2 SV and 6 SV.

A significant increase of the mass of dry matter was observed in both treatments 28 AV and 28 SV, respectively from 8.5 to 9.3 kg and from 8.77 to 9.71 kg.

6.2.5. Conclusions the vermifilter media

The same trends were observed for the wet weight of the mesocosms, the variability of wet weight between replicates within one treatment, the dry weight, the weight of water and the water content: an increase during the experiment, which was higher for higher input of fresh manure and which was higher in treatments without earthworms. This in contradiction wih the observations of Aira and Dominguez (2008) who observed that the carbon decrease after pig slurry input was higher without earthworms, and that it did not depend on the quantity of added pig slurry (1.5 or 3 kg).

Dry matter content decreased with experiment time, while the wet weight increased. The increase in wet weight during each period of two weeks was significant for the higher doses (6 and 28 L/day; $P<0.01$). The difference in wet weight between treatments with or without earthworms was significant only after the second period and for the high dose ($P<0.05$). In the treatments with earthworms, the initial dry matter content was lower due to the wet mass of earthworms (254±3 compared to 263±7 g dw kg^{-1} ww). The final dry matter content was similar for the lower doses and, for the high dose, higher in the treatment with earthworms (239±4 compared to 232±2 g dw kg^{-1} ww in dose 28 L/day).

The dry matter mass was more or less stable for the small doses, whatever the presence of earthworms. The input varied between 0.1 and 1.5 kg dw. Significant increases were only observed after the second period and for the high inputs (28 L/day; $P<0.05$) but without a significant effect of the presence of earthworms.

6.3. Mesocosm temperatures

Air temperature around the mesocosms varied between 10 and 20℃ with daily variations around 7 K. The mesocosm temperature varied between 11 and 21℃ with daily variations around 2 K. It was around 2 K higher than mean air temperature, except just after the liquid input because the liquid was generally colder than the mesocosms (between 10 and 13℃). Temperatures of treatments w ithout earthworms were a little lower and more heterogeneous than treatments with earthworms. At the end of experiment, the highest temperature was observed with the highest dose (28 l/day). These observations can be explained by the heat production within the vermifilter: as during composting, heat production increases with input of fresh organic matter and with oxygen availability. Moreover, higher variability of temperatures in mesocosms without earthworms can be related to a higher heterogeneity of the fresh manure percolation within the mesocosm.

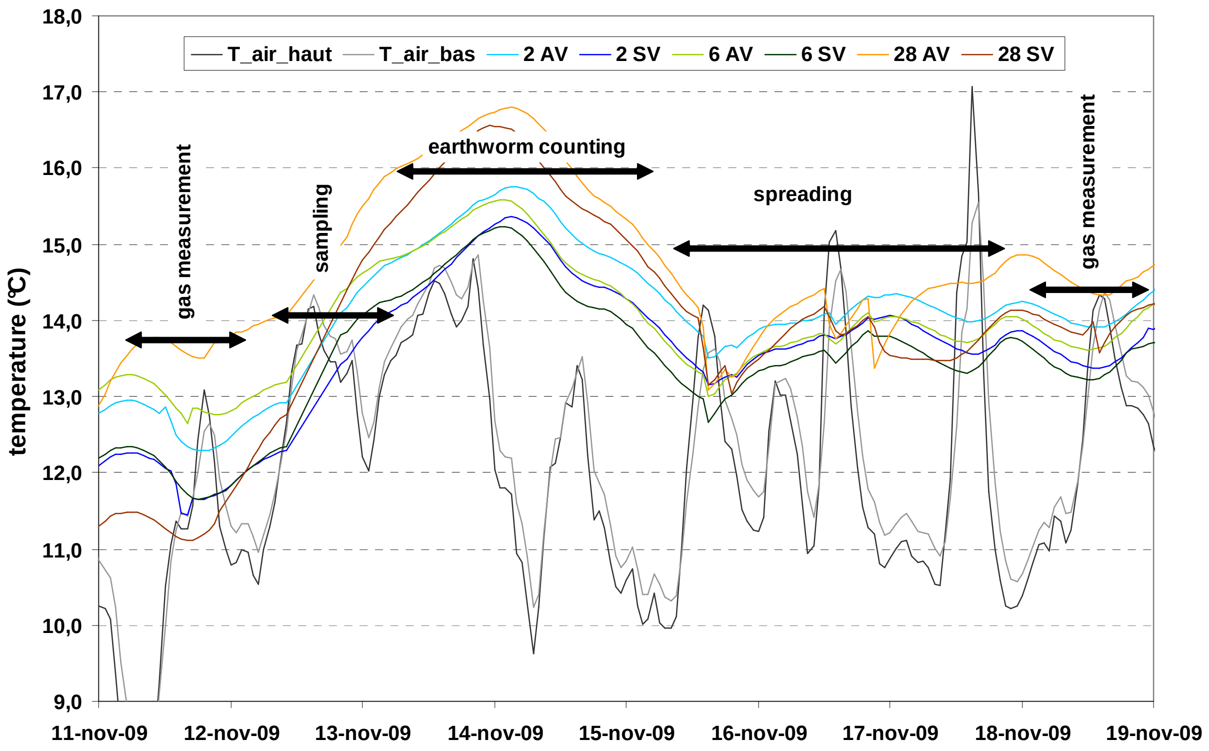


Figure 44. Average temperature between 11th and 19th November 2009

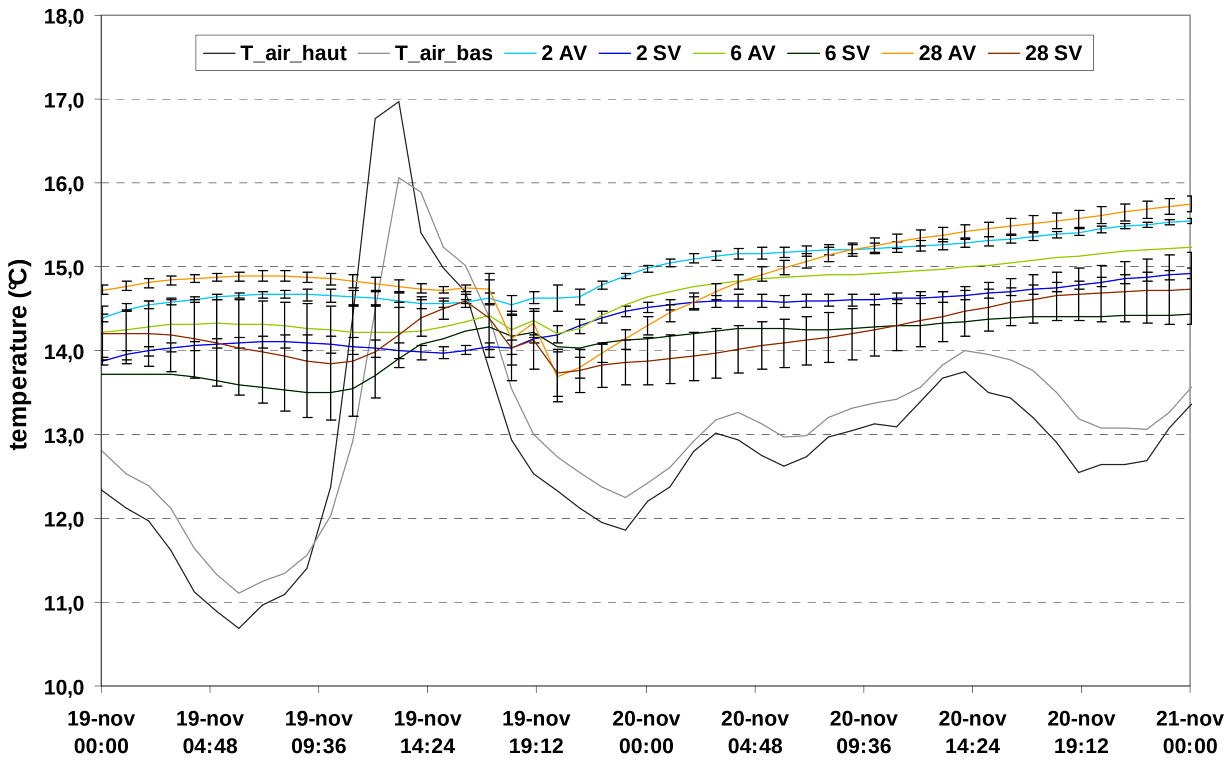


Figure 45. Average temperature and standard deviation (only for doses 2 and 28 l/day) between 19th and 20th November 2009; the variability of temperature between replicates increased with dose and in the absence of earthworms.

6.4. Gaseous emissions

6.4.1. Methane (CH_4) Emission

Table X. Methane emission

day of measurement	31 Oct '09		11 Nov '09		25 Nov '09	
dose	CH_4 emission (g C-CH_4.day^{-1}. mesocosm^{-1})	standard deviation	CH_4 emission (g C-CH_4.day^{-1}. mesocosm^{-1})	standard deviation	CH_4 emission (g C-CH_4.day^{-1}. mesocosm^{-1})	standard deviation
2 AV	0.0039	0.0001	< det.level	< det.level	-0.0059	0.0016
6 AV	-0.0023	nd (1 value)	-0.0043	nd (1 value)	-0.0049	0.0020
28 AV	-0.0051	0.0007	-0.0091	0.0015	-0.0097	0.0023
2 SV	0.0038	0.0010	-0.0023	nd (1 value)	-0.0042	< det.level
6 SV	< det.level	< det.level	-0.0055	0.0020	-0.0062	0.0027
28 SV	< det.level	< det.level	0.0832	0.0505	0.0639	0.0307

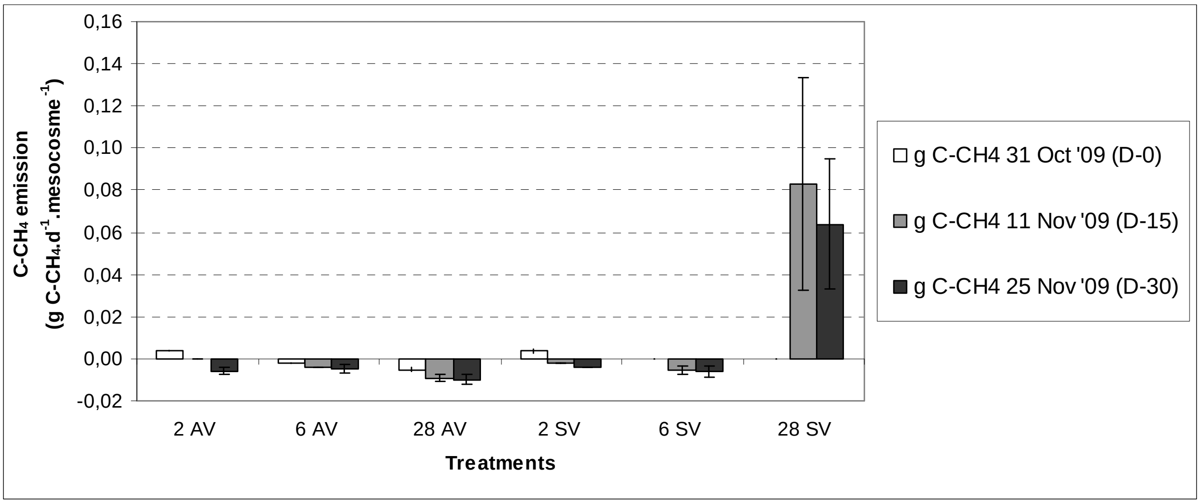


Figure 46. Methane Emissions

On first day, before the first spreading, methane emission was only observed in treatments 2 AV and 2 SV. It was around 4 mg C-CH_4.day^{-1}. mesocosm^{-1}. Other treatments with earthworms showed a methane sink. Emission or sink were not detected in treatments without earthworm.

All treatments with earthworms showed a methane sink during the second period. The methane sink increased following the dose: at the end of experiment, the methane sink of dose 2 l/day was 6 mg C-CH_4.day^{-1}. mesocosm^{-1}, dose 6 l/day was 5 mg C-CH_4.day^{-1}. mesocosm^{-1} and dose 28 l/day was 10 mg C-CH_4.day^{-1}. mesocosm^{-1}.

Treatment 2 SV and 6 SV showed same phenomena as treatments with earthworms, with a methane sink that increased with the dose.

Methane emission was clearly observed in treatment 28 SV. It reached 64 mg $C\text{-}CH_4.day^{-1}.\ mesocosm^{-1}$.

6.4.2. Ammonia emission

On the first day, only treatment 2 SV showed an ammonia emission 1.4 mg $N\text{-}NH_3.day^{-1}.\ mesocosm^{-1}$.

Clear ammonia emissions were observed with dose 28 l/day during the first and the second period. Ammonia emission was higher in treatments without earthworms than with earthworms. At the end of experiment, the ammonia emission was 2.5 mg $N\text{-}NH_3.day^{-1}.\ mesocosm^{-1}$ for treatment 28 AV and 9.4 mg $N\text{-}NH_3.day^{-1}.\ mesocosm^{-1}$ for treatment 28 SV.

Ammonia sink was detected during the second period in treatment 2 AV: 1.5 mg $N\text{-}NH_3.day^{-1}.\ mesocosm^{-1}$.

Table XI. Ammonia emission

day of measurement	31 Oct '09		11 Nov '09		25 Nov '09	
dose	NH_3 emission (g $N\text{-}NH_3.day^{-1}.\ mesocosm^{-1}$)	standard deviation	NH_3 emission (g $N\text{-}NH_3.day^{-1}.\ mesocosm^{-1}$)	standard deviation	NH_3 emission (g $N\text{-}NH_3.day^{-1}.\ mesocosm^{-1}$)	standard deviation
2 AV	< det.level	< det.level	< det.level	< det.level	-0.0007	< det.level
6 AV	< det.level	< det.level	< det.level	< det.level	< det.level	< det.level
28 AV	< det.level	< det.level	0.0015	0.0004	0.0025	0.0005
2 SV	0.0014	< det.level	< det.level	< det.level	< det.level	< det.level
6 SV	< det.level	< det.level	< det.level	< det.level	< det.level	< det.level
28 SV	< det.level	< det.level	0.0056	0.0025	0.0094	0.0039

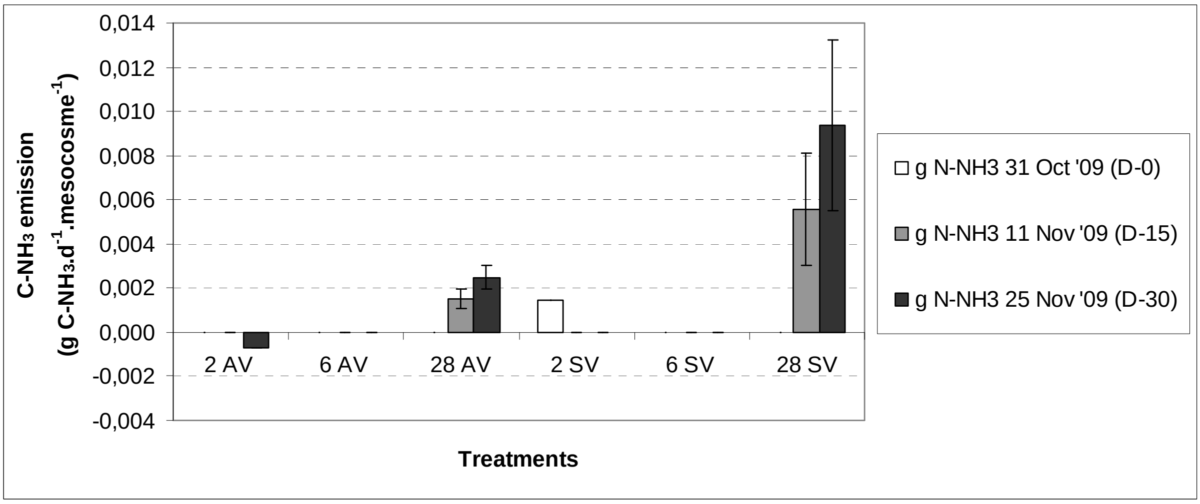


Figure 47. Ammonia emission

6.4.3. Carbon dioxide emission

On the first day, before the first spreading, carbon dioxide emission was higher in treatments with earthworms compared to treatments without earthworms, despite a lower mass of dry matter. We suggest that this difference can be attributed to the earthworm population.

During the last period, the carbon dioxide emission in doses 2 l/day and 6 l/day was similar in treatments with earthworms or without earthworms: carbon dioxide emission of treatments with earthworms was in the range 4.66 - 5.25 g $C\text{-}CO_2.day^{-1}$. $mesocosm^{-1}$, while it was in the range 4.81 - 5.27 g $C\text{-}CO_2.day^{-1}$. $mesocosm^{-1}$ in treatments without earthworms.

Table XII. Carbon dioxide emission

day of measurement	31 Oct '09		11 Nov '09		25 Nov '09	
dose	CO_2 emission (g $C\text{-}CO_2.day^{-1}$. $mesocosm^{-1}$)	standard deviation	CO_2 emission (g $C\text{-}CO_2.day^{-1}$. $mesocosm^{-1}$)	standard deviation	CO_2 emission (g $C\text{-}CO_2.day^{-1}$. $mesocosm^{-1}$)	standard deviation
2 AV	5.18	2.28	5.09	0.70	4.66	0.32
6 AV	7.44	0.81	5.37	0.76	5.25	0.37
28 AV	5.59	0.22	7.77	0.90	11.10	1.42
2 SV	4.04	0.65	2.85	0.32	4.81	0.29
6 SV	4.03	0.30	5.18	0.48	5.27	0.21
28 SV	4.02	0.18	8.26	4.27	20.31	7.54

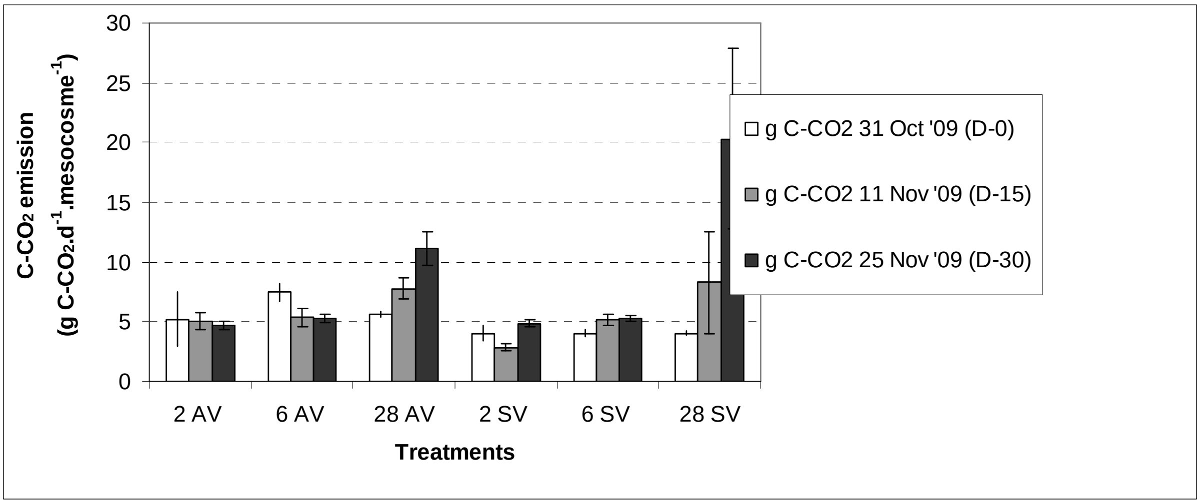


Figure 48. Carbon dioxide emission

In the case of dose 28 l/day, carbon dioxide emission during the last period of treatment with earthworms was lower than treatment without earthworm: respectively 11.1 g C-CO_2.day^{-1}. mesocosm^{-1} and 20.3 g C-CO_2.day^{-1}. mesocosm^{-1}. This difference is in contradiction with the hypothesis that respiration of 28 AV should be higher than 28 SV because of anoxic conditions of treatment 28 SV and earthworm population of treatment 28 AV.

6.4.4. Water emissions

There is no clear trend in water emission. Temporal variations and variability between the replicates of a same treatment were both high. Therefore, either this protocol was not suited to accurate measurements of water emissions, or neither the earthworm presence nor the input dose had a strong influence of the water emission.

Table XIII. Water emission

day of measurement	31 Oct '09		11 Nov '09		25 Nov '09	
dose	water emission (g water.day^{-1}. mesocosm^{-1})	standard deviation	water emission (g water.day^{-1}. mesocosm^{-1})	standard deviation	water emission (g water.day^{-1}. mesocosm^{-1})	standard deviation
2 AV	33.13	13.18	9.01	5.10	23.29	2.39
6 AV	28.48	7.10	3.51	2.63	29.25	5.07
28 AV	16.37	5.41	9.76	2.11	20.67	9.47
2 SV	28.37	13.24	3.96	3.53	23.12	5.97
6 SV	38.52	13.87	2.50	nd (1 value)	26.33	13.62
28 SV	20.23	13.17	4.09	1.97	22.12	2.76

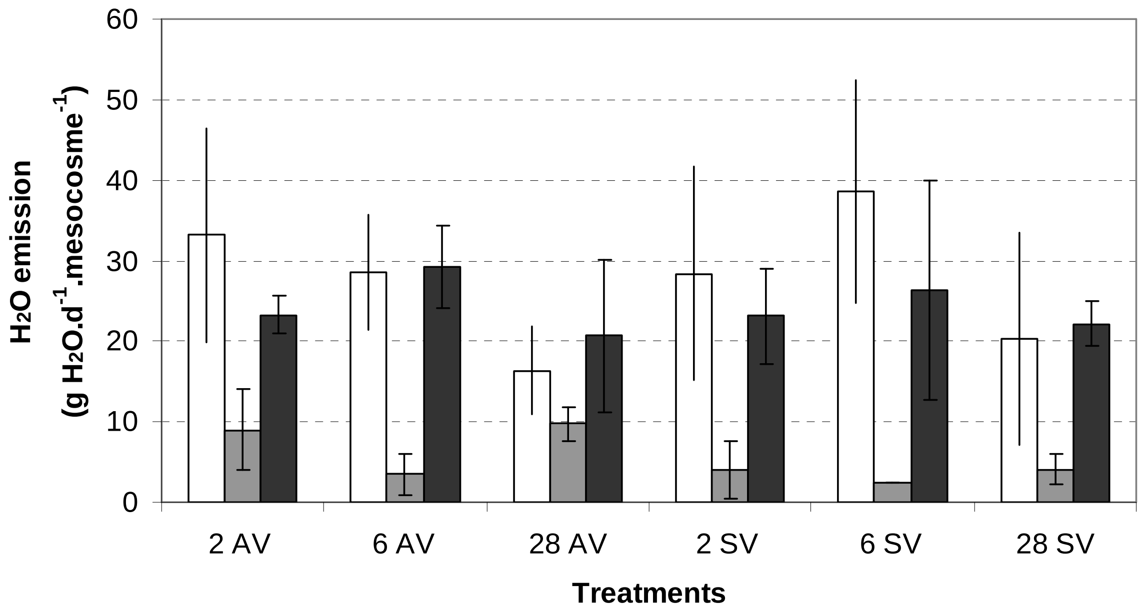


Figure 49. Water emission

Nevertheless, the higher water emission on the first day can be attributed to the mixing operations the day before measurements that stimulated the microbial transformations like in a composting process.

6.4.5. Nitrous oxide emission

For dose 2 l/day nitrous oxide emission of treatment with earthworms were in the range 8.9 to 9.6 mg N-N_2O.day^{-1}. mesocosm^{-1}. They were higher than for treatment without earthworms that were in the range 2.2 to 12 mg N-N_2O.day^{-1}. mesocosm^{-1}.

Table XIV. Nitrous oxide emission

day of measurement	31 Oct '09		11 Nov '09		25 Nov '09	
dose	N_2O emission (g N-N_2O.day^{-1}. mesocosm^{-1})	standard deviation	N_2O emission (g N-N_2O.day^{-1}. mesocosm^{-1})	standard deviation	N_2O emission (g N-N_2O.day^{-1}. mesocosm^{-1})	standard deviation
2 AV	0.0089	0.0061	0.0081	0.0011	0.0096	0.0009
6 AV	0.0134	0.0025	0.0109	0.0019	0.0185	0.0025
28 AV	0.0095	0.0009	0.0681	0.0065	0.1787	0.0492
2 SV	0.0022	0.0008	0.0049	0.0007	0.0123	0.0016
6 SV	0.0019	0.0001	0.0347	0.0035	0.0444	0.0040
28 SV	0.0024	0.0002	0.1088	0.0712	0.4385	0.1689

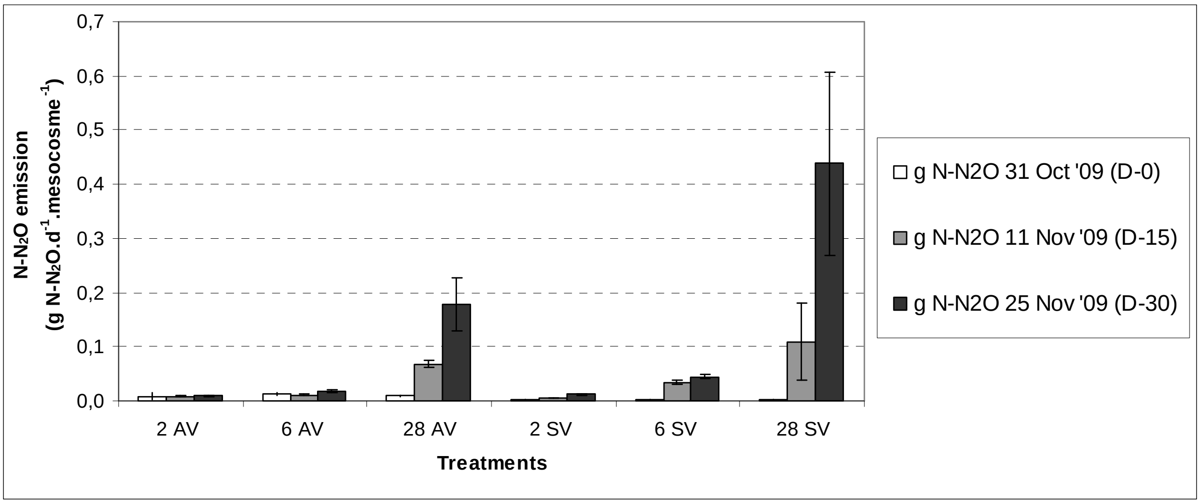


Figure 50. Nitrous oxide emission

On the contrary, for dose 6 l/day nitrous oxide emission of treatment with earthworms were in the range 13 to 19 mg $N\text{-}N_2O.day^{-1}$. $mesocosm^{-1}$, that was smaller than for treatment without earthworms where emissions were in the range 1.9 to 44 mg $N\text{-}N_2O.day^{-1}$. $mesocosm^{-1}$.

For dose 2 l/day nitrous oxide emission of treatment with earthworms were in the range 8.9 to 9.6 mg $N\text{-}N_2O.day^{-1}$. $mesocosm^{-1}$. They were higher than for treatment without earthworms that were in the range 2.2 to 12 mg $N\text{-}N_2O.day^{-1}$. $mesocosm^{-1}$.

On the contrary, for dose 6 l/day nitrous oxide emission of treatment with earthworms were in the range 13 to 19 mg $N\text{-}N_2O.day^{-1}$. $mesocosm^{-1}$, that was smaller than for treatment without earthworms where emissions were in the range 1.9 to 44 mg $N\text{-}N_2O.day^{-1}$. $mesocosm^{-1}$.

For dose 28 l/day there was a clear reduction of nitrous oxide emission in treatment with earthworms: it was in the range 9.5 to 180 mg $N\text{-}N_2O.day^{-1}$. $mesocosm^{-1}$, while it was in the range 2.4 to 440 mg $N\text{-}N_2O.day^{-1}$. $mesocosm^{-1}$for the treatment without earthworms.

Emission of nitrous oxide increased with the dose and with the duration of experiment. The increase was higher in treatments with accumulation of fresh manure at the surface (6 SV, 28 AV, and 28 SV).

6.5. Earthworm populations

6.5.1. Earthworm abundance

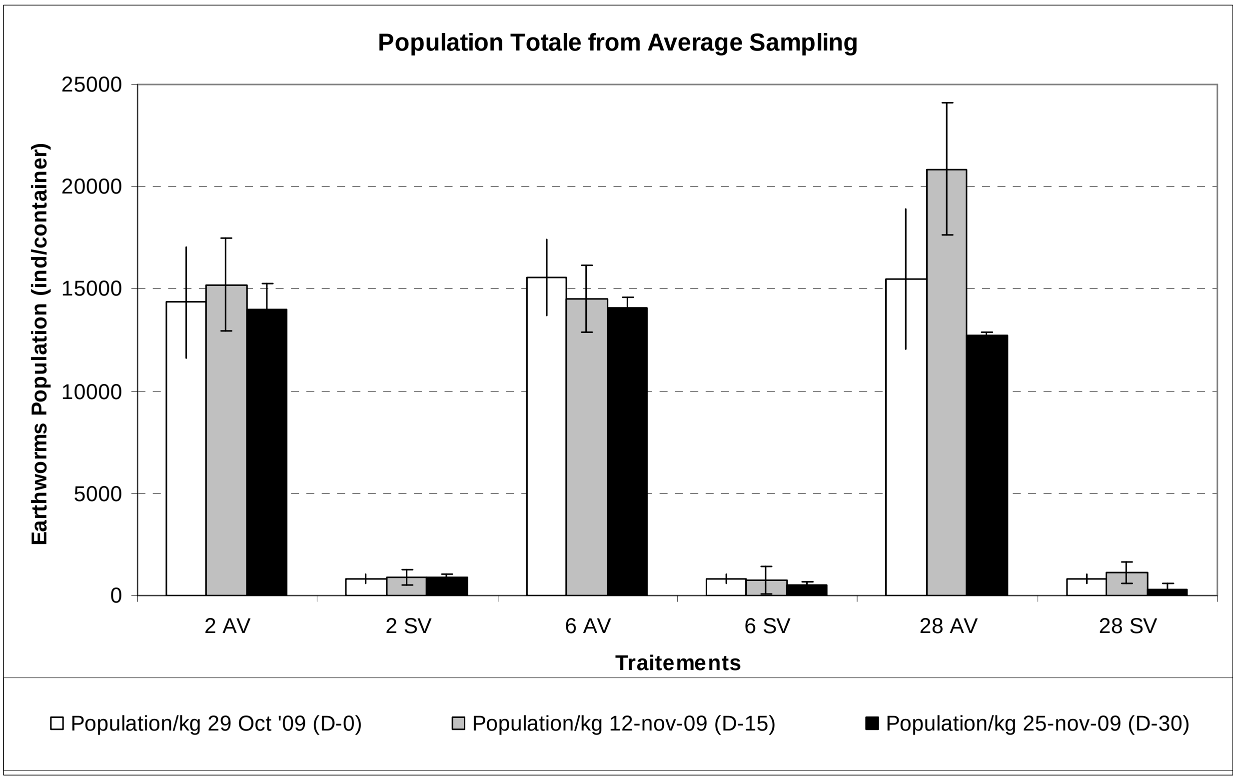


Figure 51. Total population of Earthworm from Average Sampling (the population is deduced from the population of the average samples and the wet weight of the mesocosms)

Table XV. Total population from average sampling

sampling date	29 Oct '09		12 Nov '09		25 Nov '09	
dose	population abundance (ind.mesocosm^{-1})	standard deviation	population abundance (ind.mesocosm^{-1})	standard deviation	population abundance (ind.mesocosm^{-1})	standard deviation
2 AV	14330	2690	15190	2266	13957	1314
2 SV	822	227	862	372	877	137
6 AV	15550	1836	14490	1649	14060	499
6 SV	822	227	7480	652	530	163
28 AV	15470	3426	20860	3255	12690	198
28 SV	822	227	1104	536	291	272

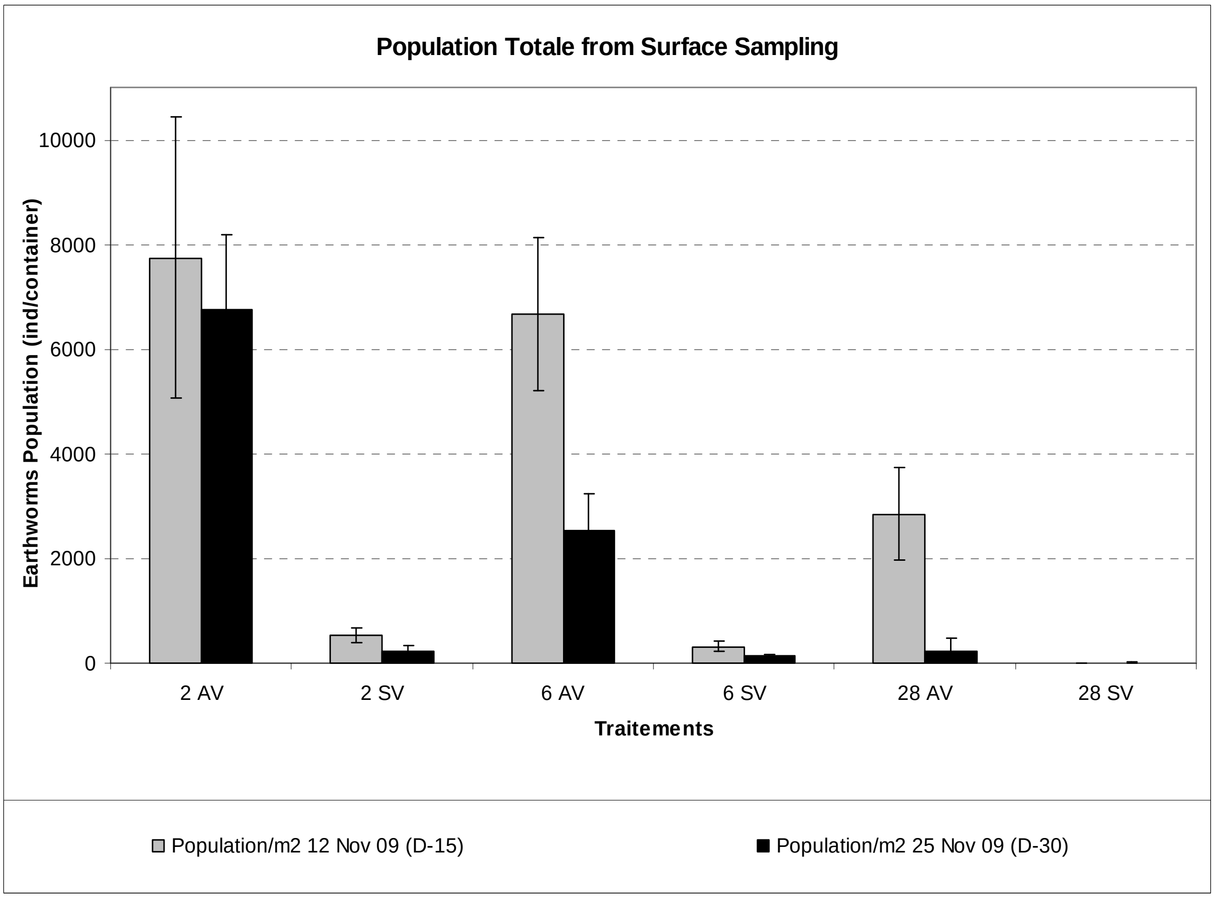


Figure 52. Total population of Earthworms from surface Sampling (the population is deduced from the population of the surface samples and the surface area of the mesocosms)

Table XVI. Total population from surface sampling

sampling date	29 Oct '09		12 Nov '09		25 Nov '09	
dose	population abundance (ind.mesocosm^{-1})	standard deviation	population abundance (ind.mesocosm^{-1})	standard deviation	population abundance (ind.mesocosm^{-1})	standard deviation
2 AV	nd[(a)]	nd	7746	2684	6748	1449
2 SV	nd	nd	533	139	236	94
6 AV	nd	nd	6665	1457	2539	699
6 SV	nd	nd	322	104	129	26
28 AV	nd	nd	2845	884	225	246
28 SV	nd	nd	nd[(b)]	nd	6	10

(a) surface sampling was not defined the day of installation
(b) surface sampling could not be achieved because of the liquid accumulated in the surface due to clogging

The result showed that the dominant population was juvenile earthworms during all the experiment.

On the first day, the population of the treatments with earthworms was around 15 000 individuals/mesocosm and it was not significantly different between doses.

After the first period of 15 days, the population of dose 28 l/day was around 21000 individuals/mesocosm and it was the highest of all the experiment. The population of the other dose remained around 15000 individuals/mesocosm.

After the second period, the population of dose 2 and 6 l/day remained around 14 000 individuals. For the dose 28 l/day it dropped from 21000 to 12700 individuals/mesocosm.

The mesocosm population calculated from surface sampling was always lower than from average sampling. The difference was highest for the dose 28 l/day because of the anoxic conditions of the surface.

For the treatments without earthworm, the evolution of the population show a similar trend as for the treatments with earthworms: the variations between periods were small for dose 2 and 6 l/day, while for dose 28 l/day, the abundance increased during the first period and decreased during the second one.

6.5.2. Earthworm biomass

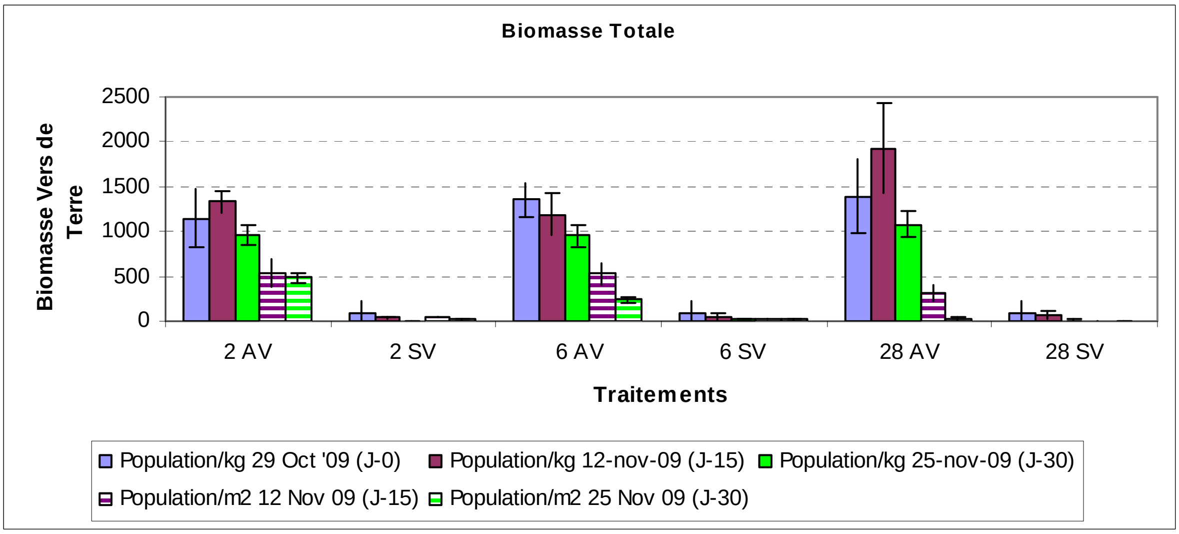


Figure 53. Earthworm biomass

Evolution biomass dose 2 and 28 l/day of treatment with earthworm had same phenomena. Biomass increased in mid experiment and decreased in end experiment. Evolution biomass dose 6 l/day of treatment with earthworm was different. It decreased from begin to end experiment.

Biomass on surface of treatment with earthworm had same phenomena for all experiment. It decreased from begin to end experiment. The biomass population dose 28 l/day of treatment with earthworm decreased very strict from 300 to 20 g/m².

Biomass total and surface of treatment without earthworm had same phenomena for all dose. The earthworm biomasses decreased from begin to end experiment.

Table XVII. Earthworm biomass from average sampling

sampling date	29 Oct '09		12 Nov '09		25 Nov '09	
dose	population biomass (g.mesocosm^{-1})	standard deviation	population biomass (g.mesocosm^{-1})	standard deviation	population biomass (g.mesocosm^{-1})	standard deviation
2 AV	1146	325	1330	123	953	109
2 SV	92	122	44	11	nd	nd
6 AV	1351	179	1193	240	950	129
6 SV	92	122	52	45	26	4
28 AV	1393	413	1928	504	1076	147
28 SV	92	122	62	44	8	8

Table XVIII. Earthworm biomass from surface sampling

sampling date	29 Oct '09		12 Nov '09		25 Nov '09	
dose	population biomass (kg.mesocosm^{-1})	standard deviation	population biomass (kg.mesocosm^{-1})	standard deviation	population biomass (kg.mesocosm^{-1})	standard deviation
2 AV	nd[(a)]	nd	535	161	487	55
2 SV	nd	nd	41	4	17	7
6 AV	nd	nd	527	119	236	41
6 SV	nd	nd	21	5	13	7
28 AV	nd	nd	310	91	21	26
28 SV	nd	nd	nd	nd	0.1	0.10

[(a)] *surface sampling was not defined the day of installation*

6.5.3. Conclusions concerning earthworms

Earthworm population and biomass were highly correlated (R^2=0.955) with a mean weight of 84 mg earthworm^{-1}. The juveniles were most abundant (80% on average). Values from average samples indicated much higher populations for the entire mesocosm than values from surface samples. The density of earthworms was higher close to the surface, except after the second period with the high dose (28 AV), but many earthworms were located inside the vermifilter. Similar variations, such as population decrease in treatment 28 AV, were indicated by each sampling method but results were loosely correlated (R^2=0.506). This low correlation can be explained by the heterogeneous distribution of earthworms within each mesocosm.

The population was around 15 000 individuals per mesocosm. It was stable with the doses 2 and 6 L/day. With dose 28 L/day, this population increased after the second week until around 20 000 individuals per mesocosm, then it dropped to 13 000. This shows that the worm population can rapidly increase when feed is adequate (first period, treatment 28 AV), and that it does not decrease rapidly when, during some weeks, feed is limited (treatment 2 AV or 6 AV), but it decreases rapidly when the environment becomes toxic (mortality and population decrease during second period, treatment 28 AV).

The population in the surface layer was in the range 10 000 – 40 000 individuals m^{-2} with doses 2 and 6 L/day, and it decreased to 1 000 individuals m^{-2} after the second period in treatment 28 AV. The population rise during the first period of treatment AV could not be observed because the surface sampling was not meaningful at the beginning of the experiment.

7. *Discussion of hypothesis*

7.1. Representativity of the observations

The stability of earthworm population in most treatments indicate that the biological processes inside the mesocosms were probably close to those inside the large vermifilter. Therefore we assume the representativity of the measurements. However, to make sure this hypotthesis, an experiment during one year would be necessary, with earthworm counting in both mesocosms and large vermifilter.

In treatments without earthworms, we found a small earthworm population. During the preparation of the material without earthworms, the earthworms were removed manually from the vermicompost. The earthworms that were removed were clearly visible. However, some earthworms could remain in the material because they were either very small (in juvenile level) or in cocoons. These phenomena could be hardly avoided. During the experimentation of one month, some of the invisible earthworms could change from cocoon to juvenile, and grow from juvenile to adult. This population could be identified during the counting process. We assume that this population did not influence significantly the physical fluxes and the biological transformation in the mesocosms “without earthworms” because the number of earthworms was much less than in the mesocosms “with earthworms”.

7.2. Confirmation of the existence of an optimal input of liquid and organic matter of the mesocosms

The experiment described in the previous chapter showed that an optimal input exist from a statistical point of view, but it was not possible to clearly show a decrease in either population abundance or biomass due to either excess of organic load or insufficient nutrient input.

The experiment described in the present chapter clearly confirmed this result. It showed that the population was clearly modified by the high dose. The high input (700 g DM day^{-1} kg $earthworms^{-1}$) leads to an increase of about 30% in the population and biomass during the first period. The population decreased during the second period, and dying earthworms were observed. These results show that the high level of organic input was close to the feeding needs of the earthworm population. Observed feeding need was between 200 and 700 g dry matter slurry per day for 1 kg wet weight earthworms, and for vermifilter temperatures varying between 10 and 20℃. This is higher than values given in the literature (Fayolle et al., 1997; Ndegwa et al., 2000; Clarke et al., 2007). During the second period, the removal of fine particles (either excess of fresh organic particles, or earthworm castings) was too low for this high input and the environment became anoxic after some weeks.

In short term, the ideal dose of slurry input depends on the ingestion capacity of the earthworms. In the longer term, it depends on the removal of the excreted organic matter, which should not fill the pores and let the vermifilter become anoxic. If the water flow percolating through the macroporosity can remove the fine particles, it is not necessary to remove the vermifilter material. If it can not remove these particles, a periodic removal of vermifilter material becomes necessary. It can be assumed that, when the free air space decreases due to accumulation of fine particles, the organic load can be maintained but the hydraulic load should be decreased in order to avoid a too high water content.

7.3. Heat transfer

The temperature decreased just after spreading because the added liquid was colder than the mesocosm,. The decrease was higher in the treatments with a high dose. Water is the main factor to low down the temperature. Temperature of mesocosms can decrease because of water evaporation (latent heat) and because of the water output where the temperature is higher than the water input (convective and sensible heat). This case was same as the phenomena of water application in India compost production (Tamrakar and Maharjan, 2006).

On the contrary, the temperature increased during the periods without spreading (gas measurement and sampling period). The temperature increase was higher in the treatments with a high dose. It can be related to a higher dose of fresh organic matter that will be degraded by the aerobic microbes. Processes of aerobic metabolism release heat. According to Higgins and Walker (2001), the products of the metabolic activities are water, carbon dioxide, ammonia and heat. The treatment with earthworms had higher temperature but lower carbon dioxide emissions than treatments without earthworms. Nagavallemma et al. (2004) found a different result. Their treatment with earthworms had lower temperatures than the treatment without earthworms. However, their treatments did not use the input of organic matter diluted in liquid.

The presence of water plays an important role in the temperature of mesocosm. In the present experiment, the liquid in treatments without earthworms did not pass fluently mesocosm, in the case of high doses. The increase in liquid content of the mesocosms without earthworms was higher than mesocosms with earthworms. This additional liquid probably helped to reduce oxygen diffusion and heat production and then to decrease temperature (Tamrakar and Maharjan, 2006).

7.4. Effect of earthworms on mixing the input matter and on liquid circulation

A clear effect of earthworm activity is to ingest the fresh organic matter added on the surface, mix it with existing organic matter and maintain the permeability of the surface. Ingestion and burrowing are shown by the absence of clogging, only observed in dose 28 L/day of treatment with earthworms. On the contrary, it was not possible to add all of 28 L/day pig slurry without making artificial holes in treatments without earthworms. Another effect of mixing in the gut of earthworms is to associate the nitrogen-rich liquid and particles that arrive at the surface to the carbon-rich substrate of the vermifilter, then, to reduce the mobility of nitrogen, because digestion increases the stability of organic matter. Therefore, a reduction in ammonia and nitrous oxide emission in treatments with earthworms is observed, compared to treatments without. Then, a visible abundance of earthworm population near the surface can be considered as an indicator of effective mixing of manure input and avoiding the manure excess that leads to N_2O, NH_3. CH_4 and CO_2 emissions.

The effect of earthworms on liquid circulation and global free air space is shown by the evolution of vermifilter wet weight and dry matter. The increase in wet weight is lower when there are earthworms, and qualitative observations during the spreading showed that the circulation of the liquid through the vermifilters was more rapid with earthworms. The rise of dry matter is lower in treatments with earthworms due to higher dry matter content of the liquid output. As the volume variations of the vermifilters were generally not significant ($P<0.05$) while clear increases in wet weights were observed, the increase in wet weight and dry weight in treatment without earthworms and high input of fresh slurry (28 SV) can be interpreted by a decrease in free air space. Two processes can explain this evolution. Close to the surface, the particulate fraction of the manure input filled the pores, leading to clogging. Within the vermifilter, the particulate fraction filled the free air space and accumulated water between the fine particles. In treatments with earthworms, the manure particles were removed from the surface by the earthworms, and the higher liquid circulation within the vermifilter made easier the removal of the casting particles excreted within the porosity. As this effect is an indirect consequence of earthworm activity on their physical environment, that has consequences on other biological processes (Figure 54), earthworms can be considered as “ecosystem engineers” (Lavelle et al., 1997) within the vermifilter.

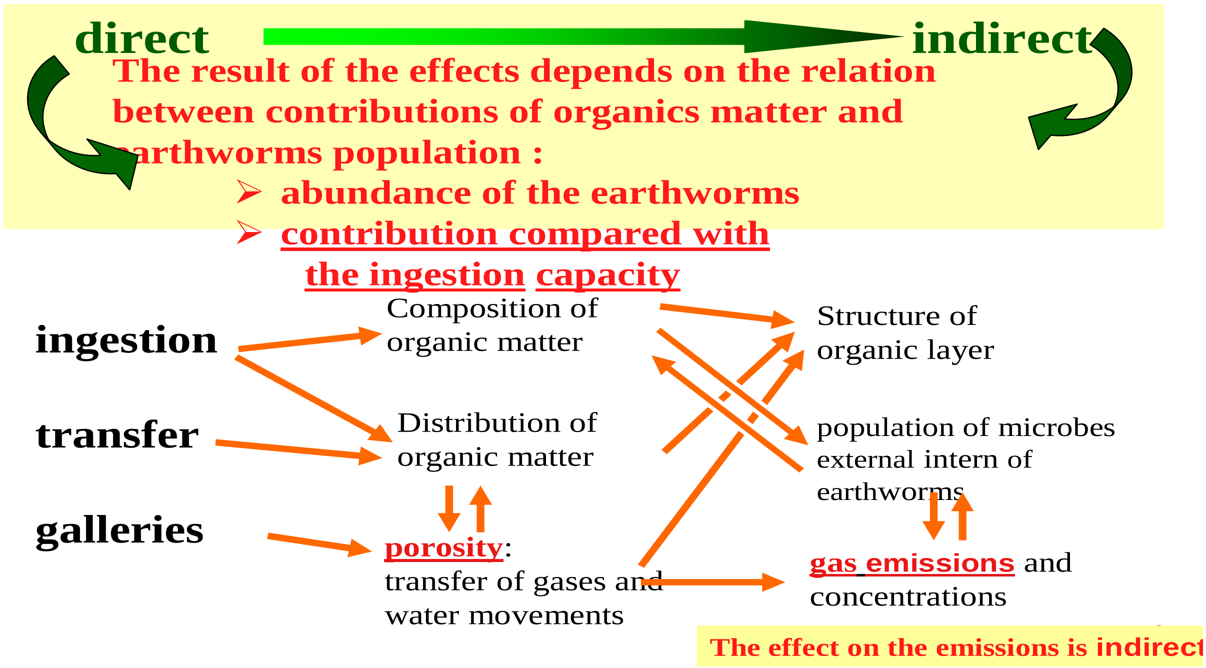


Figure 54. Direct and indirect effect of earthworm to gaseous emissions.

7.5. Effect of earthworms on maintaining a connected free air space inside the porous media and on the resulting gas emissions

In treatments with earthworms the higher free air space can be either connected, allowing a higher gas diffusion and higher oxygenation through the vermifilter, or disconnected, leading to anaerobic zones within the vermifilter. Anaerobic zones result from low oxygen diffusion through the liquid phase compared to the high oxygen requirement of the microbes transforming the fresh manure. In the first case, the liquid circulates in macropores through the vermifilter; in the second case, the liquid input pushes out the liquid remaining in the vermifilter (the so-called “piston-flow”). The major process is discussed on the basis of gas emissions and vermifilter temperatures.

Temperatures of treatments with earthworms were higher than temperatures of treatments without. Therefore, higher heat production inside the vermifilter can be assumed. It can be explained by higher oxygen diffusion and consumption within vermifilters with high earthworm abundance because aerobic metabolism is more exothermic than anaerobic metabolism. Surprisingly, the CO_2 emission results did not confirm temperature differences. The CO_2 emission increased with the dose of manure input, and with the accumulation of manure particles near the surface in treatments without earthworms, i.e. with the quantity of bioavailable carbon at the surface. As a matter of fact, the CO_2 emission can be explained by either respiration in aerobic conditions when the vermifilter is sufficiently oxygenated or by fermentation when the organic matter fills the pores and the vermifilter becomes anoxic. When the dose is high (28 L/day) the anoxic conditions close to the surface, where the liquid manure is added, can explain higher CO_2 emission through increased fermentation.

The differences in CH_4 emission lead us to assume that air diffusion is higher in treatments with earthworms. The negative emissions (sink of CH_4) can be explained by the air diffusion through the vermifilter and methane oxidation by the microbes as it is commonly observed in agricultural soils and earthworm casts. The differences are negligible with the small doses (2 and 6 L/day): the free air space is high and the water circulation rapid with or without the presence of earthworms. The differences are high with the high dose (28 L/day): the earthworms maintain a connected free air space that allows air diffusion through the vermifilter and methane oxidation that increases with increased nitrogen inputs. However, without earthworms the vermifilter becomes anoxic and methane is emitted.

Therefore, the hypothesis that the effect of earthworms on gaseous emissions is mostly indirect is confirmed. The effect results from their impact on the free air space and on mixing of the added fresh organic matter.

As CH_4 emission contributes to the global warming impact of slurry based systems, a visible abundance of earthworm population near the surface can be considered as an indicator of maintaining a connected free air space within the vermifilter and a clear reduction of CH_4 emissions compared to liquid manure systems.

7.6. Resulting effect of earthworms on gaseous emissions

7.6.1. Confirmation of methane sink by earthworm casts

The most important feature of methane emission was the clear sink of methane observed in treatments with earthworms. The sink observed after the second period varied between -3.3 and -9.7 mg $C\text{-}CH_4$ day^{-1} $mesocosm^{-1}$. The methane sink was significantly different from 0 in treatment 28 AV after the first and second period ($P<0.01$). We assume that it is due to the stimulation of the methanotrophic community (Héry et al., 2008). The methane emission was significantly different from 0 in treatment 28 SV after the second period (64±31 mg $C\text{-}CH_4$ day^{-1} $mesocosm^{-1}$; $P<0.05$). This indicated anoxic conditions. As already observed in slurry experiments, methane emission starts rapidly when fresh organic matter is accumulated. Therefore, the abundance of earthworms is a simple indicator that can be used to certify that the liquid remains aerobic in “fresh slurry” systems. A small methane sink was also observed with the low doses in treatments without earthworms, when no clogging was observed, but with undetectable emissions in some replicates (null values). This sink can be explained by the casts deposited in the substrate prior to the experiment (Moon et al., 2010). The highest variability was observed the first day, with values ranging from a small sink (-5.5 mg $C\text{-}CH_4$ day^{-1} $mesocosm^{-1}$) to a small emission (4.4 mg $C\text{-}CH_4$ day^{-1} $mesocosm^{-1}$).

Methanotrophic bacteria are capable of converting methane to carbon dioxide and therefore serve as an important methane sink (Wilshusen et al. 2004). They use the methane as the source of energy in the process of methane oxidation:

$$CH4 + 2O2 \rightarrow CO2 + 2H2O$$

Compost is a growth medium for methanotrophic bacteria (Mancebo et al. 2010). According to Moon et al. (2010), earthworm casts have a methanotrophic contribution to methane removal. In the present experiment, all treatments with earthworms showed a sink phenomena. It confirms the results of Hery et al. (2008) who observed that earthworms could stimulate the growth and activity of methanotrophic bacteria, therefore increasing methane oxidation. Our result indicate that the presence of earthworms is important for methane sink phenomena in vermifiltration.

7.6.2. Negligible ammonia emission

Ammonia emissions were observed only for the treatments with high slurry input. Even for this treatment they were low (less than 10 mg $N\text{-}NH_3$ day^{-1} $mesocosm^{-1}$) compared to the nitrous oxide emission. Low ammonia emissions are generally observed when temperature is low and when the slurry is diluted.

Ammonia emission releases during composting period. It will increase with temperature and air humidity (Burton and Turner 2003). According to (Beck-Friis et al. 2001), the effect of compost temperature on ammonia emission is not clearly understood. In our observations, higher temperature did not increase the NH_3 emission (Figure 45). Treatments with earthworms had higher temperature than treatments without earthworms but they showed lower ammonia emission.

Microbial activities influence ammonia emission (Figure 55). Beck-Friis et al. (2001) observed that the microbial activities decrease ammonia emission. Earthworms and vermicompost have a positive effect on microbial activity (Kale et al., 1992; Atiyeh et al., 2001). Treatments with earthworms could decrease ammonia emission.

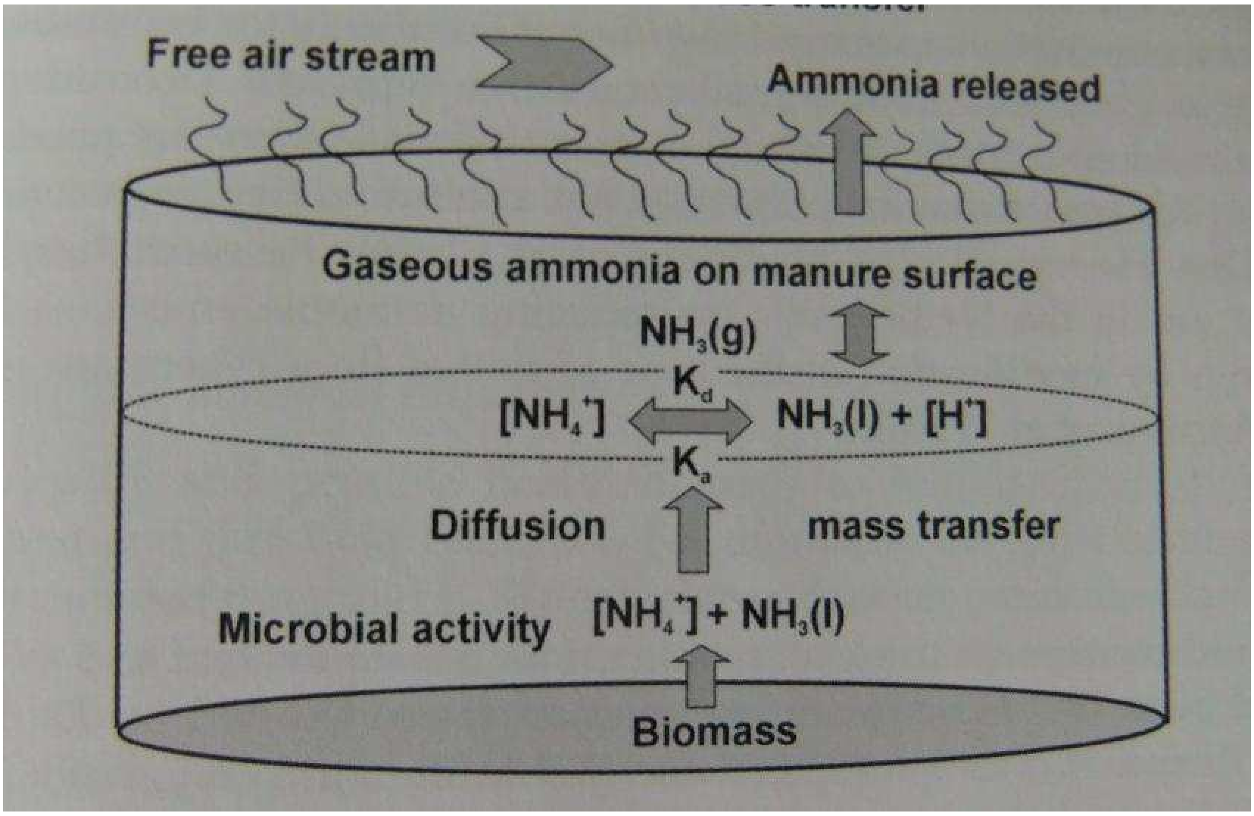


Figure 55. Mechanisms of ammonia emission in composting (Burton and Turner 2003)

7.6.3. Carbon dioxide emission

Carbon dioxide emission varied between 2.8 and 20.3 g C-CO_2 day^{-1} $mesocosm^{-1}$. The difference in carbon dioxide emission between treatments with and without earthworms on the first day of measurement was low but similar to emissions previously observed with earthworms (Binet et al., 1998). The initial emission was not correlated with the dry weight of the mesocosms but the increase in emission followed the increase in dry weight. Therefore, it can be concluded that CO_2 emission depends on the input of fresh organic matter in this kind of system.

The variability between replicates was high, so the differences between dates or between treatments were hardly significant (P>0.01). However, results show that for the low doses after the first and second period, the emissions of treatments with earthworms were a little higher than for treatments without (5.1±0.6 and 4.5±1.1 g C-CO_2 day^{-1} $mesocosm^{-1}$ for treatments 2 to 6 AV and 2 to 6 SV respectively). On the contrary, for the high dose the emissions of treatments with earthworms were lower than for treatments without (9.4±2.1 and 14.3±8.6 g C-CO_2 day^{-1} $mesocosm^{-1}$ for treatments 28 AV and 28 SV respectively, including the first and second periods).

Therefore, it is suggested that in the case of vermifiltration, the hypothesis that the emission of CO_2 will be increased by the earthworms is false, despite the probable increase of aerobic metabolism showed by higher temperatures. It should be mentioned that the CO_2 emission can be different from the CO_2 production by the fauna and microbial activities. As a matter of fact, the differences in inorganic carbon between input and output liquids previously observed by Li et al. (2008) show that a part of the CO_2 flux can be due to the transfer of liquids.

During pig slurry application periods, the vermicompost temperature decreased. On the contrary, vermicompost temperature increased when there were no slurry applications. Although respiration process released CO_2 and heat, water of pig slurry application shortly decreased temperature after spreading.

The treatments with earthworm had higher temperature (Figure 44) than without earthworms ones during the period of non-spreading and it had higher CO_2 emission (Figure 48). It indicated that there were respiration processes by the fauna vermicompost although the vermifilters input was stopped.

7.6.4. Nitrous oxide emission

As usually noted in the literature, nitrous oxide emissions were highly variable, mean values ranging from 2 to 438 mg N-N_2O day^{-1} $mesocosm^{-1}$, even between the replicates of the same treatment. Differences between dates were not significant for the low doses with earthworms (2 AV, 6 AV: 12±4 mg N-N_2O day^{-1} $mesocosm^{-1}$).

An increase during the experiment could be observed in all treatments. It was significant for the high dose and in treatments without earthworms (2 SV, 6 SV, $P<0.1$; e.g. from 35±3 mg N-N_2O day^{-1} $mesocosm^{-1}$ after the first period in treatment 6 SV to 44±4 mg N-N_2O day^{-1} $mesocosm^{-1}$ after the second period).

With the lowest dose, a higher emission in treatment with earthworms could be observed. It corresponds to a substrate poor in nitrogen. It can be related to previous observations with vermicomposting (Frederickson and Howell, 2003).

A clear decrease in nitrous oxide emission in treatments with earthworms was observed for doses 6 L/day and 28 L/day. For the medium dose, the difference was significant ($P<0.01$). For the high dose (28 L/day), the effect of earthworms at a given date was not significant because of variability ($P>0.1$) but for the three replicates compared at each date, all observations with earthworms but one had lower emissions than mesocosms without earthworms (respectively 123±68 mg N-N_2O day^{-1} $mesocosm^{-1}$ and 274±215 mg N-N_2O day^{-1} $mesocosm^{-1}$ for 28 AV and 28 SV). These observations of the effect of earthworms confirm the previous results of Contreras-Ramos et al. (2009).

Therefore, the hypothesis that the earthworms should induce higher N_2O emissions was true with the low dose and false for the normal or high doses. The decrease induced by the earthworms show that three processes should play a major role: (i) the dilution of the added nitrogen inside the vermifilter when there are earthworms, while the nitrogen remains close to the surface in treatments without earthworms; (ii) the reduction in nitrous oxide emissions through the effect of epigeic earthworms on the structure of the upper organic layer (Ellenberg et al. cited by Borken et al., 2000); (iii) the increase in organic matter stability after digestion of the fresh input by the earthworms.

In both cases, either low or high nitrogen input, we therefore assume that the effect of earthworms on nitrous oxide emissions was due to their feeding strategy and its effect on an increased nitrogen turnover within the vermifilter ((Bohlen and Edwards 1995); Neilson et al., 2000; Sampredo and Dominguez, 2008).

7.6.5. A new hypothesis to explain the effect of earthworms on either increase or decrease of nitrous oxide emission

In the case of organic matter processing with earthworms, there is a contradiction in the literature between authors who show an increase in nitrous oxide emissions due to earthworms (e.g. Frederickson and Howell, 2003; Hobson et al., 2005) and authors who show a decrease induced by earthworms (e.g. Contreras-Ramos et al., 2009). The question of extrapolating these results to all cases of vermi-technologies has been discussed by Edwards & Arancon (2008). Our result can help to further discuss this contradiction and propose a new hypothesis to resolve it.

The observations of the present experiment reproduced both cases (increase or decrease) on the basis of the same initial material. It shows that the contradiction of the literature is not a fundamental contradiction, based on the incompatibility of both situations. With the lowest dose, our observations support the results of the first ones (increase), while with the medium and high doses, they support the observations of the last ones (decrease): a clear decrease in nitrous oxide emission in treatments with earthworms was observed for doses 6 L/day and 28 L/day. For the medium dose, the difference was significant ($P<0.01$). For the high dose (28 L/day), the effect of earthworms at a given date was not significant because of variability ($P>0.1$) but for the three replicates compared at each date, all observations with earthworms but one had lower emissions than mesocosms without earthworms (respectively 123±68 mg N-N_2O day^{-1} $mesocosm^{-1}$ and 274±215 mg N-N_2O day^{-1} $mesocosm^{-1}$ for 28 AV and 28 SV; i.e. 34±19 mg N_2O m^{-2} h^{-1} and 76±59 mg N_2O m^{-2} h^{-1}; or 0.4 to 0.9 % of daily nitrogen input).

The increase in nitrous oxide emission by earthworms in environments with low inputs of fresh organic nitrogen can be explained by a gut content richer in available nitrogen and carbon compared to outside substrate (Drake & Horn, 2007). This richer environment induces a microbial activity of denitrification higher inside the gut than outside. Drake & Horn (2007) did not clarify the origin of the nitrogen and the decrease of C/N ratio in nitrogen-poor soils, i.e. the mass balance of nitrogen that explains earthworm growth. Enhanced biological nitrogen fixation, as shown by Striganova et al. (1993) and Umarov et al. (2008), is probable because of the anoxic and carbon rich conditions that prevail in the earthworm gut. In our case, an accurate mass balance of nitrogen or the analysis of isotopic ratio in different organic fractions could show if this hypothesis is true or not.

The low dose treatment applied 2 L.day^{-1} pig slurry. This dose was equivalent to a pig slurry input that has 38 g DM/m^2/day dry matter and 3 g N/m^2/day total Nitrogen Kjeldahl (Table III.). We suggest that if the quantity of pig slurry that is applied to vermifilter is less than this dose, vermifilter will release nitrous oxide and emission level will increase.

The decrease in nitrous oxide emission by earthworms in environments with high inputs of fresh organic matter can be explained by a gut content poorer in available nitrogen compared to the fresh organic matter input. The decrease induced by the earthworms can be explained by three processes: (i) the dilution of the added nitrogen inside the vermifilter when there are earthworms, while the nitrogen remains close to the surface in treatments without earthworms; (ii) the reduction in nitrous oxide emissions through the effect of epigeic earthworms on the structure of the upper organic layer (Ellenberg et al. cited by Borken et al., 2000); (iii) the increase in organic matter stability after digestion of the fresh input by the earthworms. Therefore, the denitrification activity inside the gut will be lower than the denitrification activity within the layer of fresh organic matter that remains at the surface, in treatments without earthworms and with high dose. The feeding activity of the earthworms induces the removal of the fresh organic matter input rich in nitrogen, in steady-state systems using vermi-technologies.

In the case of optimal (6 $L.day^{-1}$) and high dose (28 $L.day^{-1}$), these doses showed that earthworms can decrease nitrous oxide emission. The application of 6 $L.day^{-1}$, similar to the input of the large vermifilter, gave 114 g $DM/m^2/day$ dry matter and 8 g $N/m^2/day$ total Nitrogen Kjeldahl (Table III.) in the mesocosms. We suggest that the medium dose (8 g $N/m^2/day$) can be near the threshold between increase and decrease of nitrous oxide emission. If pig slurry application is more than it, earthworms can reduce nitrous oxide emission.

As a matter of fact, the results presented in the literature are based on emissions expressed relatively to a state-variable such as the area, or the mass of substrate, or the earthworm population (Drake & Horn, 2007; Frederickson and Howell, 2003; Contreras-Ramos et al., 2009). Our observations were higher than these previously observed emissions. However, these experiments did not use high nitrogen inputs per replicate. When expressed relatively to a flux-variable such as the nitrogen input, the emissions observed in the work described here were low, 0.4 – 0.9 % of nitrogen input. Expressing emissions relatively to state-variables is common in natural studies because the food chain is based on the resources naturally present in the environment. However, if the environment is modified to ensure high agricultural production per unit area and time, emissions should be studied on both basis: state-variable and flux variable.

In both cases, either low or high nitrogen input, we assume that the effect of earthworms on nitrous oxide emissions was due to their feeding strategy and its effect on an increased nitrogen turnover within the vermifilter (Bohlen and Edwards, 1995; Neilson et al., 2000; Sampredo and Dominguez, 2008). We therefore propose the new hypothesis that there is a threshold of the input of available organic nitrogen that will determine whether earthworms will increase or decrease the nitrous oxide emission, as compared to the same environment without earthworms. This threshold depends on the food chain that transforms the fresh organic input into stable organic matter (abundance of different functional groups, fluxes of organic matter of different stabilities within the substrate) and its evolution with varying seasons and input level of organic nitrogen. In a nitrogen-poor environment, without organic nitrogen inputs, earthworm gut stimulates biological nitrogen fixation, from which enhanced nitrous oxide emission will be observed. In a nitrogen-rich environment, with inputs of animal manure that can be considered as a steady-state on a monthly time step, a low C/N ratio (we suggest less than 10) of the organic input, and an abundant population of earthworms (we suggest above 500 earthworms kg^{-1} dry matter of substrate), the feeding strategy of the earthworms induce lower nitrous oxide emissions compared to the same environment without earthworms.

7.7. Potential impact of vermifiltration of pig fresh manure on global warming

This work confirmed previous experiments showing evidence of nitrous oxide emissions and methane sinks induced by earthworm populations, two gases implicated in the impact of animal farming on climate change (FAO, 2006a). Previous work suggested that earthworms can be an important contributor to biogenic emissions of nitrous oxide (Drake & Horn, 2007) but that vermitechnologies can not increase significantly anthropic emissions of nitrous oxide (Clive & Arancon, 2008). Nevertheless, as animal production is expected to increase (FAO, 2006b), it is important to discuss whether vermifiltration can be associated or not to the sustainable development of pig production.

The present work and additional considerations let us assume that vermifiltration will contribute to reduce the global warming potential of the most common pig production system. Usually, pigs are reared on partly or totally slatted-floor soils, producing liquid manure, inducing emissions of NH_3 and CH_4 from the liquid manure, and nitrous oxide emissions after spreading the manure. NH_3 emissions and excess of nitrogen input to the crops will induce indirect emissions of nitrous oxide (Basset-Mens et al., 2006). As the liquid manure is rich in ammoniacal nitrogen, its impact on carbon sequestration is closer to inputs of mineral fertilizer than to inputs of solid manure such as compost. If vermifiltration is used, NH_3 emission will be minimized following the frequent flushing of animal excreta. CH_4 emission will be replaced by CH_4 sink. Energy use should decrease compared to transport of liquid manure or to its treatment in nitrification-denitrification plants. N_2O emission from agricultural soils is expected to decrease because stable organic matter is applied, that should less disturb the carbon and nitrogen cycles in the soil. Carbon sequestration should increase because earthworm casts are rich in stable soluble and particulate organic compounds, that can migrate to deep layers of the soil and be associated into organo-mineral micro-aggregates.

Therefore we suggest that a visible abundance of earthworms near the surface can be used as a bioindicator of low energy input, and low greenhouse gas and ammonia output in systems using fresh slurry with water recycling.

8. *Conclusions*

Earthworms have a clear effect, mostly indirect, on gaseous emissions during vermifiltration of animal pig fresh slurry. The high earthworm population was associated with a methane sink, the absence of ammonia emissions and reduced nitrous oxide emissions.

Earthworm population can increase in few weeks when feed is sufficient. Population decrease was negligible when feed was small, but it was high when the environment became anoxic because of excess of fresh organic matter.

In low dose, the organic matter input needs longer time, e.g. six months like compost maturation, to change the environment of vermifilter. Their several parameters were same as the optimal dose ones. In high dose, the input organic matter only need short time to change the vermifilter. Most of their result were different to other treatments.

9. *Knowledge application to design and management*

Optimal input of fresh organic matter depends on the substrate and the earthworm population. As it should be rapidly transformed to avoid the apparition of anoxic conditions, it also depends on the climatic conditions. Therefore, the cold periods should be used to design the minimum size as a function of the expected population and the substrate.

When starting a new vermifilter with a low population, a progressive increase in dose should allow the progressive development of earthworm population and the colonization of the substrate by the microbial population suitable for earthworms.

Different ratios of hydraulic load and organic load should be used to adapt to variable conditions observed at the surface of vermifilter. If surface permeability decreases, the hydraulic load should be reduced to avoid surface clogging. If fresh organic matter accumulates at the surface, it indicates that the earthworm population is not active enough to transform it. The organic load should be reduced until surface accumulation stops.

Earthworm abundance is easy to control at the surface of the vermifilter when climatic conditions are suitable. Therefore, it can be used as a bioindicator to certify low energy input, and low greenhouse gas and ammonia output in systems using fresh manure with water recycling. This from a practical point of view. However, if the objective is to quantify the earthworm population in the vermifilter, this bioindicator is not sufficient, an average sample and a measurement of the weight of the vermifilter are necessary.

Part 2: Bio recycling systems: optimal interactions between subsystems including vermifiltration, macrophyte lagooning, and constructed wetlands

Chapter 3: Spatial interactions: biological filtration of liquid manure and water recycling through vermifiltration and macrophyte lagooning

1. Résumé du chapitre 3 : interactions spatiales, filtration biologique d'un effluent liquide et recyclage de l'eau par lombrifiltration et lagunage à macrophytes

Les effets négatifs des élevages de porcs intensifs sont la pollution de l'eau, les émissions de gaz polluants et l'épuisement de ressources naturelles. Augmenter l'efficacité du recyclage peux réduire les deux impacts sur l'environnement que sont la raréfaction des ressources naturelles et l'émission de polluants. Le système de transformation de Guernévez emploie une combinaison de sous-systèmes biologiques pour réduire la concentration des éléments chimiques dans l'eau, puis réutiliser l'eau pour évacuer les effluents animaux, et pour produire des plantes. Ce système se compose d'une chasse d'eau installée dans la porcherie, un tamis, un lombrifiltre, des marais filtrants et des lagunes à macrophytes. L'eau est recyclée en continu dans le système.

La littérature scientifique indique que le lombrifiltre, les marais filtrants et les lagunes réduisent la concentration des éléments chimiques dans l'eau. Si le système est conçu afin d'avoir un intrant optimal dans chaque sous-système, alors la production maximum des vers de terre, du lombricompost, et de la végétation sera réalisée parce que l'eau et les nutriments ne sont pas des facteurs limitants dans les sous-systèmes. Cependant, la baisse de concentration des éléments chimiques entre l'entrée et la sortie d'eau ne devrait pas être identique pour tous les éléments (N, P, K, etc.). Le recyclage de l'eau devrait conduire à une augmentation de la concentration des éléments chimiques qui sont les moins retenus dans le système.

Les chapitres 1 et 2 de ce rapport ont étudié le sous-système constitué par le lombrifiltre. Elles ont montré que le « preferendum » du lombrifiltre peut être caractérisé par un « intrant optimal » de lisier frais de porc. La population de vers de terre et les transformations induites de l'effluent organique sont maximisées au voisinage de cet intrant optimal. Les émissions de gaz polluant ne sont pas augmentées.

Ce chapitre 3 analyse les conséquences des interactions spatiales entre les sous-systèmes. Les objectifs spécifiques de ce chapitre sont (i) d'analyser dans quelle mesure la composition du liquide est modifiée par le principe de recyclage de l'eau et des éléments inutilisés, (ii) d'analyser dans quelle mesure le recyclage modifie la composition des produits qui peuvent être exportés par les sous-systèmes.

La méthode est basée sur une surveillance à long terme des concentrations dans l'eau qui sort de chaque sous-système. Les résultats confirment que le lombrifiltre, les marais filtrants et les lagunes ont le potentiel de réduire la DCO, l'azote, le phosphore et le potassium. Les efficacités d'abatement sont pour la DCO de 96 %, pour l'azote total

de 95 %, pour le phosphore de 75 %, pour le potassium de 67 %, et pour l'NH_4^+ de 95 %. Ces niveaux d'abatement contrastés induisent un changement de la stoechiométrie avec une concentration en potassium accrue en comparaison d'un effluent d'élevage issu d'un système sans recyclage. Le bilan de matière des sous-systèmes de lagunes et de marais filtrants a été estimé. Il montre que la sédimentation et la volatilisation sont des fonctions plus efficaces pour l'abatement des éléments que la production de plantes. Pourtant nous n'avons pas observé de nets changements de concentration de la composition des plantes et des sédiments après deux ans de recyclage.

La production de plantes est habituellement variable en raison des saisons et des opérations de plantation ou de récolte. Le système associe donc des sous-systèmes à flux continu de nutriments (ex. la porcherie) et des sous-systèmes à flux variables (ex. les lagunes à macrophytes). Les seconds accumulent de la matière durant les périodes défavorables à la production des plantes. Le chapitre 4 qui suit dans ce rapport analysera la compatibilité entre compartiments à flux continu de nutriments et compartiments à flux variables. Des interactions temporelles apparaissent entre les périodes de faible croissance des plantes et d'accumulation d'éléments nutritifs dans les sous-systèmes et les périodes de la croissance élevée des plantes et d'utilisation des nutriments.

2. Abstract

Negative effects of intensive piggery system are water pollution, gas emissions and resource use. Increasing the recycling efficiency of systems can decrease both impacts on environment that are ressource depletion and polluting emissions. The Guernévez transformation system uses a combination of biological subsystems to reduce the concentration of chemical elements in the water used for flushing the animal effluents, and to produce plants. This system consists of a mechanical flushing installed in the piggery, a sieve, a vermifilter and integrated constructed wetlands and lagoons with macrophytes. The water is continuously recycled within the system.

The scientific literature indicates that the vermifilter, the constructed wetlands and the lagoons reduce the concentration of chemical elements in the water. If the system is designed in order to have an optimal input in each subsystem, then maximum production of earthworms, vermicompost, and vegetation will be achieved because water and nutrients are not limiting factors in all subsystems. However, the abatement of various chemical elements (N, P, K, etc.), i.e. the concentration reduction between input and output water, should not be the same. When recycling the water, the concentration of chemical elements that are less reduced should increase.

The chapters 1 and 2 of this report studied the vermifilter subsystem. They showed that the "preferendum" of the vermifilter can be defined using an "optimal input" of pig fresh manure. The earthworm population and the induced transformations of organic effluent are maximized close to this optimal input. The polluting gas emissions are not increased.

This chapter 3 analyses the consequences of spatial interactions between the subsystems. The specific objectives of this chapter are (i) to analyze to what extent the composition of the liquid is modified by the principle of recycling the water and the unused elements, (ii) to analyze to what extent the recycling modifies the composition of the products that can be exported by the subsystems.

The method is based on a long term monitoring of the concentrations in the water that flows out each subsystem. The results confirm that the vermifilter, the constructed wetlands and the lagoons have potential to COD, nitrogen, phosphorus and potassium removal. Removal efficiencies were COD 96 %, total nitrogen 95 %, phosphorus 75 %, potassium 67 %, and NH_4 95 %. These different abatement levels induced a change in the stoechiometry with increased concentration of potassium compared to an animal wastewater without recycling. An estimate mass balance of the lagoon and wetland subsystems showed that sedimentation and volatilization are more effective functions for chemical element removal than plant uptake. However, we did not observe clear differences in the composition of plants or sediments after two years of recycling.

Plant production is usually variable because of seasons, and because of planting or harvesting operations. The system associates subsystems with steady-state fluxes and transformations (e.g. the piggery) and subsystems with variable fluxes (e.g. macrophyte lagoons). In the second category nutrients accumulate during the periods unfavourable to plant growth. The following chapter 4 of this report will analyze to what extent subsystems with a continuous input of nutrients are compatible with subsystems with variable transformation processes. Temporal interactions appear between periods of small plant growth and nutrient accumulation in the subsystems and periods of high plant growth and nutrient use.

Keywords:

Constructed wetland, Nitrogen, Water quality, Recycling

3. Introduction

Animal production is important for human life. It provides the protein and lipid needed. On another side, animal production has negative environmental impact. The feed production can reduce natural ressources such as soils, water, phosphate fertilizers. The effluents from animal production can cause the environment pollution. For example, piggery intensive system has a lot of liquid effluents that will make air and water pollution. Treatment systems are needed to reduce these negative effects. Expensive treatment systems are not compatible with agricultural activities. Minimizing transport and producing useful byproducts can improve the economic feasibility of treatment.

Piggery Experimental Farm of Guernévez, Saint Goazec, France designed integrated treatment system that can reduce the negative effects of animal effluents (Morand et al., 2011). The system uses a combination of physical, chemical and biological treatments. Park (2009) found that the combination of different systems can reduce the pollution effect of wastewater. In the case of recycling algae harvested from green tides, Charlier et al. (2007) shows that the combination of a physical treatment (pressing) and biological treatments (hydrolysis, biogas production, composting) is more efficient than a single treatment.

Guernévez recycling system for piggery wastewater is started by the effluent machine evacuation which is below animal floor. Treatment is continued by sieve. The animal wastewater will cross the vermifilter where it is treated by biochemical treatment.

The animal wastewater will flow through four lagoons. The last part of the system is storage lagoon. The water in storage lagoon will be reused to evacuate the effluents that are below animal building. Each day the wastewater will pass several times on the same parts and same processes.

The vermifilter subsystem consist of wood chips and earthworms. The animal effluents liquid will pass vermifilter and its chemical element will be filtered by vermicompost. Earthworms and microorganism will help to transform the organic matter and to reduce the concentration of chemical elements. As shown by Subler et al. (1998) and Ghosh et al. (1999) vermicompost presents some advantages compared to compost in terms of nutrient content, nutrient availability and growth factors. The study of Li et al. (2008) showed that the design should be based on nitrogen availability and not on carbon availability in the case of animal effluents.

Constructed wetlands subsystems use gravels and water as media. Several plants are planted in the constructed wetlands. The plants can absorb diluted nutrients and be used as animal feed or biomass. When used in controlled environments where they can be harvested, plants can help to avoid the environmental problems associated to eutrophication of rivers, lakes, and coastal waters. They will also induce other processes that can reduce the concentrations of chemical elements. Sedimentation and aerobic or anaerobic microbial transformations are processes that influence the decrease in chemical elements in constructed wetlands. Studies based on slurry added to small size combinations of lagoons with free floating plants (FFP) and constructed wetland with horizontal subsurface flow (HSF) gave efficiencies used to design the constructed wetlands according to nitrogen abatement (Morand et al., 2011).

Most literature on biological treatment systems concerns open systems, often designed according to COD abatement. Li et al. (2008) observed that in the case of animal wastewater, the design of vermifiltration should not be based on COD. In chapter 1 and 2, we confirmed that there is an optimal input of a complex liquid, that will maximize the population of earthworms, even in the case of a pilot based on water recycling. Matos et al. (2010) showed for constructed wetland that the abatement of nitrogen (N) is higher than the abatement of potassium (K). If this result applies to our case, the concentration of potassium in water will increase more than the concentration of nitrogen after recycling within the system. If the concentration of K becomes higher and if the knowledge of abatement related to open systems can be used, the same abatement will induce a higher mass removal. Therefore, it is theoretically possible that a steady-state level is reached, where the concentration of some elements in the water is higher than in open systems, and where the mass removal of all elements corresponds to the mass input of the system, due to the animal effluents. However, either the values of abatement, the crop yields or the composition of the crops can be different in the recycling system and in an open system because the stoechiometry of the different nutrients will change and it will induce differences in biological processes.

Therefore, the specific objectives of this chapter are (i) to analyze to what extent the composition of the liquid is modified by the principle of recycling the water and the elements that were not stored or volatilized by the treatment subsystems, (ii) to analyze to what extent the recycling modifies the composition of the products that can be exported by the subsystems.

4. Hypothesis

The removal of nitrogen (N), phosphorus (P) and potassium (K) in the system should be different because nitrogen can be lost by volatilization, phosphorus can be stored in the solid organic matter accumulated in different parts of the system, and potassium should accumulate in the water. As the system design was based on nitrogen, high concentrations of potassium should be observed and toxic levels can be reached that will require the removal of the liquid. The weight of chemical elements which is in sediment and plants can help to know the conversion process of chemical elements in constructed wetlands. These processes will help to know the major processes of decrease in chemical element.

If concentrations of potassium are quite different in the liquid of a closed system compared to an open system, the plants or the organic matter produced by the closed system should be richer in potassium and other nutrients that would accumulate in the water. It is possible that this higher concentration in plants will limit the necessity to remove the liquid of the closed system.

5. Material and methods

5.1. Experimental design

The experiment was conducted at Piggery Experimental Farm of Guernévez, Saint Goazec, France from 6 August 2008 to 11 December 2008.

The complete experimental design has been described in introduction. The pilot system has been designed to recycle all the piggery wastewater. The wastewater that exits from piggery building will pass several phases to reduce its chemical elements until the final constructed wetland. The water that is in this final constructed wetland (storage lagoon) will be pumped to the piggery building and be used for flushing the pig effluents (Figure 6 and Figure 12).

The water samples were taken every 15 days for all parts of experiments and were stored in plastic bottles. There were 9 positions of water sampling. The samples were analyzed in small laboratory also located in Guernévez.

The water samples positions were in four the tank; after piggery, sieve, vermifilter and preparation tank for lagoon. Five samples positions are after first to fifth lagoon (Figure 56).

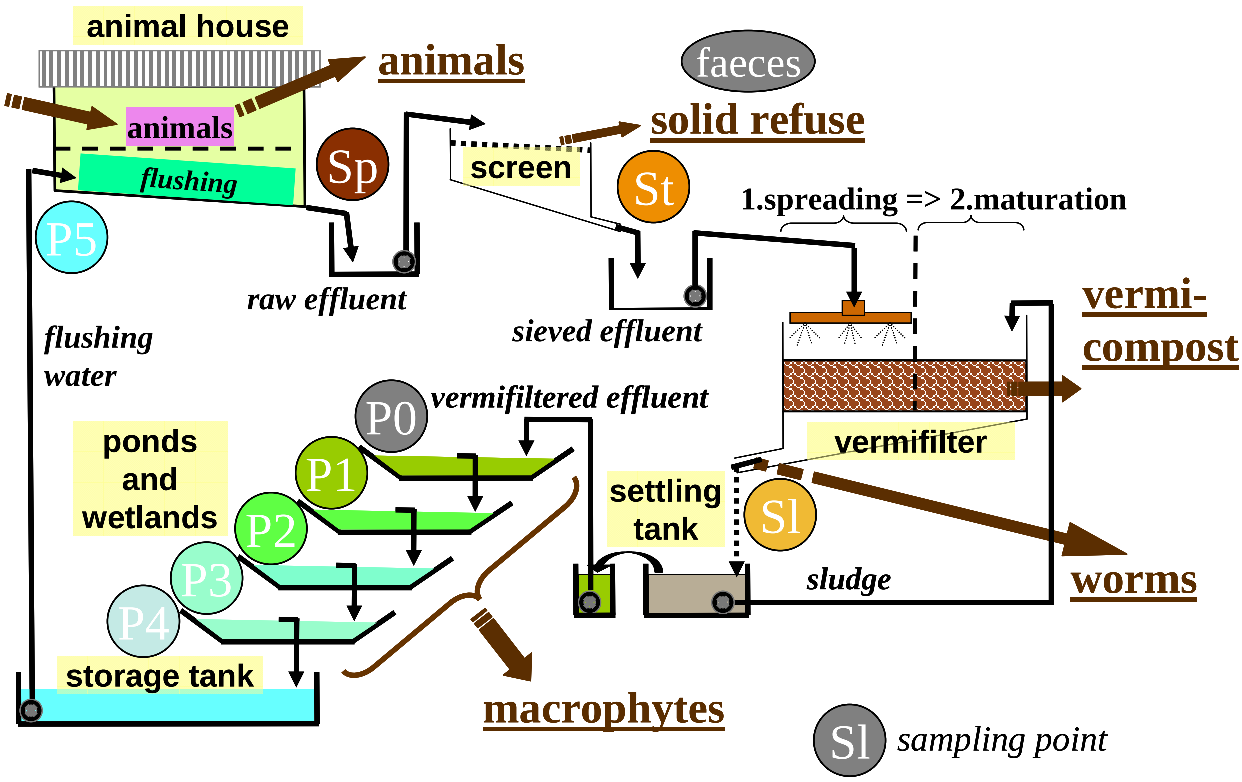


Figure 56. Position of the sampling points (subsystems without plants are from Sp to P0; vegetated subsystems are from P1 to P5)

5.2. Measurements

Water samples were analyzed for COD, total nitrogen, phosphorus, K, NH_4^+, NO_3^-, NO_2^-, dry matter, suspension matter, temperature and pH. This experiment used Hach Lange kits and a colorimeter (Hach Lange, Lasa 100) to determine COD, total nitrogen, phosphorus, K, NH_4^+, NO_3^-, NO_2^-. More details on the chemical analysis (reagents, dilutions, etc.) are given in Tureau (2010). The rest of water samples were stored in plastic bottles and frozen for further controls.

Some samples of sludge were taken as solid samples. The places where the sediment samples were taken were: (i) settling tank after vermifiltration, (ii) first and (iii) third lagoon. These samples were analyzed by a laboratory using standard methods (Agrilabo, Morlaix) for several parameters such as organic matter, total nitrogen, organic nitrogen, phosphorus, potassium, organic carbon, Cu and Zn.

The plants were harvested in several times. After harvesting, the plants were weighed, in the case of floating plants, they were drained before weighing. Some harvests of plant were sampled and analyzed for nitrogen, phosphorus and potassium using standard methods (Agrilabo, Morlaix).

5.3. Mass balance estimate

The mass balance was estimated for a period of 100 days, using the observed harvest of plants, averages of observed concentrations in plants and water.

The transfer between subsystems was calculated assuming that the flow of water was always equal to 800 L/flushing and 6 flushing/day (4800 L/day).

The terms of sedimentation and volatilization were deduced from the difference between input and output water and the plant uptake, because the mass of sludge produced during a chosen period was not measured.

5.4. Data processing

The data were calculated by Excel program. The efficiency (different concentration level between inlet and outlet) in every part of the pilot system was used to analyze the evolution of chemical elements.

The quantity of harvested plants and the result of chemical element analysis were used for the calculation of the quantity of chemical elements that are in plants and lagoon sediment. These two types of solids can explain the decrease and conversion of chemical elements like P or K in the system. In the case of N, volatilization can also explain the concentration decrease.

6. Results

6.1. Concentration of nutrients in the water

6.1.1. COD evolution

COD concentration decreased from piggery outlet (Sp) to storage lagoon one (P5). The decrease in COD was very high from piggery outlet to vermifilter (Sl). The decrease value was 2552 mg.L^{-1} COD and its efficiency was 57.7 %.

The decrease in COD continued from first lagoon inlet (P0) to the outlet of the storage lagoon (P5). The decrease value in this part was lower but the efficiency higher. It was 578 mg.L^{-1} COD and its efficiency was 72.3 %.

Therefore, vermifilter and similar organic treatments are more adapted to achieve a significant mass decrease of concentrated effluents on a limited area, whereas constructed wetlands need more area but can achieve a high retention on diluted effluents.

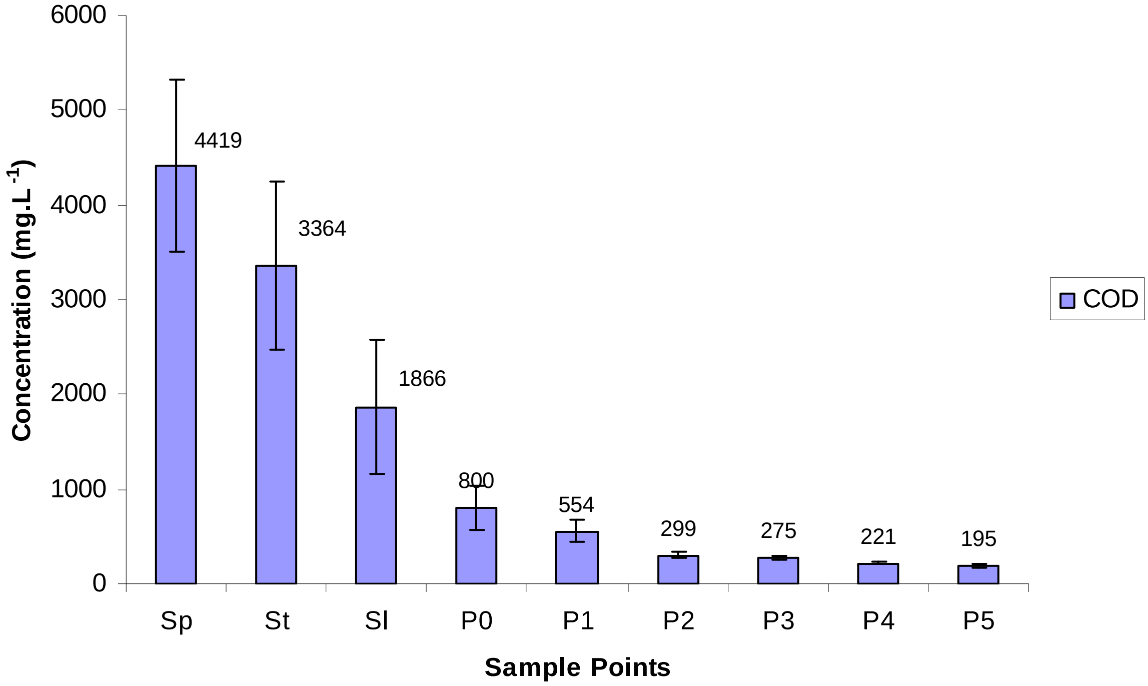


Figure 57. Concentration of COD in levels from Piggery (Sp) to storage basin (P5)

6.1.2. Total N, total P and total K evolution

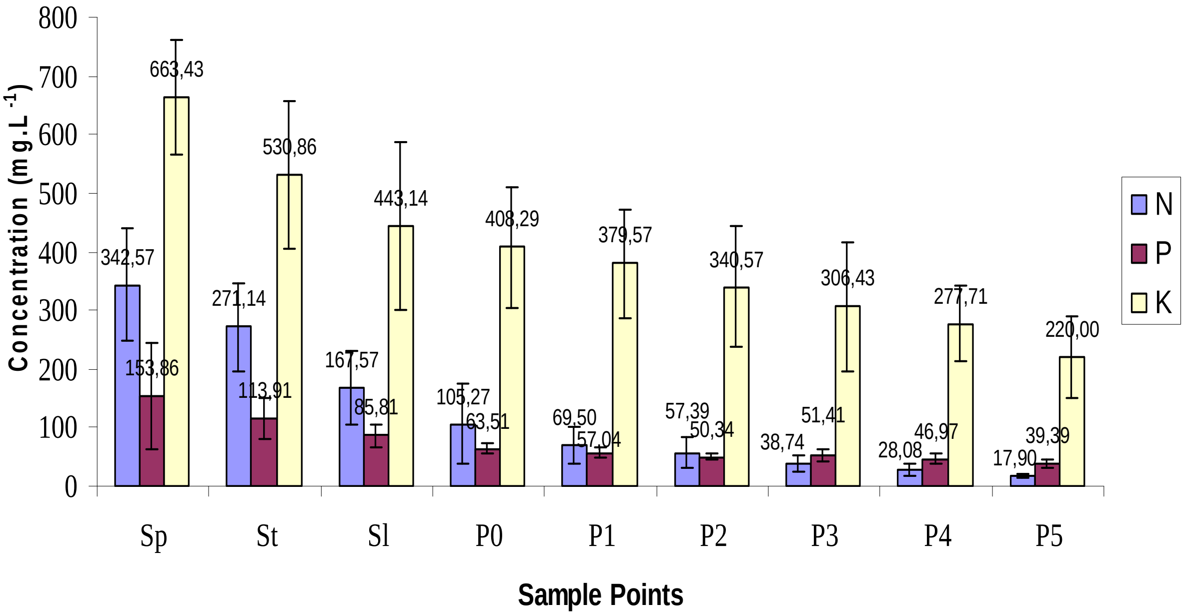


Figure 58. Concentration of total N, total P and total K from Piggery (Sp) to storage basin (P5)

N, P, and K concentrations decreased from piggery outlet (Sp) to storage lagoon one (P5). The decrease was very high from piggery outlet to vermifilter (Sl; respectively 175, 68, and 220 mg.L^{-1}). The decrease in N, P, K continued from first lagoon inlet (P0) to the outlet of the storage lagoon (P5). The decrease in this part was lower than in first part (respectively 87, 25, and 188 mg.L^{-1}). The efficiency of P or K removal was around 40% for both vermifilter and combination of constructed lagoon. Concerning N, the efficiency was much higher for the constructed lagoons than for the vermifilter (respectively around 80% and 50%). Te removal efficiencies for the pilot system, considered from the piggery to the storage lagoon, were 95%, 75%, 67% respectively for N, P, and K.

The efficiency of K removal was lower than the efficiency of N removal as previously observed in similar biological systems. It resulted in a progressive increase in K concentration during the first trimester of funtionning. However, on a yearly basis, it reached a plateau that tend to increase a little in winter and decrease in summer.

6.1.3. NH_4 evolution

NH_4 decreased from piggery outlet to storage lagoon ones. Ammonium decreased highly from piggery outlet to vermifilter ones. The decrease value was 68 mg.L^{-1} NH_4 and its efficiency was 59.8 %. Ammonium decreased lowly in the part of lagoons. From inlet first lagoon to outlet last ones, the decrease concentration of ammonium was 23 mg.L^{-1} NH_4 and its efficiency was 59.2 %.

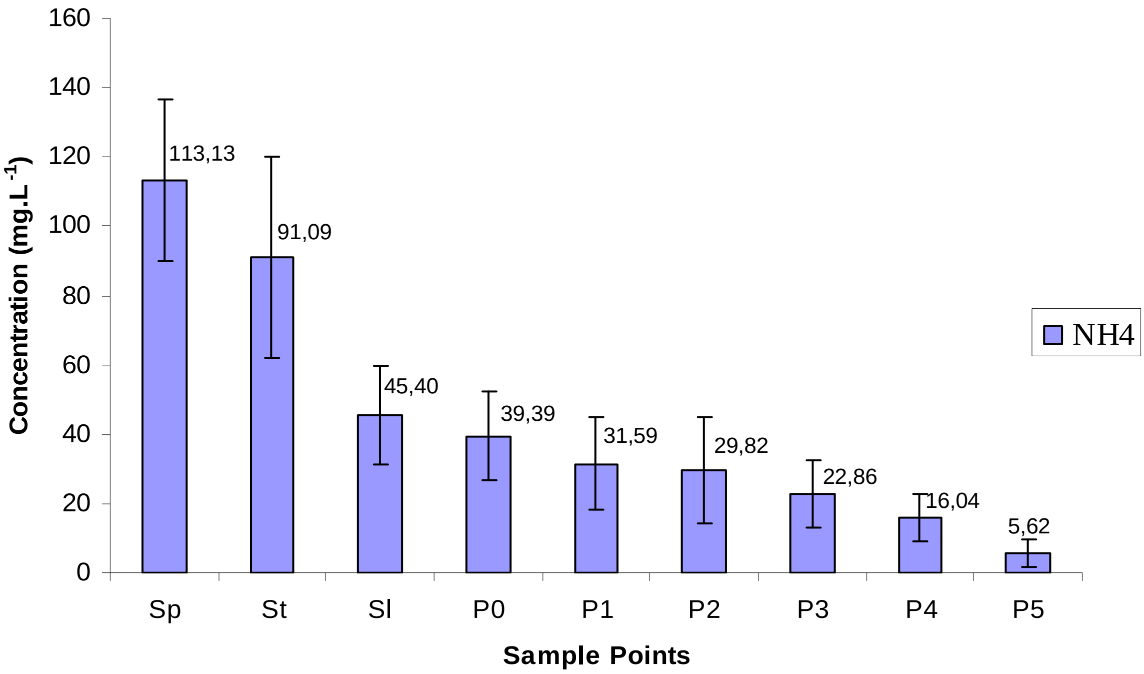


Figure 59. Concentration of NH_4 in levels from Piggery (Sp) to storage basin (P5)

6.1.4. Evolution of NO_3 and NO_2

Nitrate concentration increased from piggery outlet to vermifilter ones. Then it decreased from vermifilter outlet to settling tank ones. There was no clear trend of concentration change from inlet of first lagoon to outlet storage ones although the concentration increased in storage lagoon. The increase value from piggery outlet to vermifilter ones was 4.5 $mg.L^{-1}$ NO_3 and its efficiency was therefore negative (-140 %). The last result of lagoon system, the concentration increased 0.5 $mg.L^{-1}$ NO_3 from inlet first lagoon to outlet last ones and its efficiency was 17 %. The highest concentration nitrate was 7.74 $mg.L^{-1}$ in outlet vermifilter.

The change in nitrite concentrations was same as nitrate. The concentration increased from piggery outlet to vermifilter ones. There was not trend of change concentration between inlet first lagoon to outlet storage ones although the concentration increased a little in storage lagoon.

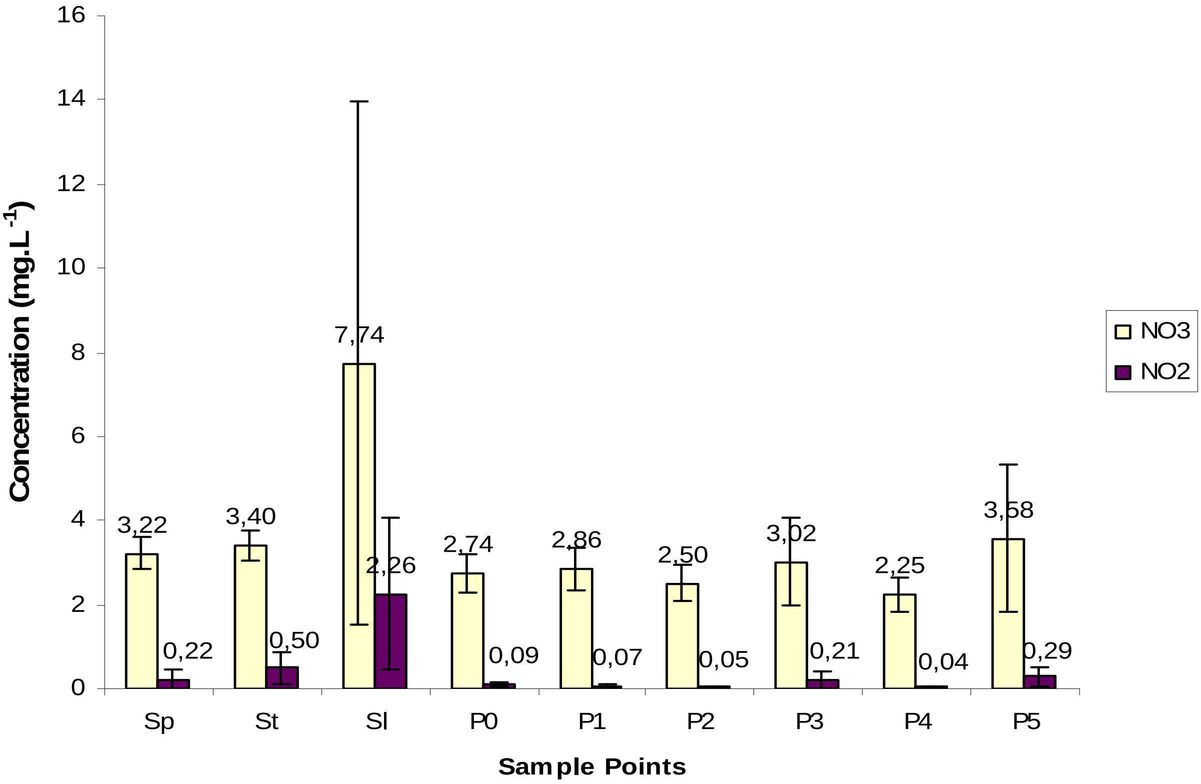


Figure 60. Concentration of NO_3 and NO_2 in levels from Piggery (Sp) to storage basin (P5)

6.2. Concentration of nutrients in sludge and plants

Concentrations of P and K in sludge increased in the pilot system based on water recycling, compared to the concentrations previously observed in the open system (Table XIX.). On the contrary, the concentrations of N remain similar, considering the dry matter difference. Therefore, it can be assumed that recycling the water will induce higher concentrations of P and K in the water and therefore a higher capacity of the biological system to export P and K through the organic products.

Table XIX. Concentrations of dry matter and nutrients observed in sludge of either open system ("station expérimentale") or recycling system ("prototype")

parameter	*unit*	open system			recycling system
		1A	1B	1C	P1
dry matter	*kg.m^{-3}*	68	73	67	92
total N	*kg.m^{-3}*	1,9	2,2	2	3,1
total P	*kg.m^{-3}*	0,39	0,52	0,48	2,49
total K	*kg.m^{-3}*	0,17	0,17	0,17	0,50

Concentrations of most nutrients in plants harvested in the pilot system based on water recycling, did not increase compared to the concentrations observed in the open system (Table XX.) except in the case of phosphorus. It can be explained by the abundance of available potassium and other elements (Cu, Zn) in the slurry that was added to the open system. On the contrary, in the case of P, the recycling induce an increase in the soluble P that could increase its availability in the pilot system based on recycling.

Table XX. Concentrations of dry matter and nutrients observed in plants harvested either in open system ("station expérimentale") or in recycling system ("prototype")

		floating macrophytes			rooted macrophytes		
parameter	unit	*Eicchornia crassipes*	*Pistia stratiotes*	*Lemna spp.*	*Glyceria aquatica*	*Phragmites australis*	*Juncus inflexus*
				open system			
dry matter	% wet weight	4,2	4,9	5,3	23,8	25,0	30,8
total N	% dry weight	5,6	4,3	6,4	1,6	2,9	1,7
total P	% dry weight	1,4	0,7	1,8	0,3	0,3	0,2
total K	% dry weight	4,6	4,7	2,6	1,6	2,5	1,5
Cu	mg/kg DW	26	84	54	3	10	4
Zn	mg/kg DW	170	327	286	24	73	30
				recycling system			
dry matter	% wet weight	4,4	5,3	8,3	30,2	45,4	42,1
total N	% dry weight	4,4	3,8	6,1	1,2	1,7	1,5
total P	% dry weight	4,3	3,6	3,8	1,3	0,7	0,8
total K	% dry weight	4,0	3,6	1,7	1,4	0,8	1,3
Cu	mg/kg DW	15	7	6	2	2	2
Zn	mg/kg DW	173	53	45	6	23	22

6.3. Nutrient retention in sludge and plants

During experiment, the chemical element concentration of water decreased from piggery outlet to storage lagoon. The decrease of chemical elements could be influenced by three process; volatilization, sedimentation and plant uptake.

Nitrogen level decreased from piggery outlet to storage lagoon. The highest decrease was in vermifilter (49.7 kg N stored or volatilized). In the lagoon system, there was nitrogen decrease for all levels. The weight of nitrogen uptake by plants was between 0.2 and 4.5 kg N. The weight sedimentation or volatilized was therefore estimated between 0.4 and 16.9 kg N, higher than plant uptake. In the storage lagoon, the weight of nitrogen exported after plant uptake was 4.5 kg N. In this lagoon, it was heavier than sedimentation.

Table XXI. N fluxes in the prototype

N					Time :	100 days		Out	flow total :	480000	L
observations							calculations				
	Concentration		Decrease		Area (m²)	Harvest observation (kg)	Outflow (kg N)	Plants			Lagoon mud
	average $mg.L^{-1}$	std. dev. $mg.L^{-1}$	%	$mg.L^{-1}$				Kg plants /100 days	Analyze N (g/kg brut)	kg N	(kg N + Volat)
ST	271.1	75.0	26.3	71.4			34.3				34.3
SL	167.6	63.2	61.8	103.6	40		49.7				49.7
P0	105.3	67.9	59.2	62.3			29.9				29.9
P1	69.5	31.9	51.5	35.8	38	98.0	17.2	114	2.4	0.3	16.9
P2	57.4	27.3	21.1	12.1	102	90.0	5.8	91	7.2	0.7	5.2
P3	38.7	14.7	48.1	18.6	44	992.2	8.9	451	2.6	1.2	7.8
P4	28.1	9.9	38.0	10.7	174	40.0	5.1	40	3.7	0.2	5.0
P5	17.9	4.8	56.9	10.2	181	3082.9	4.9	1722	2.6	4.5	0.4

In general, phosphorus concentration decreased from piggery outlet to storage lagoon. As usually observed in pre-treatment of pig slurry, the highest decrease of phosphorus was observed in sieve outlet (19 kg P). The phosphorus stored in the vermifilter and the settling tank (vermicompost and vermicasts) was respectively 13 and 11 kg P. In the lagoon system, the highest decrease in phosphorus was 4 kg P/subsystem. The plant uptake was small except in P5, it was between 0.3 kg and 10 kg. For parameter sedimentation, several parts indicated the phosphorus decrease such as in first, second and fourth level. The decrease weight was around 2 kg. The sediment of third and fifth lagoon indicated a phosphorus release: negative values (between -3 and -6 kg P) were calculated. It can be explained by the phosphorus that was stored in the subsystem during the cold season and that was used during the warm season by the plants.

Table XXII. P fluxes in the prototype

P					Time :	100 days		Out	flow total :	480000	L
observations							calculations				
	Concentration		Decrease		Area (m²)	Harvest observation (kg)	Outflow (kg P)	Plants			Lagoon mud
	average mg.L^{-1}	std. dev. mg.L^{-1}	%	mg.L^{-1}				Kg plants /100 days	Analyze P (g/kg brut)	kg P	kg P
ST	113.9	34.6	35.1	39.9			19.2				19.2
SL	85.8	19.1	32.7	28.1	40		13.5				13.5
P0	63.5	9.0	35.1	22.3			10.7				10.7
P1	57.0	8.5	11.3	6.5	38	98.0	3.1	114	6.0	0.7	2.4
P2	50.3	6.7	13.3	6.7	102	90.0	3.2	91	7.4	0.7	2.5
P3	51.4	9.9	-2.1	-1.1	44	992.2	-0.5	451	5.6	2.5	-3.1
P4	47.0	7.8	9.5	4.4	174	40.0	2.1	40	6.3	0.3	1.9
P5	39.4	7.1	19.3	7.6	181	3082.9	3.6	1722	5.6	9.7	-6.1

Potassium concentration decreased from piggery outlet to storage lagoon. Like for phosphorus, the highest retention was achieved by the sieve (64 kg K). The potassium was more stored by the vermifilter than by the settling tank (respectively 42 and 17 kg K). It was heavier than the retention by the lagoon subsystems. These ones were between 6 and 13 kg K. In lagoon subsystems P1 to P4, most of sediment potassium weight was heavier than plant assimilation ones that had weight between 1.5 and 10 kg K. The potassium sediment in storage lagoon (P5) was negative (-10 kg K). In this subsystem the potassium of plant uptake was 38 kg and was heavier than in lagoon sedimentation.

Table XXIII. K fluxes in the prototype

K					Time :	100 days		Out	flow total :	480000 L	
observations							calculations				
	Concentration		Decrease		Area (m^2)	Harvest observation (kg)	Outflow (kg K)	Plants			Lagoon mud
	average $mg.L^{-1}$	std. dev. $mg.L^{-1}$	%	$mg.L^{-1}$				Kg plants /100 days	Analyze K (g/kg brut)	kg K	kg K
ST	530.9	124.6	25.0	132.6			63.6				63.6
SL	443.1	143.4	19.8	87.7	40		42.1				42.1
P0	408.3	102.7	8.5	34.9			16.7				16.7
P1	379.6	92.7	7.6	28.7	38	98.0	13.8	114	20.7	2.4	11.4
P2	340.6	102.4	11.5	39.0	102	90.0	18.7	91	62.3	5.7	13.1
P3	306.4	109.8	11.1	34.1	44	992.2	16.4	451	22.1	10.0	6.4
P4	277.7	65.3	10.3	28.7	174	40.0	13.8	40	37.7	1.5	12.3
P5	220.0	70.4	26.2	57.7	181	3082.9	27.7	1722	22.1	38.0	-10.3

7. Discussion of hypothesis

7.1. Removal of macronutrients

7.1.1. Decrease in nitrogen

The result showed that the system filtration reduced nitrogen concentration. When the pig slurry passes the sieve, this equipment would separate solid and liquid phase. The solid phase will be leaved in the sieve and the liquid will flow to the tank. The nitrogen concentration of liquid that pass sieve will be less than the outlet piggery. The pig slurry of outlet piggery consists of liquid and solid phase, the concentration of pig slurry influenced by these phases. The pig slurry after sieve consists of liquid and less solid phase. Its concentration is influenced by liquid phase. The solid phase which is in minimum quantity doesn't influence the concentration. In this step, the physical treatment influences the reduction of nitrogen concentration.

When the pig slurry passes vermifiltration, the earthworms will ingest the pig slurry. In earthworm ingestion system, there are reactions that reduce nitrogen concentration. The result of earthworm ingestion will be the feces (earthworm casts) that have less quantity nitrogen than before ingestion. The chemical component of organic matter after ingestion will be more stable. The organic matter which is in the porosity between woodchips, will adsorb dissolved nitrogen. In this step, the layer of organic matter will retain nitrogen and it will rest in compost. The liquid that exits from vermifilter will have less concentration of nitrogen than its inlet. In this step, the biochemical processes influence the nitrogen reduction.

The decrease of nitrogen after vermifilter has been already observed by Taylor et al. (2003). Li et al. (2008) found the efficiency of nitrogen removal by vermifilter was 83 %. The pigs were different in this experiment compared to the experiment of Li et al. (2008). Here the pigs were gestating sows whereas they were growing-finishing pigs in the case of Li et al. (2008). Gestating sows ingest and excrete less nitrogen compared to growing pigs. A difference in the nature and the concentration of nitrogen in the pig effluent can explain the difference in N removal efficiency. The other difference is that in the present case the worm casts can flow out the vermifilter and be collected in the settling tank. The objective is to avoid clogging of the vermifilter. It was not the case during the experiment of Li et al. (2008). Therefore, the lower removal efficiency can also be explained by a difference in the output of organic particles, that is probably higher in the present case.

Although total nitrogen concentration decreased, the nitrate and nitrite concentration increased from piggery outlet to vermifilter ones. This phenomenon was associated to a decrease in ammonium concentration. It shows that there was significant nitrification fluxes in this subsystem despite the short residence time of the water, around one day. The nitrogen lossed by the vermifilter and the settling tank was high. We could not indicate whether it was stored or lost through denitrification because we did not measure the mass budget of the vermifilter.

The liquid will pass several constructed wetlands. In this part of the pilot system, there are plants which help filtration system. In the first and third constructed wetland, the nitrogen concentration will decease by sedimentation and absorption of plants. The plants can reduce the liquid flow and stimulate the microorganism to fix and degrade organic matter. In the second and fourth lagoon, the nitrogen will decrease through the plant uptake. Nitrogen efficiency in these subsystems was above 70 %. It was higher that the results of Knight et al. (2000) and Verhoeven and Meuleman (1996) who observed an efficiency around 50 %.

In the part of lagoons, there were no significant changes in nitrate or nitrite concentrations. However, there were small differences between lagoos. If we compare among the parts of lagoons with more details, the second and fourth lagoons had the nitrate and nitrite concentration less than the first and third ones. The phenomenon showed the aerobic and anaerobic processes that were found in the constructed wetland with water media provoke the nitrification process followed by denitrification in the horizontal subsurface wetlands.

The efficiency of nitrate removal in the lagoons was small because nitrate concentrations were small compared to total nitrogen concentrations. Therefore the efficiencies were lower than the nitrate removal that were found by Reilly et al. (2000) 80 % and Kadlec (2010) 67 %.

7.1.2. Decrease in phosphorus

Phosphorus concentration decreased from piggery outlet to vermifilter ones. This phenomenon was same as nitrogen decrease. In the sieve, the separation between solid and liquid was effective to decrease the phosphorus concentration. The outlet concentration of sieve was only influenced by liquid phase.

In vermifilter, the earthworm ingestion helped decrease phosphorus concentration. The results of earthworm ingestion were phosphorus in form more stable that were in compost pores and difficult to be evacuated by liquid. Taylor et al. (2003) found the vermifilter can reduce phosphorus concentration. The phosphorus efficiency was around 40 % and it was less than the result of Li et al. (2008), around 60 %. Like the difference in nitrogen removal efficiency, it can be explained either by a difference in the nature and concentration of P inf the effluent, or by the lower content of particles (worm casts) in the experiment of Li et al. (2008).

In the lagoons, phosphorus concentration decreased from inlet first lagoon to outlet fourth. The decrease phenomenon in the lagoon is different than the nitrogen decrease. The nitrogen concentration always decreased from inlet first lagoon to outlet fourth ones. Phosphorus concentration in the lagoon with water media was higher than gravel ones. In this case, the lagoon with gravel was more effective to reduce phosphorus. Yousefi and Mohseni-Bandpei (2010) found the lagoon with gravel media could reduce phosphorus. There was indication that the anaerobic processes inside the gravel lagoon were more intensive to decrease phosphorus than the aerobic of water lagoon. Precipitation of phosphorus in stable mineral or organic compounds is supposed to be the most important process for phosphorus removal (Schulz et al. 2003, Newman et al. 2000). When the concentration in precipitates increases, the concentration in soluble phosphorus will also increase. Therefore, the phosphorus concentration in the storage lagoon remained high at equilibrium compared to the nitrogen concentration because nitrogen was volatilized in the first parts of the system.

In part of lagoon, the efficiency of phosphorus removal was 26 % and was lower than the result of Zemanova et al. (2010) 50-70% and Lu et al. (2009) 59%. But, the total efficiency phosphorus removal or in integrated wetland (vermifilter and lagoons) was 70 % and it was lower than the efficiency that was found by Park (2009) 99 %.

7.1.3. Decrease in potassium

Potassium concentrations were very high in this experiment. Potassium concentration of piggery outlet were twice higher than nitrogen concentration. According to Petersen et al. (2007), there is a relationship between the pig feed and chemical elements of its effluents. In this case, the pig feed could contribute to the high potassium concentration in outlet piggery. However most of the increase is probably explained by the lower efficiency in K removal and the water recycling.

Potassium concentration decreased from inlet piggery to outlet last lagoon. After vermifilter, the potassium efficiency was 33 % and it was lower than the result of Li et al. (2008) 54%.

Potassium decrease was observed by Venterink et al. (2002) but wasn't found in the experiment of Pierce et al. (2010). The potassium decrease process was same as nitrogen ones. The decrease was high from piggery to vermifilter and was low among lagoons. In last of lagoon, the potassium concentration was still higher than other macronutrients.

7.1.4. Removal of COD

COD concentration decreased from piggery outlet to storage lagoon. The decrease phenomenon of COD was same as the nitrogen ones. COD decreased very high from piggery outlet to vermifilter ones (2552 $mg.L^{-1}$ COD). The physical treatment in sieve could reduce COD concentration although its efficiency (57.7 %) was less than the part of lagoon.

In the vermifilter, earthworms had role of biology treatment. Their ingestion system could reduce the COD concentration of liquid which passed vermicompost. We found the concentration COD of outlet were lower than its inlet. Taylor et al. (2003) also found that vermifiltration could reduce the COD concentration.

The COD concentration decreased in all parts of lagoon. The level decrease of COD concentration in several lagoons was lower than the part of sieve-vermifilter. The mechanism of absorption by plant uptake and microorganism fixation influenced the decrease of COD concentration in lagoon. The wetland system is effective for COD removal (Ayaz, 2006). In this part, the efficiency was 72.3 % and it was almost same as the observation of Verhoeven and Meuleman (1996) 80–90% and Vrhovsek et al. (1996) 80 %.

7.1.5. Role of precipitation and evaporation

Precipitation and evaporation influence removal process in constructed wetlands. Precipitation influences the liquid quantity in inlet of constructed wetland (Dunne et al. 2005) and evaporation influences the liquid lost from wetland system to atmosphere (Figure 61).

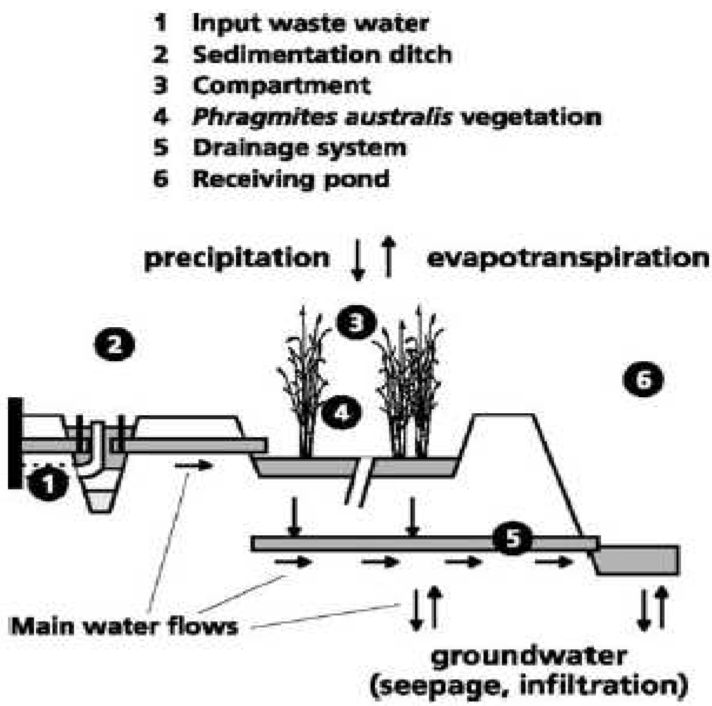


Figure 61. Precipitation and evaporation in wetland (Meuleman et al. 2003)

The influence of precipitation and evaporation on removal process in our case is a little different because the water is recycled. The duration of observation was shorter than one year that is short to have an accurate knowledge of the system. We observed some influence of the climate. Precipitation influenced the constructed wetland system after some events of heavy rain (Arias et al. 2003). However, our results correspond to the hypothesis of Raisin et al. (1997) who explained the contribution of precipitation was insignificant in small area of wetland.

In the inlet, the liquid supply was much higher than the input due to the climate on a daily time step. There was hypothesis that the system evaporates more liquid than precipitation input on a yearly basis. During rainy period the water accumulated slowly in the storage lagoon. On the contrary, the level of water in the storage lagoon decreased during the summer period. It could be expected from these variations a dilution of nutrients during winter and an increase in concentrations during summer. The contrary was observed with potassium: concentration increase during winter and decrease during summer. It can be explained by the plant uptake that was small during winter (small plant growth) and high during summer (maximu plant growth).

7.2. Variability of concentration measurements

During experiment, the variability of concentrations among seasons was small compared to the variability at daily time step due either to the time of feed input and excretion, or to the variations of animal number in the pig house. In the same part of the system, e.g. vermifilter or wetland output, the concentrations did not show high differences between the warm and cold season.

The result showed there were significant differences among parts. A clear decrease in concentrations of chemical elements was observed from first to last part of experimental system. Therefore we conclude that most variability was due to system design and piggery output and not to the climate.

7.3. Nitrification-denitrification, sedimentation and plant uptake

According to experiment result, the macronutrient concentration decreased from piggery outlet to storage lagoon. The processes that can reduce the chemical concentration of nitrogen are nitrification-denitrification, sedimentation and plant uptake. Nitrification-denitrification is usually considered as the major process of nitrogen removal.

The direct plant uptake is usually low for chemical elements removal in constructed wetland (Zemanova et al. 2010). The results showed the nitrogen quantity in the sediment is more than 1 kg and is higher than the nitrogen quantity in plants that only has less than 1 kg. The comparison of concentrations in plants and sludge in either the open system or the pilot system based on recycling, showed that nitrogen concentrations were not modified by the recycling. Two hypothesis can be suggested: (i) nitrogen level in the storage lagoon was so small that it did not change the nitrogen input in the vermifilter and the constructed wetland; (ii) if recycling increases the nitrogen concentration in water (e.g. if the number of subsystems decreases) the losses through denitrification will increase.

For phosphorus, several lagoons indicated that phosphorus quantity in sedimentation were more than 2 kg and were higher than plant uptake that were only less 1 kg. Several water lagoons had negative value in their sediment quantity and had phosphorus exported after plant uptake. This case indicated that lagoon sediment released phosphorus and the plant uptake used this phosphorus. The comparison of concentrations in sludge and plants with or without recycling showed a clear increase of P concentration in both sludge and plants. It can be related to the higher P availability because the P that is recycled is the most soluble fraction. Therefore, recyling will induce a clear increase in the capacity of the biological treatment system to export more phosphorus.

Potassium removal had same phenomenon as nitrogen. Most removal of potassium was explained by lagoon sediment or were more than 10 kg. The potassium quantity exported by harvested plants was less than 6 kg. The water lagoon indicated unstable situation. In some lagoons the potassium quantity of sediment was lower than plant uptake ones, in other lagoons the potassium quantity of sediment was higher than plant uptake ones. The release of liquid to the environment was not considered necessary because of too high concentrations of potassium. Some liquid could flow out of the system in winter after heavy rainfall events, but the mass of potassium released during these events was considered negligible. The comparison of concentrations in sludge and plants with or without recycling showed a clear increase of K concentration in the sludge but not in the plants. It can be suggested that the K concentration was already high enough in the open system, thus the plant could not uptake more in the recycling system. Therefore, recyling will induce an increase in the capacity of the biological treatment system to export more potassium when organic matter is exported. If only small quantities of organic matter are produced, more plants should be harvested (through a higher area or a longer growing season) in order to export the potassium.

8. Conclusions

The mix treatment system could reduce the chemical elements by 95%, 75%, 67% for N, P, K respectively. Concentrations of COD, N and K always decreased from piggery outlet to storage lagoon. Phosphorus did not always decrease. In some free floating macrophyte lagoon (e.g. P3 and P5), an increase in average P concentration could be observed. It was not significant considering the temporal variations, but it can be explained by the release from the P accumulated in the sediments during the cold season. The potassium concentration level was higher than nitrogen ones because of the water recycling.

The Guernévez recycling used mix system treatment. Physical treatment is found in animal building and sieve. Biological and chemical treatments are found in vermifilter as well as in lagoon system.

The system indicated that the output by organic matter was more efficient to reduce the concentration of chemical element because most the weight of its chemical elements was heavier than the uptake by plants.

Most exportation was achieved on the concentrated effluent processed by the sieve and the vermifilter. However, to achieve a diluted water for the flushing of the piggery, the highest removal efficiencies were achieved by the combination of constructed wetlands that alternated aerobic (freewater) and anaerobic (subsurface flow) constructed wetlands.

Recycling increased the capacity of the system to export more P and K through the solid output. It was not necessary to release liquid because of nutrient accumulation of the water.

9. Knowledge application to design and management

Design and management of a system combining vermifiltration and lagooning must ensure that the production and harvesting of plants and organic matter compensate for the input of non-volatile elements such as phosphorus or potassium on a yearly basis. Previous results did not show clearly that the concentration of harvested products increased with higher concentrations of phosphorus and potassium in the circulating liquid. Therefore, the composition of products obtained in similar conditions but in open system can be used to choose the size of the plant production subsystems.

Optimal transfer between subsystems depends on harvesting objectives and nutritional requirements of the plants. Design and harvesting periods can be based on results observed in open systems. Nutrient requirements of plants can be based on relationships between yield and composition (Khiari et al., 2001).

Not only the total lagooning area but also the number of lagooning levels has an influence on the functioning. There were five lagooning levels (three free water and two subsurface flow wetlands) and three organic levels (sieve, vermifilter, and settling tank) in our system. This number achieved significant reduction of nutrients, pathogens and endocrine disruptors (cf. Appendix 2). As we observed, floating macrophytes harvested at third level of lagoon could be used for pig feeding. Similarly, it can be assumed that emerged macrophytes harvested at levels two or four can be used as bedding material in livestock houses or for composting operations.

Chapter 4: Temporal interactions: seasonal effect on plant growth and concentration decrease of nitrogen and COD

1. *Résumé du chapitre 4 : interactions temporelles : effet de la saison sur la croissance des plantes et l'abatement d'azote et de DCO*

Les effluents de porcherie sont riches en éléments chimiques et provoquent des pollutions de l'environnement. Ils devraient être réutilisés et non rejetés dans l'environnement. Les marais filtrants permettent des intrants plus élevés et une production végétale supérieure par unité de surface en comparaison de l'épandage sur des parcelles de terrain cultivées. Cependant, la croissance des plantes dépend du climat. La diminution de concentration dans l'eau et les transformations des composés organiques et minéraux sont supposées dépendre également de la saison.

L'objectif de ce chapitre est d'analyser l'effet saisonnier sur la croissance des plantes et sur la diminution de concentration.

La méthode mise en œuvre utilise un suivi à long terme de la concentration en eau et de la croissance de plantes pour trois combinaisons de lagunes à macrophytes flottantes et de marais filtrants avec des hélophytes, où les plantes étaient régulièrement récoltées. Les résultats montrent qu'une forte croissance des plantes peut être réalisée tandis que l'efficacité de traitement varie entre 63 et 93% pour l'azote total. La saison est un facteur important dans le fonctionnement du marais filtrant. Cette expérience a confirmé les résultats de la littérature indiquant que l'efficacité de filtration varie avec les saisons mais qu'elle varie moins que la croissance des plantes.

Par conséquent, on peut conclure que le recyclage de l'eau dans le système complet est associé à la réutilisation des nutriments à l'intérieur de chaque lagune ou marais filtrant, entre des périodes où les nutriments s'accumulent et des périodes où les concentrations des nutriments peuvent diminuer en raison d'un développement rapide de la végétation. La conception des systèmes associant des sous-systèmes continus, tels que la porcherie, et des sous-systèmes discontinus, tels que les marais filtrants, devrait tenir compte de cette succession de périodes contrastées. Le recyclage de l'eau est un moyen de vérifier que la compensation entre périodes contrastées conduit à des concentrations stables.

2. *Abstract*

Piggery wastewater that is rich in chemical elements and provoke environment pollution should be reused and not released in the environment. Constructed wetlands allow higher inputs and crop production compared to spreading on cultivated plots by surface unit. However, plant growth depends on climate. The concentration decrease in the water and the transformations of organic and mineral compounds should also depend on the season.

The objective of this chapter 4 is to analyze the seasonal effect on plant growth and concentration decrease.

The method used long term monitoring of water concentration and plant growth for three combinations of lagoons with harvested floating macrophytes and constructed wetlands with harvested helophytes. The results show that high plant growth can be achieved while treatment efficiency ranged between 63 and 93% for total nitrogen. The season is an important factor in the functioning of constructed wetland. This experiment confirmed previous literature indicating that the filtration efficiency varies with the seasons but that it varies less than the plant growth.

Therefore, it can be concluded that recycling the water throughout the whole system is associated to recycling inside each lagoon or constructed wetlands between periods where nutrients accumulate and periods where the stock of nutrients can decrease because of a rapid development of vegetation. Design of systems associating continuous subsystems, such as the piggery, and discontinuous subsystems, such as the constructed wetlands, should take into account this succession of contrasted periods. Recycling the water is a key-point that allows to control the effective compensations between constrasted periods and the result in stable concentrations.

Keywords:

Constructed wetland, Nitrogen, Water quality, Season

3. Introduction

The wastewater of animal housing or urban and rural living sewage are mainly organic polluted, without high levels of toxic compounds or elements. Animal farms use huge amounts of water and produce effluents that are generally spread on soils in Europe or directly released into rivers in some countries. When the fertilizers contained in the effluents are less or equal to the crop requirements, the animal farm contributes to the fertility of the region. On the contrary, when the fertilizers spread exceed these requirements, the excess contaminates the soils and waters and induce eutrophication. One environmental and economical problem of pig farms is wastewater because its high weight reduces the possibility of transporting high quantities to region where fertilizers are needed for crop production. The wastewater that leave piggery has high levels of nutrients. Usually, its level of chemical elements is higher than the standard level of water pollution limit. Thus, it should not be released into the rivers.

Many biological methods were developed to treat wastewaters. Nowadays, people mainly focus on the pollutant removal effects of the treatment methods. During the process, C and N were changed into CO_2, N_2, or other gases. However, with the increasing problem of resource limit in the world, we regard that people should pay more attention on the transformation of the pollutants in the wastewater into all kinds of beneficial productions, other than the purification of water. Especially in most developing nations, they just begin to treat the solid and liquid wastes. If the traditional concept of “treat” could be more inclined to “transformation”, it can bring massive beneficial products, even decreasing the emission of greenhouse gases.

In extensive biological systems used to treat wastewater of small towns or farms, various macroscopic species can be used as bio-monitors or as actors adapted to a range of decreasing concentrations (e.g. earthworms, macrophytes, etc.). They can be used either to restore the initial biodiversity of natural ecosystems in polluted regions, or to increase the biodiversity compared to anthropic ecosystem with only biophysical treatments. Of course, the macroscopic species itself are the productions transferred by the pollutants. The maximization of various productions (compost, plants, and animals) from the elements contained in that water is preferred. However, extensive biological systems have an optimum input of nutrients and a tolerance range: below, the organisms do not grow, above, the system becomes toxic and the organisms die.

Ponds and constructed wetlands can help to reduce the chemical elements (Sevrin-Reyssac, 1999). This way is less expensive, easy in the application and effective. Several researches found that they reduce the chemical elements (Greenway, 2005; Hunt et al., 2006). The level of several chemical elements such as nitrogen (Romero et al., 1997) and nitrate (Reilly et al., 2000) could be reduced by constructed wetland.

Therefore, knowledge is needed to give the values of optimal loads in various regions, as a function of the species and the climate. Knowledge of nutrient reduction is critical to manage environment protection while knowledge of plant growth is critical to manage crop production and optimize harvesting operations.

Piggery Experimental Farm of Guernévez, Saint Goazec, France applies constructed wetland to reduce the chemical element of piggery wastewater. The wastewater of piggery flowed in four level constructed wetlands. The function of several level of constructed wetland was the wastewater of piggery could be filtered several times and the level chemical element in outlet would be below the limit of standard pollution. However, the output of nutrients from the piggery is more or less stable throughout the year, whereas the transformation of nutrients by the combination of constructed wetlands depends on the season.

The chemical processes in wetland depend on plant growth and development, which is influenced by the seasons. The experiment of (Jing and Lin 2004) showed different result of ammonia removal in several seasons. The objective of this chapter is to analyze the effect of different plant species and different seasons on nutrient reduction and plant growth.

4. Hypothesis

The seasonal effect on plant growth should be higher than on nutrient removal because the physical and microbial processes involved in nutrient removal do not stop during the cold season.

If the nutrient removal during the cold period is due to accumulation in the lagoon, the plant uptake during the warm season should compensate for this accumulation in order to have a stable composition of the liquid on a yearly time step.

5. Material and methods

5.1. System design

The experiment was located at Piggery Experimental Farm of Guernévez, Saint Goazec, France. The experiment had 4 levels (Figure 62). Every level had 3 series (A, B and C). Levels 1 and 3 were lagoons with free floating plants. Levels 2 and 4 were horizontal subsurface flow constructed wetlands, fulfilled with gravels. Total constructed wetlands in this experiment were 12. Constructed wetland 2C had two parts, they were alternatively used during one week.

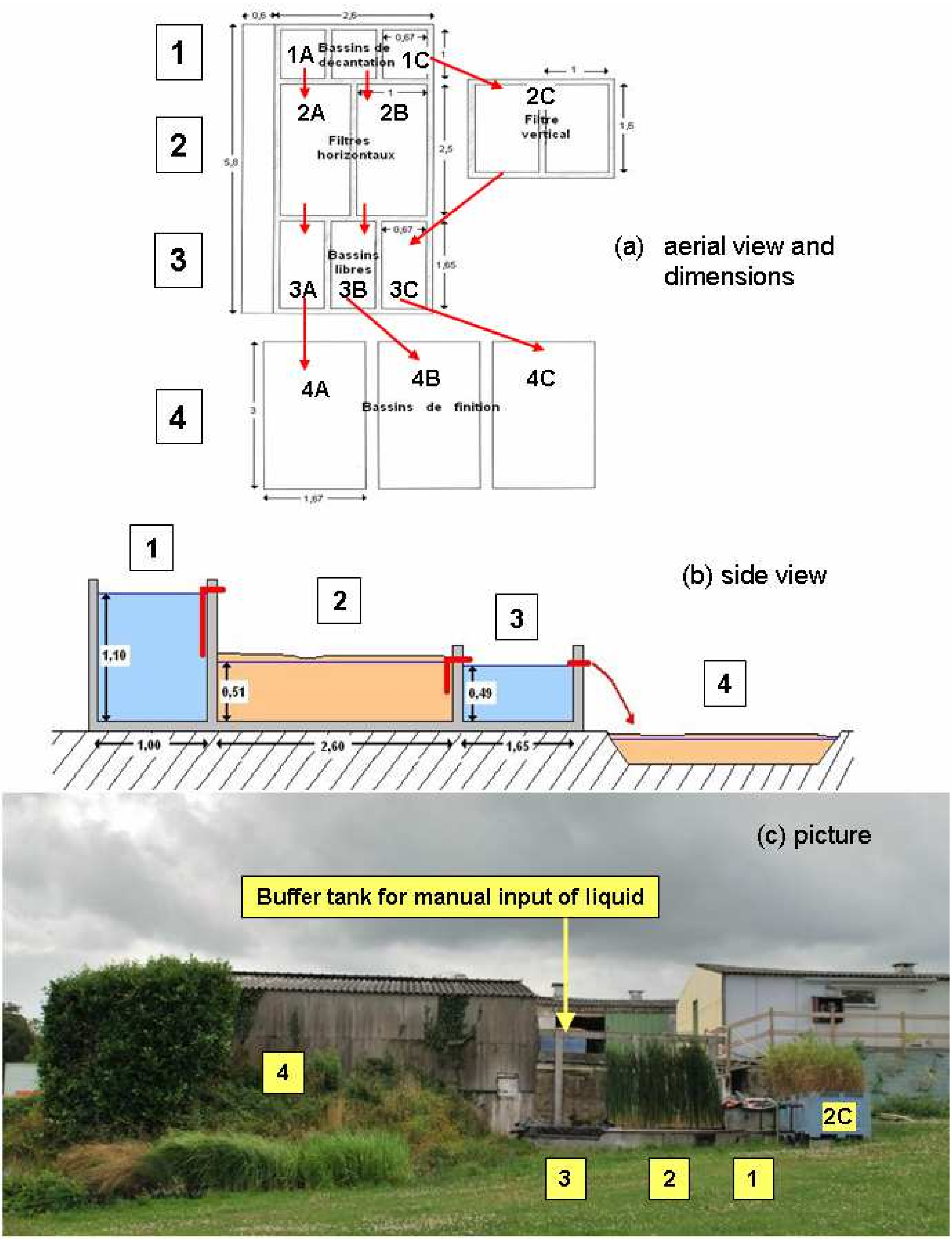


Figure 62. "Station expérimentale" with 3 combinations of 4 levels

Each series of constructed wetland had several combinations of plants. The plants were changed specially in levels 1 and 3 because floating plants such as *Eicchornia* *crassipes* or *Pistia stratiotes* could not survive the cold season. For levels 2 and 4, the plants were not changed.

Constructed wetland in level 1 contained 770 L water that had a hydraulic residence time (HRT) of 4.6 days. Continuous-horizontal flow constructed wetland in level 2 which had a volume of 1300 L. It contained 520 L water, the HRT being 3.1 days. The alternative-horizontal constructed wetland had a volume of 1000 L. It contained 400 L water, the HRT being 2.5 days the weeks with input, and 7 days the weeks without input. In third level, the volume of constructed wetland was 539 L. It contained 539 L, the HRT being 3.2 days. In the fourth level of constructed wetland, the total volume was 1750 L. It contained 700 L water, the HRT being 4.2 days.

The plants used in levels 1 and 3 were Hydrocotyle, Azolla, Water Hyacinth, Canadian waterweed and Pistia. In level 2 and 4, we planted Phragmites, Typha, Iris, Carex, Acorus, Glyceria and Mentha. In the levels 1, 2 and 3, every wetland had only one plant. In level 4, we made combinations of plants.

5.2. Experimental design

The experiment was conducted from January 2006 to August 2006 in first phase and from January 2007 to March 2008 for the second phase.

40 L pig slurry were diluted in 1000 L water in a plastic container. This liquid was fed to the constructed wetland. Every day, 500 L were added to the 3 series, 167 L being added to each series. The liquid could flow by gravity from level to the next one.

The water samples were taken every 15 days for all constructed wetlands and stored in plastic bottles. The weight of plants was measured at each harvest, around each week for floating plants. The height of rooted plants was measured regularly, depending on the growth, and they were weighed at harvest, either in summer or in autumn.

There were 13 positions of water samples for each sampling time. The samples were analyzed in a laboratory also located in Guernévez. A sample was frozen (-18℃) for further controls or additional analysis.

Water samples were analyzed for COD, total nitrogen, phosphorus, K, NH_4^+, NO_3^-, NO_2^-, and dry matter. The analysis used the Hach-Lange method based on reagents and colorimetric measurements.

6. Results

6.1. Removal of COD

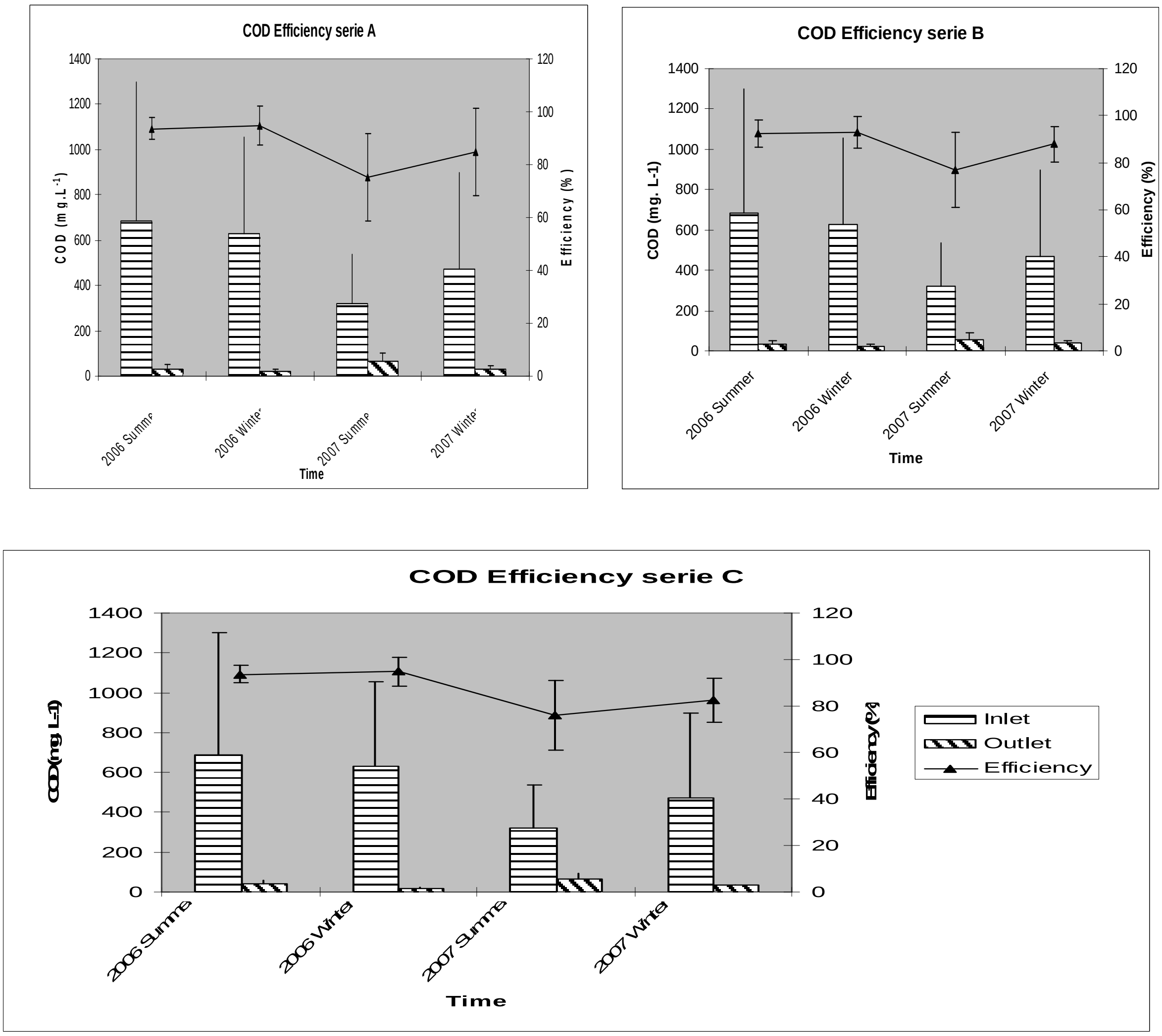


Figure 63. COD for inlet and outlet in series A, B and C.

In 2006, the average COD inlet and outlet concentration of summer was higher than winter for all treatments although the differences between two treatments were small compared to the standard deviation (Figure 63). The average COD inlet summer was 685 ± 612.7 mg.L^{-1} and winter was 625 ± 425.9 mg.L^{-1}. COD outlet summer were 34.8 ±17.5 mg.L^{-1} and winter were 20.67±9.26 mg.L^{-1} .

In 2007, the average COD inlet concentration of summer was lower than winter for all treatments but the average COD outlet summer was lower than winter. Differences between two treatments were lower than the standard deviation. The average COD inlet summer was 320.4 ± 216.1 mg.L^{-1} and winter was 471.2 ± 428.7 mg.L^{-1}. The average COD outlet summer was 63.4 ± 32.7 mg.L^{-1} and winter was 34.09 ± 9.3 mg.L^{-1}

The COD efficiency in winter was higher than in summer for all treatments although the differences between seasons were small.

6.2. Removal of total nitrogen

In 2006, average inlet total nitrogen in summer were higher than in winter for all treatments. The summer concentrations in outlet were less than winter ones. The difference between average in summer and winter were small compared with the standard deviations. Average inlet total nitrogen in summer was 56.9 ± 29.4 mg.L^{-1} and in winter was 50.9 ± 8.9 mg.L^{-1}. Average outlet summer was 11.4 ± 9.6 mg.L^{-1} and winter 19.5 ± 6.6 mg.L^{-1}.

We found different results in 2007. All average total nitrogen concentrations in the inlets and in the outlets were less in summer than in winter. Average concentration of total nitrogen in the inlet during summer was 66.4 ± 20.9 mg.L^{-1} and in winter it was 78.3 ± 37.6 mg.L^{-1}. Average concentration of total nitrogen in the outlet during summer was 12.9 ± 6.6 mg.L^{-1} and in winter was 16.6 ± 7.5 mg.L^{-1}.

Efficiency of total nitrogen removal in summer was higher than in winter both in 2006 and 2007. In 2006, efficiency of total nitrogen in summer was 76.6 ± 16.7 % and in winter it was 60.3 ± 15.5 %. In 2007, efficiency of total nitrogen removal was 81 ± 6.6 % and in winter was 76.1 ± 8.2 %. Differences between the treatments were less than the standard deviation.

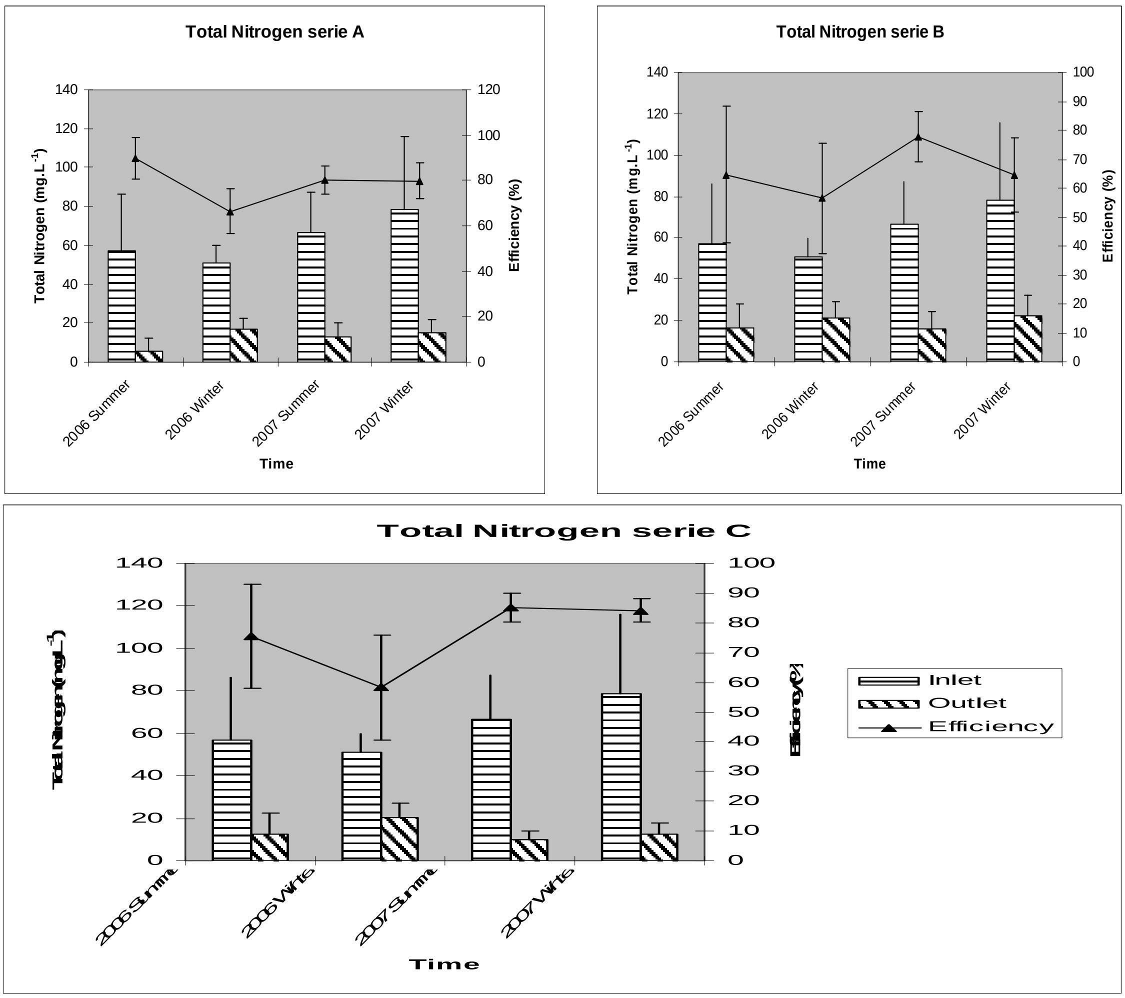


Figure 64. Concentration of total N in inlet and outlet in treatment A, B and C

6.3. Nitrate evolution

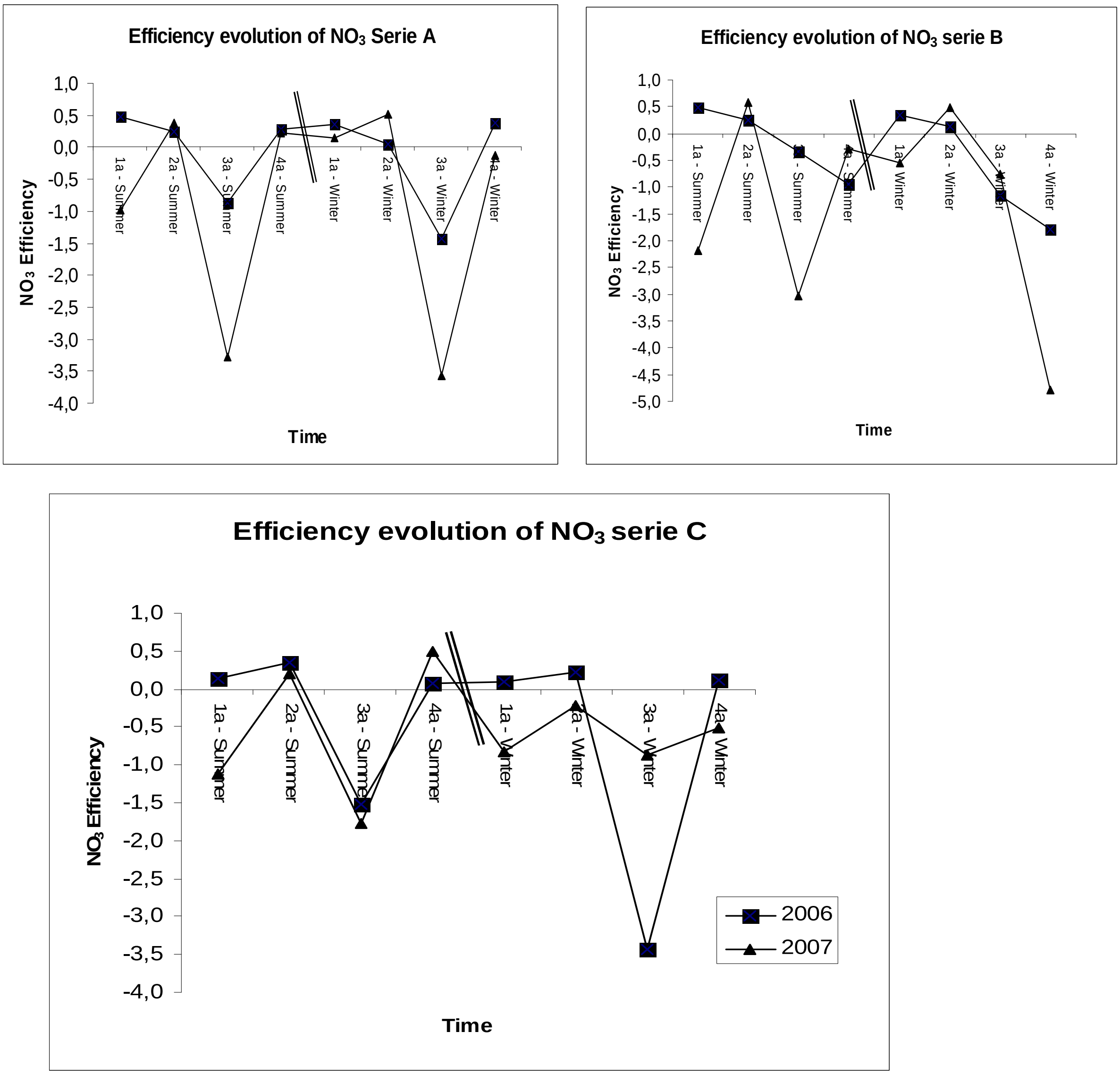


Figure 65. Evolution of efficiency of nitrate removal

Observations of efficiencies (Figure 65) show that there was no clear differences between treatments or between seasons. In 2006, the most efficiency values were positive. In the 2007, most values were negative. Efficiencies were less than 50 %. The high negative values were observed during winter.

6.4. Plant growth

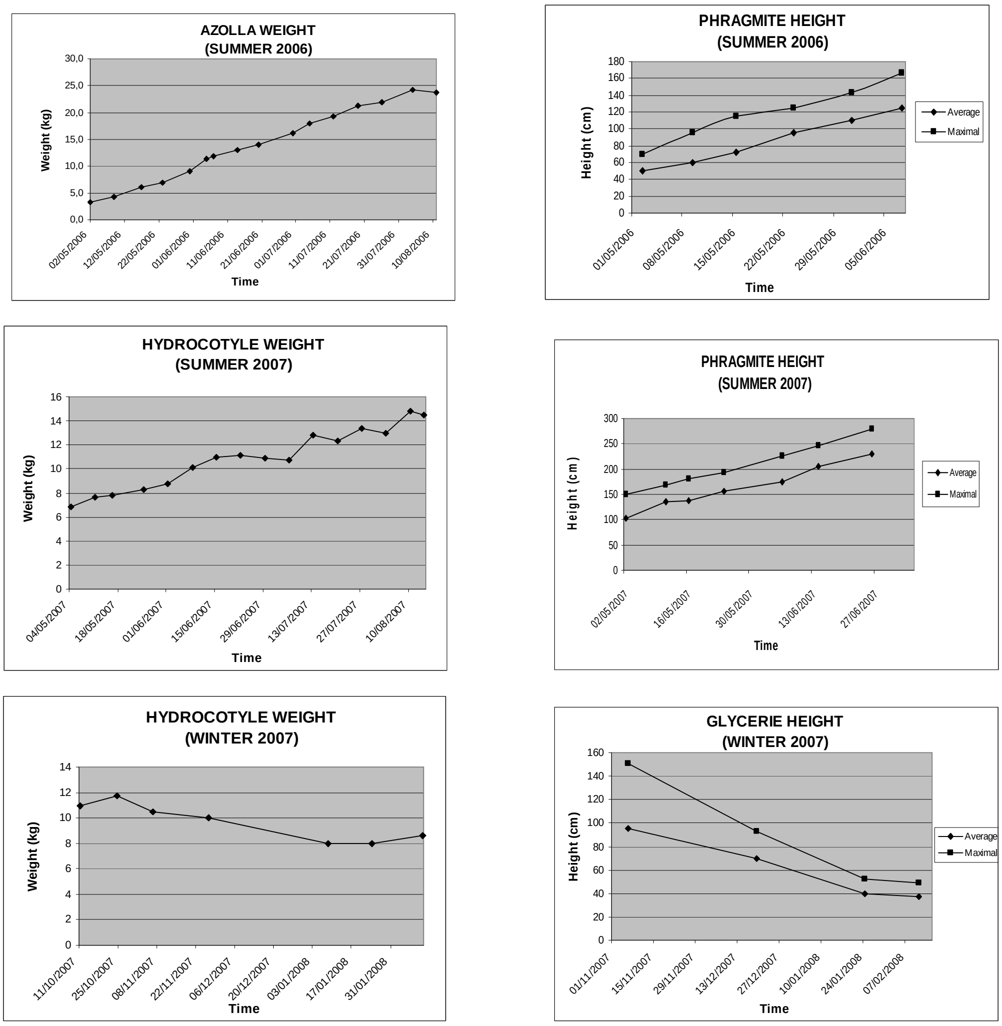


Figure 66. Plant growth

The weight and height of plant increased in summer both 2006 and 2007. In the begin season, the growth was very rapid. In the end of season, the growth of plant was slow or more stable.

On the contrary, the weight and height of plant decreased or remained almost stable in winter 2007. In another nearby experiment, we observed that the weight of Azolla increased during winter 2007.

Statistical analysis was done using the periods where the same plants were grown in the same subsystem both in winter and in summer.

Results of statistical analyses given in Table XXIV. show that the change of plant did not influence the chemical processes in the constructed wetland except in the cases of NO_3 in Hydrocotyle wetland and COD in the wetland with an association of Iris+Carex+Glycerie. Total nitrogen parameter was not influenced by the change of plant condition.

In the first level of free floating macrophyte wetland, the change of plant did not change significantly the decrease in total nitrogen and in COD. It was equally so for the difference between efficiency in summer and winter: no significant difference could be detected from this data set.

Table XXIV. Average efficiency of nutrient reduction by different plants

average	N total		COD		NO_3	
	W	S	W	S	W	S
1C Hydrocotyle	0.182 A	0.173 A	0.583 A	0.605 A	0.107 A	-0.919 B
2A Phragmites	0.196 A	0.166 A	0.448 A	0.297 A	0.044 A	0.474 A
3C Azolla	0.276 A	0.297 A	0.203 A	0.121 A	0.203 A	0.121 A
4A Iris+Carex+Glycerie	0.403 A	0.551 A	0.614 A	0.050 B	0.374 A	0.199 A

W: winter; S: Summer;
A and B refer to groups that are significantly different on the same line

7. Discussion of hypothesis

7.1. Removal of COD

In general, we found that our constructed wetland could decrease the COD concentration both in summer and in winter (Figure 63). These results were same as the result of Verhoeven and Meuleman (1996) and Knight et al. (2003) in the case of urban or livestock wastewater. Our research found that the COD outlet concentrations were lower than the COD threshold of standard pollution water of the Chambre de Commerce et d'Industrie de Paris. The COD threshold of this organization is 125 mg/l. In our experiments, the COD outlet concentrations were between 20 and 70 mg/l.

All summer COD in outlets were higher than in winter. The lower temperature is one of the limiting factors of chemical and biological processes. There is a contradiction between the observed decrease of COD in outlet and the theoretical decrease of removal processes because of lower temperatures. Two hypothesis can be suggested to explain this contradiction: (i) there is a higher turn-over of organic matter in summer that can increase dissolved COD and increase the concentration in outlet, (ii) Higher rainfall in the winter can have diluted the outlet.

The temperature in summer is more comfortable for chemical and biological processes (Kadlec and Knight, 1996). We assume that in our experiment in summer, the mineralization process was more rapid than assimilation processes. There will be accumulation of chemical element in the biomass that induced the increase in outlet COD during summer period.

7.2. Variation in removal of total nitrogen and nitrate

The system could reduce total nitrogen both in summer and winter. Day et al. (2003) explained how wetland could remove nitrogen. Temperature is one of the denitrification limiting factors (Hernandez and Mitsch, 2007a). In low temperature, denitrification process is slow, there will be accumulation of NO_3 in the system (Kadlec and Knight, 1996).

Our experiment showed the same phenomena for nitrogen. The outlet concentrations of total nitrogen in winter were higher than in summer. The piggery effluents that are rich in nitrogen and enter in the constructed wetland bring the nutrients. The nitrogen concentration in the system will increase. Because the chemical and biological processes such as plant growth in the winter are limited, there will be accumulation of nitrogen in the constructed wetland.

The nitrate evolution in the "station expérimentale" was not stable. Most values of nitrate removal efficiencies were positives during 2006 both in summer and winter. These values indicated the importance of denitrification in wetlands. This process could reduce the nitrate concentration and make the nitrate concentrations of outlet less than inlet. On the contrary, most values of nitrate efficiencies were negatives during 2007. This indicated the importance of nitrification reactions in wetland this year. The system produced nitrates and increased nitrate concentration in outlet.

During 2 years, the values of nitrification were higher than denitrification. The result (Figure 65) showed most of nitrate efficiency values were negative. Most negative values of efficiency in this system were higher than the positive values. The negative values indicated that the flux of nitrate that leaves the system is higher than the input flux because the liquid flux is quite the same. These observations indicated that the system produced more nitrates than it removed them.

Nitrification and denitrification are the principal processes that influence the decrease in nitrogen (Saunders and Kalff, 2001). Nitrogen concentration always decreased (Figure 64). In "station expérimentale", the nitrogen decrease was also induced by other factors like sedimentation or plant uptake during 2006 and 2007 because the total nitrogen always decreased although the concentration in nitrate did not always decrease.

7.3. Plants, seasons and chemical elements

Several factors influence the transformations of chemical elements in constructed wetland. Plant could influence the denitrification process through modifications of organic matter availability (Hernandez and Mitsch 2007b). The seasons influence the chemical reactions inside the lagoon. Nitrate removal was most efficient during the summer (Spieles and Mitsch, 2000). Influence of plant uptake on nitrogen removal was also maximum during the warm season.

On the other side, specially in macrophyte lagoons, the chemical elements influence the growth of plants like fertilizers influence the yield of crops. The results of Lawniczak et al. (2009) showed that plant growth in constructed wetland was mostly limited by N and not by K. Therefore, N removal should not be too high if plant growth is important for K removal.

During the warm season, plant growth was improved as well as chemical and biological processes (Kadlec and Knight, 1996). Our research also observed that the plant grew in summer, with increased weight and height, whereas in winter, the weight and height of plant decreased or was almost stable (Figure 66).

Despite the physical change in plant biology because the change of season (Figure 66), most of wetland efficiency parameters such as removal of total nitrogen, COD and NO_3 did not show strong differences (Table XXIV.). Differences in plant biology did not have a direct effect on the removal function of the constructed wetlands.

This result is in contradiction with previous results of Mitsch et al. (2005) who found that plants had a profound effect on the ecosystem function. Two hypothesis can be suggested to explain this difference: (i) either the plants did not have direct or indirect effect on the functionning in our case, (ii) or the plant continued to have a direct or indirect effect in winter through their roots and the associated rhizosphere. As we observed later a reduced efficiency in a constructed wetland without plants (series A where the plants were removed), we suggest that the second hypothesis is most probable.

7.4. Role of precipitation and evaporation

Precipitation and evaporation influence the liquid budget in constructed wetland. Precipitation has many influences in constructed wetland inlet or liquid supply (Poe et al. 2003) and evaporation influences the liquid lost (Figure 61).

Nitrogen removal in constructed wetland is dominated by nitrification-denitrification processes and by sedimentation. Nitrification in constructed wetlands can decrease during winter when the flux of oxygen supplied by plant roots decreases, inducing a decrease in the nitrogen flux lost by denitrification. Therefore a nitrogen accumulation in the constructed wetlands can be expected during the cold season. This accumulation should be compensated by denitrification fluxes higher than nitrogen input during the warm season..

Plant influences evaporation process in constructed wetland (El Hamouri et al. 2007, Brix and Arias 2005a). In this experiment, the phenomenon of relation plant-evaporation-liquid budget is same as the relation plant- evaporation-chemical element removal. Evaporation influenced directly the change in liquid budget, by compensating in summer the water accumulated in winter, but it did not influence directly the chemical element removal because the evaporation flux was small compared to the liquid input and output of the system (respectively around 5 mm/day and 50 mm/day).

8. Conclusions

Our work showed that seasonal variation in a combined wetland induced lower concentration of COD and higher concentration of total nitrogen in winter compared to summer in outlet water. This could be explained by the effect of temperature on chemical and biological process that lead to degrade organic matter, nitrogen nitrification and denitrification and absorption during plant growth.

Nitrate evolution did not show clear variations during 2 years. We assume that in this experiment, denitrification was not the first factor that influenced the total nitrogen removal. Sedimentation is supposed to be the first factor that induced nitrogen removal. The "station expérimentale" produced more nitrate than remove it because the nitrification level was higher than denitrification.

The use of these results to design wetland for livestock wastewater should depend on the macrophyte used in free water or planted wetlands. Plant growth can be used to calculate the plant uptake response but it cannot be used as the efficiency indicator or chemical element removal.

9. Knowledge application to design and management

When the climate has variable seasons, the composition of the harvested plant material will depend on time and the part harvested (global plant including roots, stems and leaves, fruits or seeds, before or after senescence). Therefore, the time of harvesting the organic products should be chosen so that the total of non-volatile nutrients that are exported compensate for the inputs.

If only plants are harvested in the lagoons, the plant uptake during the warm season should compensate for the nutrients accumulated in the lagoon during the cold season.

General discussion

1. Comparison of efficiency in "station expérimentale" and "prototype"

1.1. Size effect

Guernévez purification system has two types of experimental sites. There are the "station experimentale" and the "prototype". The sizes of two sites are different, the "station expérimentale" is small and the prototype is large.

In the «station expérimentale», there is only one species of plant in each lagoon of level 1, 2 and 3. On the contrary, there are several species of plants in the prototype in order to compare the growth in the same water. The effect of different plant species on the decrease of chemical elements could be observed in the «station expérimentale» and not in the prototype. The results of "station expérimentale" were obtained with an open system, whereas the prototype functions with water recycling. Therefore, the application of «station expérimentale» results to the prototype, especially for parameters of size and input concentration, can need some adaptation.

The plants in «station expérimentale» were changed following the season change. This condition is good to observe the season effect to plants or the plant adaptation to season. If the results are applied to prototype, the parameters of interaction among plant will be necessary to be observed. The symbiosis interaction can give the positive result such as the plant protection from destruction agent that can disturbs the plant growth and make the filtration doesn't work well. The negative interaction such as competition among plant can make decrease the filtration plant capability because the plant can't grow well. These biological interactions can induce a heterogeneity of the nutrient concentrations in the water of the constructed wetlands of the prototype. This heterogeneity can also induce different removal efficiencies in the prototype compared to the "station expérimentale".

In the pond of prototype, several free floating plants were put together in one pond. The pond was divided by borders that were made by pipe and styrofoam. This border had function to avoid the plant move to other area where the other plants live. The border can avoid the competition among plants. The border system proved that mix plants were effective to reduce the pollution of chemical element without competition both sunshine and nutrients.

In the wetland of prototype, the plants were planted together in one wetland. There were arrangement of plants. One wetland was divided into several sectors. Every sector was planted by a plant species. This method was used to avoid the competition among plants. The high plant was not planted and mixed with small plant. This method is effective because the result showed all plants in wetland could grow well. This situation is still coninuing at present.

1.2. Recycling effect

The «station expérimentale» was studied as an open system. The wastewater that entered the lagoon passed the lagoon one time. After the last lagoon, the wastewater was released on the grassland close to the "station expérimentale". In this case, the concentrations in the lagoons were influenced by the liquid that entered in the system.

Prototype is based on water recycling. After the last lagoon (P4), the water was stored in P5. This water was pumped to the animal building, then it circulated again through the several parts of the Guernévez purification system, then it returned again to the storage lagoon. The chemical element concentrations in the liquid that will re-enter depend on the inputs and outputs of all parts of the system. This result could be used to analyze the effect of recycling the water. It was shown that it induced an improved capacity of the system to export P and K, particularly through the organic products, whereas the effect on nitrogen was not observed.

2. *Hypothesis on gaseous emissions from lagooning*

When the wastewater passes the vermifilter and the lagoons, it is filtrated by several components of vermifilter and lagoons. Although the media are different, there are similar filtration and transformation processes. The indirect effects of earthworms and plants appeared to be of great importance. In vermifilter, filtration is helped by earthworm and in lagoon, it is helped by plants.

The chemical element concentrations of outlet were less than inlet. The decrease chemical elements was influenced by sedimentation, nitrification/denitrification and plant uptake. These processes converted several chemical elements to others forms either dissolved in the liquid, stored in the solids (sludge and plants), or lost as gaseous species.

The gaseous emission measurement in the field is very difficult because there are two media different of lagoons (water and gravels). The climate can change rapidly and influence volatilization processes. Uncertainty on the gaseous emissions are difficult to calculate when periods with temporal interpolation are much longer than the measuring periods.

In our work, gaseous emissions were measured in mesocosms vermifiltration and indoor system. The static chamber method was used to measure gaseous emissions. The input dose of mesocosm (6 L day^{-1}) was the dose conversion that is applied in outdoor vermifilter. The source of pig slurry that was applied to the mesocosms was the same as the pig slurry spread on outdoor vermifiltration. The gaseous emissions of N_2O, NH_3, CH_4 and CO_2 were measured.

If we assume the representativity of this experiment, the gas measurements of the mesocosms can be used to determine the gaseous emissions of other subsystems. The nitrogenous emissions of the vermifilter can be assumed to be higher or equal to the emissions of the lagoon because the concentration of nitrogen and its availability are higher in the vermifilter than in the lagoons, and because the same processes of nitrification-denitrification are supposed to occur in both sub-systems. Several factors must be verified before extrapolating mesocosm observations to other subsystems. Therefore we did not extrapolate methane, ammonia, or carbon dioxide emissions observed during the mesocosm experiment. It can not be assumed that the methanotroph population, that is increased by the earthworms, is abundant in the lagoons and constructed wetlands. Plants induce different ammonia absorption and emission exchanges with atmosphere than those occuring in the mesocosms. Plants can absorb and immobilize the carbon dioxide while these processes are neglected in the mesocosms compared to the respiration losses. Isotope experiments could help to identifiy the major fluxes of carbon and nitrogen in the recycling system.

2.1. Sample places

The size of mesocosm is 50 L of volume and 0.95 m^2 of surface area. The lagoons have 25 10^3 – 50 10^3 L of volume and 38 – 180 m^2 of surface area. Mesocosm surface is not large, when pig slurry is applied to mesocosm, liquid will be distributed evenly to all area. In contrary, every lagoon of prototype has large area. When pig slurry is applied to system, liquid will not distributed evenly to all area.

Liquid is important factor in this process because it is source chemical elements that will be converted to gaseous emission. If we want to apply the result of mesocosm emission gaseous to lagoon and the results will be not different than mesocosm, the sampling area in the lagoon must be chosen that have same condition as mesocosm.

When emissions gaseous were measured in mesocosm, the condition of mesocosm was sutured by liquid. The best area of lagoon for gaseous measurement sampling must be near inlet lagoon. Water in this area is distributed evenly. The measurement equipment will be not different than mesocosm. The emission gases in this area will the highest than others area.

The parameter is not on the equation of gaseous emissions but this parameter must be noted. If we install the measurement gaseous in other area of lagoon, the gaseous emission will be less than mesocosm because the chemical element concentration was not maximal. Then the emission gaseous will be less than mesocosm.

2.2. Size among components

Mesocosm and lagoons have different size. The size of mesocosm is 50 L of volume and 0.21 m^2 of surface area. The lagoons have between 20 10^3 – 250 10^3 L of volume and 38 – 180 m^2 of surface area. When the gaseous emission measurement of mesocosms are applied to lagoon, extrapolation can be based on area or water volume; i.e., gaseous emission per m^2 for area estimate and per L for volume estimate.

The surface sizes of lagoons are larger than mesocosm ones. The distribution liquid in surface lagoon are wider than mesocosm ones. If each part of lagoon releases gaseous, the total gaseous emissions of lagoon will be higher than mesocosm ones. Measurement gaseous emission result of vermifilter can be applied to prototype by multiply the gaseous emission result of vermifilter with the proportion of prototype and mesocosm area. Result will determine total gaseous emission on area basis.

The other part, the volumes of lagoons are bigger than mesocosm. The hypothesis for volume parameter is; when volume is bigger than the other, it will have more quantity of liquid. Because liquid has chemicals elements that will be changed to gaseous emissions, the more quantity of liquid in the system will increase the possibility of emissions. Result mesocosm can be applied in prototype is; multiply mesocosm result with the comparison volume of prototype and mesocosm. The result will determine gaseous emission on volume basis.

2.3. Dose application

Although the surface and volume of lagoons is bigger than mesocosm, the liquid concentration inlet of lagoon is always less concentrate than mesocosm ones because lagoon inlet liquid must pass more filtration parts than mesocosm. The chemicals elements that exist in lagoon are less than mesocosm, consequently, the gaseous emission that will be released by lagoon should be less than mesocosm because there are not enough source of chemical elements to be converted into gaseous phases.

The comparison between application dose of lagoon and mesocosm must be in the equation of lagoon emission. This parameter can indicate that dose application can decrease the emission in lagoon.

2.4. Calculation for N_2O

According the parameters, the equation for determining emission for lagoon on volume basis:

$E_{L,V} = E_M \times (V_L/V_M) \times (C_{L,input} / C_{M,input})$

$E_{L,V}$ = Gaseous emission of lagoon on volume basis

E_M = Gaseous emission of mesocosm

V_L = Lagoon volume

V_M = Mesocosm volume

$C_{L,input}$ = Input concentration of lagoon

$C_{M,input}$ = Input concentration of mesocosm

According to the parameters, the equation for determining emission for lagoon on area basis:

$E_{L,A} = E_M \times (A_L/A_M) \times (C_{L,input} / C_{M,input})$

$E_{L,A}$ = Gaseous emission of lagoon on volume basis

E_M = Gaseous emission of mesocosm

A_L = Lagoon large

A_M = Mesocosm large

$C_{L,input}$ = Input concentration of lagoon

$C_{M,input}$ = Input concentration of mesocosm

The experiment showed that concentration of nitrogen in the piggery outlet was around 340 mg $N.L^{-1}$. The liquid quantity that was applied to vermifilter is 800 L for one flushing. During one day, there are 6 times applications. Total liquid application of vermifilter is 4800 L per day. Every day, there will be 1.6 kg N that is released by the piggery.

Mesocosm has 0.21 m^2 area and 50 L of volume, while total vermifilter has 40 m^2 and around 0.5 m height. The total nitrogen concentration of liquid that was applied to mesocosm was around 270 mg $N.L^{-1}$. In the case of nitrous oxide, the gaseous measurements showed a mesocosm release of 12 mg N-N_2O.day^{-1}. This value is same as 0.13% of piggery output if we assume that all vermifilter has the same emission and if we extrapolate on an area basis.

Mesocosm

Area (m^2)	Volume (L)	N_2O	
		Liquid Concentration	Gaseous Emission (mg N-N_2O/day)
0.21	55	271.14	12

The emission equation on volume and area basis, on concentration basis, was used to calculate the emission in each level of prototype lagoon. The emission observed in the treatment 6 L/day was used as the gaseous emission of vermifiltration because the other treatments (2 or 28 L/day) were either too low to feed the earthworm population or too high to avoid anoxic conditions.

The example of calculation; N_2O emission of P1 (Prototype level 1) on area basis:

$$\text{Total } E_{L,A} = E_M \times (A_L/A_M) \times (C_{L,input} / C_{M,input})$$
$$= 12 \times (38/0.21) \times (69.5 \times 270)$$
$$= 557 \text{ mg.day}^{-1}$$

Emission per area

$$E_{L,A} = \text{Total } E_{L,A} / \text{Area}$$
$$= 557 \text{ mg.day}^{-1} / 38 \text{ m}^{-2}$$
$$= 14.6 \text{ mg .day}^{-1}.\text{m}^{-2}$$

Lagoon of Prototype

Level	Area (m^2)	Volume (L)	N_2O				
			Liquid Concentration	Emission Gaseous			
			Average total N during period Augt - Dec 2008	Area		Volume	
				Total (mg day^{-1})	per ($mg.day^{-1}.m^{-2}$)	Total	per Litter ($mg.day^{-1}.L^{-1}$)
P1	38	25000	69.5	557	14.6	1398	0.06
P2	102	50000	57.4	1234	12.1	2309	0.05
P3	44	25000	38.7	359	8.2	779	0.03
P4	174	50000	28.1	1166	5.9	1130	0.02
P5	181	250000	17.9	683	3.8	3601	0.01
			Total Lagoon	3998	45	9217	
			%N-Piggery outlet	0.24%		0.6%	

Table XXV. N-N_2O emission from lagooning extrapolated from vermifilter observations during mesocosm experiment

The calculation results given in Table XXV. showed lagoon had indirect effect to the reducing of N_2O emission both on area basis (m^{-2}) or volume ones (L^{-1}). Lagoon could reduce chemical element including total nitrogen. In the case of N_2O emission, total nitrogen is important factor because the several part of liquid nitrogen will be change as N_2O gas. The total nitrogen concentration in the last lagoon is lowest than the previous ones because the filtration process in the previous lagoons. The calculation result of N_2O emission per unit both area and volume in last level lagoon was lowest than the previous ones. According to calculation on area basis, the gaseous emission decreased from 14 to 3.8 $mg.day^{-1}.m^{-2}$. The calculation on volume basis, N_2O emission decreased from 0.06 to 0.02 $mg.day^{-1}.L^{-1}$.

N_2O calculation result showed emission range of N_2O are between 150 – 610 $\mu g.h^{-1}.m^{-2}$. This result is higher than result of Teiter and Mander (2005) who found the emission N-N_2O from -0.4 to 58 $\mu g.h^{-1}.m^{-2}$ on a constructed wetland for municipal wastewater with N input of 34 g $N.m^{-2}.day^{-1}$ for average annual temperature around 5℃. The result of calculation is higher than the m easurement given in the literature. Therefore it is assumed that the emission will not be above our calculation.

When this N_2O emission is compared to the nitrogen output of the piggery, it represents 0,24% of N contained in the effluent. This percentage is low compared to the loss of N_2O after spreading of liquid or solid animal manure (1.25% Bouwman, 1996).

2.5. Hypothesis for CO_2, CH_4 and NH_3

The calculation based on volume could only be applied for nitrous oxide but not for CO_2, CH_4 and NH_3 because the emissions processes are supposed to be too much different in the vermifilter and in the lagoons.

Net emission of CO_2 depends on COD removal by the microorganisms and on the primary production by microphytes and macrophytes. Because of high primary production, net CO_2 emission should be decreased in lagoon system (Mander et al. 2008).

Methane sink was observed in the mesocosm experiment due to earthworm casts. This effect should not dominate in the lagoons. On the contrary, methane emission has been observed in constructed wetland (Altor and Mitsch, 2006; Stadmark and Leonardson, 2005). It can be emitted by the sediments where anaerobic conditions can occur. In our case, we suppose that the high rate of water transfer (around 5 day residence time) and the oxygen supply within the water by the macrophytes will induce methane oxidation if it is produced by the sediments.

Ammonia emission is commonly observed during the storage of animal manure (Loughrin et al., 2006). It was only observed in the case of high dose in our mesocosm experiment but not with a dose corresponding to the continuous functioning of the large-scale vermifilter. Observed ammonia concentrations in the lagoons were lower than in the vermifilter output. Therefore, we suppose that ammonia emissions from the lagoons will be negligible compared to ammonia emissions from manure storage.

3. “Treating” or “Recycling”

The purification system of Guernévez is the treating and recycling system. The treating starts from piggery. The animal effluent that falls below the slatted-floor of the piggery was evacuated by water of the storage lagoon. The evacuation process follows the periodic operation of the pumps. The aim of evacuation process was to avoid the accumulation of pigs’ effluents in the piggery. The accumulation of effluents during several weeks can make the chemical composition of effluent change to poisonous for animals or it can increase the emission of ammonia and methane. The water that was used to evacuate the effluents would dilute the concentration of chemical elements in the effluent. These processes made the concentration of nutrients in outlet piggery lower than the initial effluent without flushing. In this phase, the treating used physical and chemical mechanisms.

When the effluent passes the sieve, the solid and liquid phase are separated. The content of chemical elements in the liquid outlet of the sieve would be lower than in the inlet ones because of the removal of solid particles. This solid can be exported or composted and reused as a fertilizer.

After the sieve, the effluent passes the vermifilter. In this part, earthworms especially their system digestive would help to transfor the chemical elements. In their digestive system, there are interactions with microorganisms increasing the stability of the released organic matter (earthworm casts) compared to the initial effluent (pig slurry). The earthworms casts can either remain in the vermifilter porosity or be carried away with the water and collected in the settling tank.

In lagoon, the plants helped to decrease the concentration of chemical elements. In the physical aspect, they would decrease the liquid velocity and induce sedimentation of chemical elements in the lagoon. Analysis showed a decrease in the concentrations of N, P, Cu, Zn (not K) when the roots of the floating plants (P1) were washed before analysis. In the biological and chemical aspect, plant would absorb the chemical element. The interaction between plants and microorganism influence the denitrification and fixation which decrease the concentrations of chemical elements. In this phase, the treating used physical, chemical and biological mechanism.

The system purification of Guernévez use integrated system. In the first part (sieve and vermifilter), the physical, biological and chemical treatment were used. In the second part (lagoon), the biological and chemical treatment was used. Park (2009) recommended integrated wetland to reduce the concentration of chemical elements from a wastewater. The variation of wetland media (water or gravel) can also break the pathogen microorganism cycle.

The system purification of Guernévez is recycling system. The water that was used for evacuate animal effluents on below piggery was from the last lagoon that called storage lagoon. As the water is not released, all the nutrients that come from the animal effluents should be exported as solids (N, P, K, etc.) or gases (N). In vermifilter, several parts chemical elements will be stored in the vermicompost. Vermicompost is organic fertilizer. There is the possibility to apply this fertilizer for agriculture plantation. The harvest of agriculture plantation will be the source of animal feed. This food chain is same as recycle process that the chemical elements will return to their source place and will follow the same process.

In lagoon part, the plant that live there such as *Azolla sp* can be used as animal feed. After vermifilter, the wastewater that was from animal will pass lagoon system. Azolla that live in the lagoon will absorb the chemical element of animal wastewater. When azolla were harvested, it will be used as animal feed. In this case, the chemical elements which are from animal will return to animal and will pass same several processes.

The plants that live in the gravel wetland have same condition as in water media. They absorb the chemical elements of wastewater. The harvest of plants can be used as litter for animals or it can be composted and be used as organic fertilizer. This fertilizer can be applied to plants that are source of animal feed. If the feed are consumed by animal, there are the recycle of chemical elements.

Conclusion

Intensive animal production has negative effect because their wastewater can make pollution of soil, water and air. The biological purification system could reduce their pollutions effects. The biological purification system of Guernévez uses the mix system with vermifiltration and constructed wetlands as media of filtration. The others aims of this biological system purification are the exportation of organic matter and plants.

Vermifilter is used to reduce the pollution effect of pig slurry. The other side, pig slurry has function as the feed source for earthworms in vermifilter. The relationship between input quantity of pig slurry and earthworm abundance in vermifilter is supposed to be a bell-shaped curve. The top of curve or optimum quantity in this experiment was around 6 $L.day^{-1}$ because most of its result parameters (average earthworm abundance, surface abundance of earthworms and earthworm biomass of average samples) were in high level. This experiment gave indications on maximum hydraulic load (around 100 $L.day^{-1}.m^{-2}$) and optimal organic input of pig organic matter (above or equal 120 g dry $matter.day^{-1}.mesocosm^{-1}$ where each mesocosms had a population around 500 g earthworms within around 30 kg of wet vermicompost).

Mesocosm vermifilters could explain the phenomena in large scale vermifilter. The quality of pig slurry and the dose influenced the change in environment and earthworm parameters. The earthworm mortality and vermifilter clogging will be found in the homogenous quality of pig slurry. The earthworm abundance phenomena in mesocosm vermifilter indicated the earthworms were in all area of vermifilter.

The earthworm abundance in vermifilter is influenced by animal wastewater. In the less and optimal dose, earthworm abundance were stable from begin to end of experiments. In high dose, the earthworms abundance was high in first week experiment and decreased in the end of experiment because of earthworm mortality and migration.

Vermifilter could indirectly reduce gaseous emission NH_3, CH_4, and N_2O. In less and optimal dose of animal wastewater, sinks of methane were clearly observed. In high dose of organic matter input, earthworms of vermifilter could reduce gaseous emission of N_2O. On the contrary, in less input of organic matter input, earthworms increased gaseous emission of N_2O. A new hypothesis was suggested to explain this observation supported by previous observations of the literature. The effect of earthworms on N_2O emission depends on the input flux of nitrogen.

Vermifilter can contribute to conserve nitrogen for agricultural uses and reduce the potential global warming associated to animal farms which are using a liquid management of the animal effluents. NH_3 emission can be minimized by the arrangement of organic matter input dose and frequent flushing. CH_4 emission will be replaced by sink CH_4. Vermifilter could decrease N_2O emission. There are three mechanisms of N_2O emission decrease (i) The dilution of added nitrogen inside vermifilter because the porosity which were made by earthworms, the added nitrogen will be rested on surface in treatment without earthworms; (ii) the reduction in nitrous oxide emissions through the effect of epigeic earthworms on the structure of the upper organic layer (Ellenberg et al. cited by Borken et al., 2000); (iii) the result of earthworms digestives system, the organic matter of fresh input that pass the earthworms will be more stable and will difficult to leave vermifilter as N_2O emission.

The mix purification system could reduce the chemical elements. Concentrations of COD, N and K always decreased from piggery outlet to storage lagoon. Phosphorus did not always decrease. In the last lagoon, an increase in P concentration was observed that could be explained by the release of P accumulated in the sediments during the cold season. The potassium concentration was higher than nitrogen concentration because the removal efficiency of potassium was less. However the output of potassium through plants and organic matter should compensate for the input by animal effluent because the concentration reached a stable level, higher when plants did not grow and lower when plant growth was maximum.

The Guernévez recycling system used mix treatment system. Physical treatment is found in animal building and sieve. Biological and chemical treatments are found in vermifilter and lagoon system.

The system indicated that the sedimentation was more efficient to reduce the concentration of chemical elements because the weight of its chemical elements was heavier than the uptake by plants.

Our work showed that seasonal variations in a combined wetland induced lower COD and higher total nitrogen in winter compared to summer in outlet water. This could be explained by the effect of temperature on chemical and biological process that lead to degradation of organic matter, nitrogen nitrification and denitrification and absorption during plant growth.

The use of these results to design wetland for livestock wastewater should be adapted depending on the macrophyte used in free water or planted wetlands. The plant growth can be used as the plant uptake response but cannot be used as the efficiency indicator or chemical element removal.

The calculation among gaseous emissions in vermifilter and several factors such as size among components and dose application could be used to estimate the N_2O emissions from lagoon. The estimates decreased from first lagoon to storage lagoon. Emissions of methane and ammonia were assumed to be negligible compared to the emissions of a liquid storage of slurry.

References

Aira M., Monroy F., Dominguez J. (2007) Eisenia fetida (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure. Microbial Ecology, 54, 662–671.

Aira, M. & J. Dominguez (2008) Optimizing vermicomposting of animal wastes: Effects of rate of manure application on carbon loss and microbial stabilization. Journal of Environmental Management, 88, 1525-1529.

Altor, A. E. & W. J. Mitsch (2006) Methane flux from created riparian marshes: Relationship to intermittent versus continuous inundation and emergent macrophytes. Ecological Engineering, 28, 224-234.

Arias, C. A., A. Cabello, H. Brix & N. H. Johansen (2003) Removal of indicator bacteria from municipal wastewater in an experimental two-stage vertical flow constructed wetland system. Water Science and Technology, 48, 35-41.

Atiyeh, R. M., C. A. Edwards, S. Subler & J. D. Metzger (2001) Pig manure vermicompost as a component of a horticultural bedding plant medium: effects on physicochemical properties and plant growth. Bioresource Technology, 78, 11-20.

Atiyeh, R. M., J. Dominguez, S. Subler & C. A. Edwards (2000) Changes in biochemical properties of cow manure during processing by earthworms (Eisenia andrei, Bouche) and the effects on seedling growth. Pedobiologia, 44, 709-724.

Ayaz, S. C. (2006) Post-treatment and reuse of tertiary treated wastewater by constructed wetlands. In 10th IWA International Specialized Conference on Diffuse Pollution and Sustainable Basin Management, 249-255. Istanbul, TURKEY.

Basset-Mens C., van der Werf H.M.G., Robin P., Morvan Th., Hassouna M., Paillat J-M., Vertès F., (2006) Methods and data for the environmental inventory of contrasting pig production systems. Journal of Cleaner Production, 15, 1395-1405.

Beck-Friis, B., S. Smars, H. Jonsson & H. Kirchmann (2001) Gaseous emissions of carbon dioxide, ammonia and nitrous oxide from organic household waste in a compost reactor under different temperature regimes. Journal of Agricultural Engineering Research, 78, 423-430.

Binet, F., L. Fayolle & M. Pussard (1998) Significance of earthworms in stimulating soil microbial activity. Biology and Fertility of Soils, 27, 79-84.

Birgand, F., Skaggs, R. W., Chescheir, G. M., Gilliam, J. W. (2007) Nitrogen Removal in Streams of Agricultural Catchments - A Literature Review. Critical Reviews in Environmental Science and Technology, 37:5, 381 – 487

Bohlen, P. J. & C. A. Edwards (1995) Earthworm effects on N dynamics and soil respiration in microcosms receiving organic and inorganic nutrients. Soil Biology & Biochemistry, 27, 341-348.

Borken W, Grundel S, Beese F (2000) Potential contribution of Lumbricus terrestris L. to carbon dioxide, methane and nitrous oxide fluxes from a forest soil. Biol Fertil Soils, 32, 142–148.

Bouché M.B. (1972) Lombriciens de France. Ecologie et systématique. INRA, Paris. 671 p.

Bouwman, A.F. (1996) Direct emissions of nitrous oxide from agricultural soils. Nutrient Cycling in th Agroecosystems, 46, 53p.

Boyle, K. E., J. P. Curry & E. P. Farrell (1997) Influence of earthworms on soil properties and grass production in reclaimed cutover peat. Biology and Fertility of Soils, 25, 20-26.

Brix, H. & C. A. Arias (2005a) Danish guidelines for small-scale constructed wetland systems for onsite treatment of domestic sewage. Water Science and Technology, 51, 1-9.

Brix, H. & C. A. Arias (2005b) The use of vertical flow constructed wetlands for on-site treatment of domestic wastewater: New Danish guidelines. Ecological Engineering, 25, 491-500.

Burton, C. H. & C. Turner (2003) Manure management. Treatment strategies for sustainable Agriculture. Wrest Park-Silsoe-Bedford-UK: Silsoe Research Institute.

Castillo, A. E., S. G. Benito & M. C. Iglesias (2005) Influence of earthworms on organic waste compostingand characterisation of vermicompost end products. Spanish Journal of Agricultural Research, 3(1), 145-150.

Charlier R. H.; Morand P.; Finkl C. W.; Thys A. (2007) Green Tides on the Brittany Coasts. Environmental Research, Engineering and Management, 3(41), 52-59. http://www.aquafuels.eu/attachments/066_Presentation%20-%20P.%20Morand%20(CNRS)%20-%20Macro-algae%20blooms%20and%20potential%20biofuels.pdf

Clarke W.P., Taylor M., Cossins R. (2007) Evaluation by respirometry of the loading capacity of a high rate vermicompost bed for treating sewage sludge. Bioresource Technology, 98, 2611–2618.

Constance, D. H. & A. Bonanno (1999) CAFO Controversy in the Texas Panhandle Region: The Environmental Crisis of Hog Production. In Culture & Agriculture, 23-26.

Contreras-Ramos S.M., Alvarez-Bernal D., Montes-Molina J.A., Van Cleemput O., Dendooven L. (2009) Emission of nitrous oxide from hydrocarbon contaminated soil amended with waste water sludge and earthworms. Applied Soil Ecology 41, 69-76.

Delgado, C. L. (2003) Rising consumption of meat and milk in developing countries has created a new food revolution. Journal of Nutrition, 133, 3907S-3910S.

Dewes, T. (1999) Ammonia emissions during the initial phase of microbial degradation of solid and liquid cattle manure. Bioresource Technology, 70, 245-248.

Drake H. L., Horn M.A. (2006) Earthworms as a transient heaven for terrestrial denitrifying microbes: a review. Eng. Life Sci., 6 (3), 261-265.

Drake H. L., Horn M.A. (2007) As the Worm Turns: The Earthworm Gut as a Transient Habitat for Soil Microbial Biomes. Annu. Rev. Microbiol., 61, 169–89.

Dunne, E. J., N. Culleton, G. O'Donovan, R. Harrington & A. E. Olsen (2005) An integrated constructed wetland to treat contaminants and nutrients from dairy farmyard dirty water. Ecological Engineering, 24, 221-234.

Edwards C.A., Arancon N.Q. (2008) Can Earthworms Harm the Planet? BioCycle. November, 2008. 53-54.

Edwards C.A., Bohlen P.J. (1996) Biology and ecology of earthworms. Chapman & Hall ed. Third edition. ISBN 0 412 56160 3.

El Hamouri, B., J. Nazih & J. Lahjouj (2007) Sub surface-horizontal flow constructed wetland for sewage treatment under Moroccan climate conditions. Desalination, 215, 153-158.

FAO (2006a) Livestock's long shadow, environmental issues and options. ISBN 978-92-5-105571-7, 416p.

FAO (2006b) Livestock report. ISBN 92-5-105421-5, 85p.

Fayolle L., Michaud H., Cluzeau D., Stawiecki J. (1997) Influence of temperature and food source on the life cycle of the earthworm Dendrobena veneta (Oligochaeta). Soil Biol. Biochem., 29 (314), 747-750.

Ferreira, F. L. A., J. de Lucas & L. A. do Amaral (2003) Partial characterization of the polluting load of swine wastewater treated with an integrated biodigestion system. Bioresource Technology, 90, 101-108.

Fiévet S. (2008) Etude du fonctionnement d'un système de lagunage à macrophytes pour l'épuration d'effluents d'élevage de la Station Porcine Expérimentale de Guernévez des Chambres d'Agriculture de Bretagne aux échelles expérimentale et pilote. Mémoire de fin d'études Master IEN 2, Ecole et Observatoire des Sciences de la Terre, 90p.

Frederickson J., Howell G. (2003) Large-scale vermicomposting: emission of nitrous oxide and effects of temperature on earthworm populations. Pedobiologia 47, 724–730.

Frederickson J., Howell G., Hobson A., Lane J. (2006) Adding value to waste derived compost: performance and environmental impact of a combined in-vessel composting and vermicomposting system. Veolia Environmental Trust, Integrated Waste Systems Research Group, The Open University, Walton Hall, Milton Keynes, MK7 6AA. 87p. http://www.wormresearchcentre.co.uk/scientific/Vermicomposting%20-%20adding%20value%20to%20compost.pdf

Friel, S., A. D. Dangour, T. Garnett, K. Lock, Z. Chalabi, I. Roberts, A. Butler, C. D. Butler, J. Waage, A. J. McMichael & A. Haines (2009) Health and Climate Change 4 Public health benefits of strategies to reduce greenhouse-gas emissions: food and agriculture. Lancet, 374, 2016-2025.

Fry F. E. J. (1947) Effects of the environment on animal activity. Univ. Toronto Study. Biol. Ser. 55, Publ. Ont. Fish. Res. Lab. 68, l-62.

Garg, P., A. Gupta & S. Satya (2006) Vermicomposting of different types of waste using Eisenia foetida: A comparative study. Bioresource Technology, 97, 391-395.

Garg, V. K. & P. Kaushik (2005) Vermistabilization of textile mill sludge spiked with poultry droppings by an epigeic earthworm Eisenia foetida. Bioresource Technology, 96, 1063-1071.

Gerke, S., L. A. Baker & Y. Xu (2001) Nitrogen transformations in a wetland receiving lagoon effluent: Sequential model and implications for water reuse. Water Research, 35, 3857-3866.

Ghosh M., G.N. Chattopadhyay, K. Baral (1999) Transformation of phosphorus during vermicomposting. Bioresource Technology, 69, 149-154.

Gilland, B. (2002) World population and food supply - Can food production keep pace with population growth in the next half-century? Food Policy, 27, 47-63.

Grant W.C. (1955) Temperature Relationships in the Megascolecid Earthworth Pheretima Hupeiensis. Ecology, 36 (3), 412-417

Greenway, M. (2005) The role of constructed wetlands in secondary effluent treatment and water reuse in subtropical and arid Australia. Ecological Engineering, 25, 501–509.

Healy, M. G., M. Rodgers & J. Mulqueen (2007) Treatment of dairy wastewater using constructed wetlands and intermittent sand filters. Bioresource Technology, 98, 2268-2281.

Hermoso, V., Clavero, M., Blanco-Garrido, F. and Prenda, J. (2009), Assessing freshwater fish sensitivity to different sources of perturbation in a Mediterranean basin. Ecology of Freshwater Fish, 18: 269–281.

Hernandez, M. E. & W. J. Mitsch (2007a) Denitrification in created riverine wetlands: Influence of hydrology and season. Ecological Engineering, 30, 78-88.

Hernandez, M. E. & W. J. Mitsch (2007b) Denitrification potential and organic matter as affected by vegetation community, wetland age, and plant introduction in created wetlands. Journal of Environmental Quality, 36, 333-342.

Héry M., Singer A.C., Kumaresan D., Bodrossy L., Stralis-Pavese N., Prosser J.I., Thompson I.P., Murrell J.C. (2008) Effect of earthworms on the community structure of active methanotrophic bacteria in a landfill cover soil. International Society for Microbial Ecology Journal, 2, 92–104.

Higgins, C. W. & L. P. Walker (2001) Validation of a new model for aerobic organic solids decomposition: simulations with substrate specific kinetics. Process Biochemistry, 36, 875-884.

Hobson A.M., Frederickson J., Dise N.B. (2005) CH4 and N2O from mechanically turned windrow and vermicomposting systems following in-vessel pretreatment. Waste Management, 25, 345-352.

Hunt,P. G., M. E. Poach, T. A. Matheny, G. B. Reddy, & K. C. Stone (2006) Denitrification in Marsh-Pond-Marsh Constructed Wetlands Treating Swine Wastewater at Different Loading Rates. Soil Sci. Soc. Am. J., 70, 487–493.

Jing, S. R. & Y. F. Lin (2004) Seasonal effect on ammonia nitrogen removal by constructed wetlands treating polluted river water in southern Taiwan. Environmental Pollution, 127, 291-301.

Jordan, T. E., D. F. Whigham, K. H. Hofmockel & M. A. Pittek (2003) Nutrient and sediment removal by a restored wetland receiving agricultural runoff. Journal of Environmental Quality, 32, 1534-1547.

Kadlec, R. H. (2003) Pond and wetland treatment. Water Science and Technology, 48, 1-8.

Kadlec, R. H. (2010) Nitrate dynamics in event-driven wetlands. Ecological Engineering, 36, 503-516.

Kadlec, R. H.; Knight, R. L. (1996) Treatment Wetlands. Lewis Publishers: CRC Press: Boca Raton, FL. 893p.

Kaiser, J. (1996) Modelling composting as a microbial ecosystem: A simulation approach. Ecological Modelling, 91, 25-37.

Kale, R. D., B. C. Mallesh, K. Bano & D. J. Bagyaraj (1992) influence of vermicompost application on the available macronutrients and selected microbial populations in a paddy field. Soil Biology & Biochemistry, 24, 1317-1320.

Kammann C., Hepp S., Lenhart K., Müller C. (2009) Stimulation of methane consumption by endogenous CH_4 production in aerobic grassland soil. Soil Biology & Biochemistry, 41, 622–629.

Kaushik, P. & V. K. Garg (2004) Dynamics of biological and chemical parameters during vermicomposting of solid textile mill sludge mixed with cow dung and agricultural residues. Bioresource Technology, 94, 203-209.

Kaviraj & S. Sharma (2003) Municipal solid waste management through vermicomposting employing exotic and local species of earthworms. Bioresource Technology, 90, 169-173.

Keffala, C. & A. Ghrabi (2005) Nitrogen and bacterial removal in constructed wetlands treating domestic waste water. Desalination, 185, 383-389.

Khiari L., L.E. Parent, N. Tremblay (2001) Selecting the High-Yield Subpopulation for Diagnosing Nutrient Imbalance in Crops. Agronomy Journal, 93, 802-808.

Kivaisi, A. K. (2001) The potential for constructed wetlands for wastewater treatment and reuse in developing countries: a review. Ecological Engineering, 16, 545-560.

Knight, R. L., V. W. E. Payne, R. E. Borer, R. A. Clarke & J. H. Pries (2000) Constructed wetlands for livestock wastewater management. Ecological Engineering, 15, 41-55.

Knight, R. L., W. E. Walton, G. F. O'Meara, W. K. Reisen & R. Wass (2003) Strategies for effective mosquito control in constructed treatment wetlands. Ecological Engineering, 21, 211-232.

Kovacic, D. A., R. M. Twait, M. P. Wallace & J. M. Bowling (2006) Use of created wetlands to improve water quality in the Midwest - Lake Bloomington case study. Ecological Engineering, 28, 258-270.

Lansing, S. L. & J. F. Martin (2006) Use of an ecological treatment system (ETS) for removal of nutrients from dairy wastewater. Ecological Engineering, 28, 235-245.

Lavelle P., Bignell D., Lepage M., Wolters V., Roger P., Ineson P., Heal O.W., Dhillion S. (1997) Soil function in a changing world: the role of invertebrate ecosystem engineers. European Journal of Soil Biology, 33 (4), 159-193.

Lavrova, S. & B. Koumanova (2007) Polishing of aerobically treated wastewater in a constructed wetland system. The University of Chemical Technology and Metallurgy,, 42, 195-200.

Lawniczak, A. E., S. Gusewell & J. T. A. Verhoeven (2009) Effect of N:K supply ratios on the performance of three grass species from herbaceous wetlands. Basic and Applied Ecology, 10, 715-725.

Lee, K. E. & R. C. Foster (1991) Soil fauna and soil structure. Australian Journal of Soil Research, 29, 745-775.

Levasseur P. (2004) Traitement des effluents porcins. Guide pratique des procédés. IFIP Institut du Porc. ISBN 2-85969-163-4. 36p.

Li, Y. S., P. Robin, D. Cluzeau, M. Bouche, J. P. Qiu, A. Laplanche, M. Hassouna, P. Morand, C. Dappelo & J. Callarec (2008) Vermifiltration as a stage in reuse of swine wastewater: Monitoring methodology on an experimental farm. Ecological Engineering, 32, 301-309.

Loughrin, J. H., A. A. Szogi & M. B. Vanotti (2006) Reduction of malodorous compounds from a treated swine anaerobic lagoon. Journal of Environmental Quality, 35, 194-199.

Lu, S. Y., F. C. Wu, Y. Lu, C. S. Xiang, P. Y. Zhang & C. X. Jin (2009) phosphorusus removal from agricultural runoff by constructed wetland. Ecological Engineering, 35, 402-409.

Mancebo, d. C. S. U., J. P. A. Hettiaratchi & O. Hurtado (2010) Compost as a Growth Medium of Methanotrophic Bacteria. Journal of Solid Waste Technology and Management, Proceedings of 25th International Conference on Solid Waste Technology and Management.

Mander, U., K. Lohmus, S. Teiter, T. Mauring, K. Nurk & J. Augustin (2008) Gaseous fluxes in the nitrogen and carbon budgets of subsurface flow constructed wetlands. Science of the Total Environment, 404, 343-353.

Matos, A. T., Abrahao, S. S., Borges, A. C., Matos, M. P. (2010) Influence of organic loading rate on the performance of constructed wetlands cultivated with grass. Engenharia Sanitaria e Ambiental. 2010, vol.15, n.1, pp. 83-92.

Meuleman, A. F. M., R. van Logtestijn, G. B. J. Rijs & J. T. A. Verhoeven (2003) Water and mass budgets of a vertical-flow constructed wetland used for wastewater treatment. Ecological Engineering, 20, 31-44.

Mitchell, A. (1994) Production of Eisenia fetida and vermicompost from feed-lot cattle manure. In 5th International Symposium on Earthworm Ecology (ISEE 5), 763-766. Columbus, Oh.

Mitsch, W. J., L. Zhang, C. J. Anderson, A. E. Altor & M. E. Hernandez (2005) Creating riverine wetlands: Ecological succession, nutrient retention, and pulsing effects. Ecological Engineering, 25, 510-527.

Moon, L. E., S. Y. Lee, S. H. Lee, H. W. Ryu & K. S. Cho (2010) Earthworm cast as a promising filter bed material and its methanotrophic contribution to methane removal. Journal of Hazardous Materials, 176, 131-138.

Morand P., Robin P., Pourcher A.M., Oudart D., Fievet S., Luth, Cluzeau D., Picot B., Landrain B. (2011) Design of an integrated piggery system with recycled water, biomass production and water purification by vermiculture, macrophyte ponds and constructed wetlands. Water science and technology, 63 (6), 1314-1320.

Morand P., Robin P., Qiu J.P., Li Y.S., Luth, Cluzeau D., Hamon G., Amblard C., Fievet S., Oudart D., Pourcher A.M., Picot B., Landrain B. (2009) Test of an extensive system combining screen, vermifilter, macrophyte lagoons, and constructed wetlands to recover nutrients from fresh manure and recycle water for flushing in pig housing. Progress in Environmental Science and Technology, Vol. II (parts A and B) 1122-1132.

Nagavallemma, K., S. Wani, S. Lacroix, V. Padmaja, C. Vineela, M. B. Rao & K. Sahrawat (2004) Vermicomposting : Recycling wastes into valuable organic fertilizer. In Global theme on Agroecosystems, 20. Andhra Pradesh, India: ICRISAT (International Crops Research Institute for the Semi-Arid Tropics.

Ndegwa P.M.,Thompson S.A., Das K.C. (2000) Effects of stocking density and feeding rate on vermicomposting of biosolids. Bioresource Technology, 71, 5-12.

Newman, J. M., J. C. Clausen & J. A. Neafsey (2000) Seasonal performance of a wetland constructed to process dairy milkhouse wastewater in Connecticut. Ecological Engineering, 14, 181-198.

Odum, E.P. (1971) Fundamentals of Ecology, 3rd ed.W.B. Saunders Company, Philadelphia.

Oudart, D. (2009) Etude du traitement du lisier de porc par lombrifiltration et lagunage à macrophytes à l’échelle du pilote. Projet de fin d’étude Septembre 2008-Février 2009, 117p.

Padmavathiamma, P. K., L. Y. Li & U. R. Kumari (2008) An experimental study of vermi-biowaste composting for agricultural soil improvement. Bioresource Technology, 99, 1672-1681.

Park, W. H. (2009) Integrated constructed wetland systems employing alum sludge and oyster shells as filter media for P removal. Ecological Engineering, 35, 1275-1282.

Petersen, S. O., S. G. Sommer, F. Beline, C. Burton, J. Dach, J. Y. Dourmad, A. Leip, T. Misselbrook, F. Nicholson, H. D. Poulsen, G. Provolo, P. Sorensen, B. Vinneras, A. Weiske, M. P. Bernal, R. Bohm, C. Juhasz & R. Mihelic (2007) Recycling of livestock manure in a whole-farm perspective. Livestock Science, 112, 180-191.

Pierce S.C., M. T. Moore, D. Larsen & S. R. Pezeshki (2010) Macronutrient (N, P, K) and Redoximorphic Metal (Fe, Mn) Allocation in Leersia oryzoides (Rice Cutgrass) Grown Under Different Flood Regimes. Water Air Soil Pollution, 207, 73-84.

Poe, A. C., M. F. Piehler, S. P. Thompson & H. W. Paerl (2003) Denitrification in a constructed wetland receiving agricultural runoff. Wetlands, 23, 817-826.

Pramanik, P., G. K. Ghosh, P. K. Ghosal & P. Banik (2007) Changes in organic - C, N, P and K and enzyme activities in vermicompost of biodegradable organic wastes under liming and microbial inoculants. Bioresource Technology, 98, 2485-2494.

Raisin, G. W., D. S. Mitchell & R. L. Croome (1997) The effectiveness of a small constructed wetland in ameliorating diffuse nutrient loadings from an Australian rural catchment. Ecological Engineering, 9, 19-35.

Reilly, J. F., A. J. Horne & C. D. Miller (2000) Nitrate removal from a drinking water supply with large free-surface constructed wetlands prior to groundwater recharge. Ecological Engineering, 14, 33-47.

Romero, J. A., F. A. Comin & C. Garcia (1997) Restored wetlands as filters to remove nitrogen. In 13th International Symposium on Environmental Biogeochemistry (ISEB), 323-332. Monopoli, Italy.

Rule, A. M., A. R. Chapin, S. A. McCarthy, K. E. Gibson, K. J. Schwab & T. J. Buckley (2005) Assessment of an aerosol treatment to improve air quality in a swine concentrated animal feeding operation (CAFO). Environmental Science & Technology, 39, 9649-9655.

Sampredo L., Dominguez J. (2008) Stable isotope natural abundances (δ13C and δ15N) of the earthworm Eisenia fetida and other soil fauna living in two different vermicomposting environments. Applied Soil Ecology, 38 (9), 1-99.

Saunders, D. L. & J. Kalff (2001) Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia, 443, 205-212.

Schulz, C., J. Gelbrecht & B. Rennert (2003) Treatment of rainbow trout farm effluents in constructed wetland with emergent plants and subsurface horizontal water flow. Aquaculture, 217, 207-221.

Sévrin-Reyssac, J., J. d. l. Noüe & D. Proulx (1999) Le recyclage du lisier de porc par lagunage. Ed. Tech.& Doc./Lavoisier.

Sharma, S., P. Pradhan, S. Satya & P. Vasudevan (2005) Potentiality of Earthworms for Waste Management and in Other Uses – A Review. The Journal of American Science, 1(1), 4-16.

Shelford, V.E. (1911) Ecological succession I. Stream fishes and the method of physiografic analysis. Biological Bulletin 21: 9–35.

Singh, R. B. (2000) Environmental consequences of agricultural development: a case study from the Green Revolution state of Haryana, India. Agriculture Ecosystems & Environment, 82, 97-103.

Spieles, D. J. & W. J. Mitsch (2000) The effects of season and hydrologic and chemical loading on nitrate retention in constructed wetlands: a comparison of low- and high-nutrient riverine systems. Ecological Engineering, 14, 77-91.

Stadmark, J. & L. Leonardson (2005) Emissions of greenhouse gases from ponds constructed for nitrogen removal. Ecological Engineering, 25, 542-551.

Striganova B.R., Pantoshderimova T.D., Tiunov A.V. (1993) Comparative estimation of nitrogen fixation activity in the intestine of various species of earthworms. Izvestiya Akademii Nauk Serya Biologicheskaya, 2, 257-263

Subler S., Edwards C., Metzger J. (1998) Comparing vermicomposts and composts. Biocycle, 39 (7), 63-66.

Sweeten, J., R. Miner & C. Tengman (2003) A Brief History and Background of the EPA CAFO Rule. 1-4. Iowa: MidWest Plan Service.

Tamrakar, A. S. & K. Maharjan (2006) Role of vermicomposting in organic farming. In Proceedings of a First National Workshop on Organic Farming. Hariharbhawan, Lalitpur: Directorate of Agriculture Extension.

Tanner, C. C., J. S. Clayton & M. P. Upsdell (1995) effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands. 1. removal of oxygen-demand, suspended-solids and fecal-coliforms. Water Research, 29, 17-26.

Taylor, M., W. P. Clarke & P. F. Greenfield (2003) The treatment of domestic wastewater using small-scale vermicompost filter beds. Ecological Engineering, 21, 197-203.

Teiter, S. & U. Mander (2005) Emission of N_2O, N_2, CH_4, and CO_2 from constructed wetlands for wastewater treatment and from riparian buffer zones. Ecological Engineering, 25, 528-541.

Tilman, D. (1998) The greening of the green revolution. Nature, 396, 211-212.

Tureau C. (2010) Traitement des effluents, issus d'élevage porcin, par lombriliftration et lagunage à macrophytes. IUT Brest, Rapport de stage DUT Génie de l'Environnement, 53p.

Umarov M. M., Striganova B. R., Kostin N. V. (2008) Specific features of nitrogen transformation in the gut and coprolites of earthworms. Biology Bulletin, 35, 6, 643-652.

Urdaneta, C., L. M. M. Parra, S. Matute, M. A. Garaboto, H. Barros & C. Vazquez. (2007) Evaluation of vermicompost as bioadsorbent substrate of Pb, Ni, V and Cr for wastewaters remediation using total Reflection X-ray Fluorescence. In 12th International Conference on total Reflection X-Ray Fluorescence Analysis and Related Methods, 1455-1460. Trento, ITALY.

US. Environmental Protection Agency (EPA). (1999) Manual : Constructed Wetlands Treatment of Municipal Wastewaters. ed. U. S. E. P. Agency, 140. Cincinnati, Ohio: National Risk Management Research Laboratory Office of Research and Development.

Venterink, H. O., T. E. Davidsson, K. Kiehl & L. Leonardson (2002) Impact of drying and re-wetting on N, P and K dynamics in a wetland soil. Plant and Soil, 243, 119-130.

Verhoeven, J. T. A. & A. F. M. Meuleman (1996) Wetlands for wastewater treatment: Opportunities and limitations. In V International INTECOL Wetlands Conference, Wetlands for the Future, 5-12. Perth, Australia.

Vrhovsek, D., V. Kukanja & T. Bulc (1996) Constructed wetland (CW) for industrial wastewater treatment. Water Research, 30, 2287-2292.

Vymazal, J. (2002) The use of sub-surface constructed wetlands for wastewater treatment in the Czech Republic: 10 years experience. Ecological Engineering, 18, 633-646.

Vymazal, J. (2005) Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. Ecological Engineering, 25, 478-490.

Vymazal, J. (2007) Removal of nutrients in various types of constructed wetlands. Science of the Total Environment, 380, 48-65.

Wilshusen, J. H., J. P. A. Hettiaratchi, A. De Visscher & R. Saint-Fort (2004) Methane oxidation and formation of EPS in compost: effect of oxygen concentration. Environmental Pollution, 129, 305-314.

Wüst P.K., Horn M.A., Henderson G., Janssen P.H., Rehm B.H.A., Drake H.L. (2009) Gut-associated denitrification and in vivo emission of nitrous oxide by the earthworm families megascolecidae and lumbricidae in New Zealand. Applied and Environmental Microbiology, 75 (11), 3430–3436.

Yousefi, Z. & A. Mohseni-Bandpei (2010) Nitrogen and phosphorusus removal from wastewater by subsurface wetlands planted with Iris pseudacorus. Ecological Engineering, 36, 777-782.

Zemanova, K., T. Picek, J. Dusek, K. Edwards & H. Santruckova (2010) Carbon, Nitrogen and Phosphorus Tranformations are Related to Age of a Constructed Wetland. Water Air and Soil Pollution, 207, 39-48.

Appendix 1: results of cluster analysis

This appendix presents all data and results of the statistical analysis which were given in Chapter 1, particularly in Table IV.

```
*******************************************************************
************** Input data for cluster analysis without hierarchy **************
*******************************************************************
************** Sampling method: average ******************************
************** Sampling date: 16th September 2009 **********************
************** Analysed variable: abundance ****************************
*******************************************************************


Title 'ANALYSE DE CLUSTER DES VERS DE TERRE 16 SEPT 09' ;
Title2 'ECHANTILLON MIX SANS HIRARQY';

data versdt;
      input popup$ v1-v2;
cards;
 2          2833.04566138396        5671.00810828066
 4          4400.34348730001        4107.62671719564
 6          6884.34562005671        5383.70545615851
 10         3510.8715512516         4010.99807533682
 14         3623.51393261595        5594.61764427177
 18         1715.53846153846        3187.07592891761
 22         4324.31298510594        3244.77017910013
 28         5563.52873231302        3549.24326483512
;


Proc fastclus maxclusters=3 out=resultat maxiter=10;
      var v1-v2;
       title 'Donnes de vers de terre pour 3 cluster' ;
run ;


proc sort data=resultat out=resultat ;
by cluster;
proc print data=resultat;
var popup;
by cluster;
run;
```

****
************** Raw results of cluster analysis ****************************
****
************** Analysed variable: abundance ****************************
************** Sampling method: average ******************************
************** Sampling date: 16th September 2009 **********************
****

Initial Seeds

Cluster	V1	V2
1	1715.54	3187.08
2	4400.34	4107.63
3	6884.35	5383.71

Minimum Distance Between Initial Seeds = 2792.605

Iteration	Criterion	Relative Change in Cluster Seeds 1	2	3
1	826.5	0	0.1574	0
2	781.5	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 781.48

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	1	.	0	2	2607.3
2	6	988.5	1781.5	1	2607.3
3	1	.	0	2	3019.5

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	1599.0879	942.5451	0.751840	3.029660
V2	1052.2874	1032.4269	0.312422	0.454381
OVER-ALL	1353.5862	988.5081	0.619056	1.625061

Pseudo F Statistic = 4.06
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	1715.54	3187.08
2	4042.60	4363.04
3	6884.35	5383.71

Cluster Standard Deviations

Cluster	V1	V2
1	.	.
2	942.55	1032.43
3	.	.

```
------------------------------------------ Cluster=1 ------------------------------------

                                          OBS    POPUP

                                           1      18

------------------------------------------ Cluster=2 ------------------------------------

                                          OBS    POPUP

                                           2      2
                                           3      4
                                           4      10
                                           5      14
                                           6      22
                                           7      28

------------------------------------------ Cluster=3 ------------------------------------

                                          OBS    POPUP

                                           8       6
```

```
*******************************************************************
**************  Input data for cluster analysis without hierarchy **************
*******************************************************************
**************  Sampling method: average  ******************************
**************  Sampling date: 30th September 2009  **********************
**************  Analysed variable: abundance  ***************************
*******************************************************************
```

```
Title 'ANALYSE DE CLUSTER DES VERS DE TERRE 30 SEPT 09' ;
Title2 'ECHANTILLON MIX SANS HIRARQY';

data versdt;
       input popup$ v1-v2;
cards;
 2          4497.57847533632        2328.16036661601
 4          3273.56280410885        4225.64359039583
 6          4786.27147646463        3630.65107348577
 10         3791.19397064657        3880.29619243745
 14         4115.23925854167        4238.30105117123
 18         3756.58279695728        4453.69195186776
 22         5825.44260894056        5083.81577621078
 28         3667.95331226797        4033.76553094656
;


Proc fastclus maxclusters=3 out=resultat maxiter=10;
      var v1-v2;
        title 'Donnes de vers de terre pour 3 cluster' ;
run ;


proc sort data=resultat out=resultat ;
by cluster;
proc print data=resultat;
var popup;
by cluster;
run;
```

****

************** Raw results of cluster analysis ***************************

****

************** Analysed variable: abundance ***************************

************** Sampling method: average ******************************

************** Sampling date: 30th September 2009 **********************

****

Initial Seeds

Cluster	V1	V2
1	4497.58	2328.16
2	3273.56	4225.64
3	5825.44	5083.82

Minimum Distance Between Initial Seeds = 2258.021

Iteration	Criterion	Relative Change in Cluster Seeds 1	2	3
1	457.8	0.2954	0.1998	0
2	300.5	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 300.54

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	2	667.1	667.1	2	1502.4
2	5	263.5	451.3	1	1502.4
3	1	.	0	2	2295.8

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	809.3204	285.0627	0.911384	10.284654
V2	795.8066	455.8299	0.765651	3.267145
OVER-ALL	802.5919	380.1589	0.839745	5.240037

Pseudo F Statistic = 13.10
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	4641.92	2979.41
2	3720.91	4166.34
3	5825.44	5083.82

Cluster Standard Deviations

Cluster	V1	V2
1	204.137	921.000
2	301.924	218.325
3	.	.

```
------------------------------------------ Cluster=1 ------------------------------------

                                          OBS    POPUP

                                           1       2
                                           2       6


------------------------------------------ Cluster=2 ------------------------------------

                                          OBS    POPUP

                                           3      4
                                           4      10
                                           5      14
                                           6      18
                                           7      28


------------------------------------------ Cluster=3 ------------------------------------

                                          OBS    POPUP

                                           8      22
```

```
*******************************************************************
**************  Input data for cluster analysis without hierarchy **************
*******************************************************************
**************  Sampling method: average  *******************************
**************  Sampling date: 7th October 2009  *************************
**************  Analysed variable: abundance  ****************************
*******************************************************************


Title 'ANALYSE DE CLUSTER DES VERS DE TERRE 07 OCT  09' ;
Title2 'ECHANTILLON MIX SANS HIRARQY';

data versdt;
        input popup$ v1-v2;
cards;
 2           1226.73544010093          3916.86729566414
 4           4784.18592538598          4181.44565439361
 6           4510.15167185772          2753.59280225702
 10          4986.98847307228          3456.43520096122
 14          4595.04567384768          4922.06300898311
 18          4948.64170508576          3531.44838907254
 22          4674.32456732942          4983.1533159154
 28          4391.34547249411          3974.68805005568
;


Proc fastclus maxclusters=3 out=resultat maxiter=10;
       var v1-v2;
         title 'Donnes de vers de terre pour 3 cluster' ;
run ;


proc sort data=resultat out=resultat ;
by cluster;
proc print data=resultat;
var popup;
by cluster;
run;
```

****

************** Raw results of cluster analysis ***************************

****

************** Analysed variable: abundance ***************************

************** Sampling method: average ******************************

************** Sampling date: 7th October 2009 *************************

****

Initial Seeds

Cluster	V1	V2
1	1226.74	3916.87
2	4674.32	4983.15
3	4510.15	2753.59

Minimum Distance Between Initial Seeds = 2235.597

Iteration	Criterion	Relative Change in Cluster Seeds 1	2	3
1	452.8	0	0.2112	0.2596
2	293.6	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 293.59

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	1	.	0	2	3437.0
2	4	380.9	583.7	3	1284.5
3	3	356.6	580.3	2	1284.5

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	1244.5181	211.1295	0.979443	47.644393
V2	747.9474	480.8924	0.704726	2.386682
OVER-ALL	1026.7061	371.3712	0.906546	9.700495

Pseudo F Statistic = 24.25
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	1226.74	3916.87
2	4611.23	4515.34
3	4815.26	3247.16

Cluster Standard Deviations

Cluster	V1	V2
1	.	.
2	165.837	512.531
3	264.927	429.083

```
------------------------------------------- Cluster=1 -------------------------------------

                                           OBS     POPUP

                                            1        2


------------------------------------------- Cluster=2 -------------------------------------

                                           OBS     POPUP

                                            2       4
                                            3       14
                                            4       22
                                            5       28


------------------------------------------- Cluster=3 -------------------------------------

                                           OBS     POPUP

                                            6       6
                                            7       10
                                            8       18
```

```
*******************************************************************
**************  Input data for cluster analysis with hierarchy *****************
*******************************************************************
**************  Sampling method: average  *******************************
**************  Sampling date: 16th September 2009  **********************
**************  Analysed variable: biomass  *****************************
*******************************************************************


Title 'ANALYSE DE CLUSTER BIOMASS DES VERS DE TERRE 16 SEPT 09' ;
Title2 'ECHANTILLON MIX AVEC HIRARQY';

data versdt;
      input biom$ v1-v2;
cards;
 2        263.095507087191      469.559471365639
 4        455.028111723764      497.878588346755
 6        673.216534845545      384.39631131842
 10       299.427188013886      262.218999175144
 14       385.759096655156      433.408035629679
 18       237.316153846154      239.429079159935
 22       585.820857783578      577.929621899723
 28       481.873375685822      303.206781767343


;


proc cluster method=single CCC outtree = tree data=versdt ;
     var v1-v2;
     id biom;
run;

proc tree;
    id biom;
        title 'L'arbre de Groupe;
run;
```

****

************** Raw results of cluster analysis ***************************

****

************** Analysed variable: biomass *****************************

************** Sampling method: average ******************************

************** Sampling date: 16th September 2009 **********************

****

Initial Seeds

Cluster	V1	V2
1	237.316	239.429
2	455.028	497.879
3	673.217	384.396

Minimum Distance Between Initial Seeds = 245.9358

Iteration	Criterion	Relative Change in Cluster Seeds 1	2	3
1	84.0776	0.1345	0.1885	0
2	79.1230	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 79.123

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	2	33.0801	33.0801	2	264.2
2	5	110.7	194.2	3	249.5
3	1	.	0	2	249.5

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	155.9831	108.8554	0.652130	1.874636
V2	120.3833	90.464995	0.596633	1.479132
OVER-ALL	139.3249	100.0835	0.631414	1.713068

Pseudo F Statistic = 4.28
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	268.372	250.824
2	434.315	456.396
3	673.217	384.396

Cluster Standard Deviations

Cluster	V1	V2
1	43.919	16.115
2	119.707	100.821
3	.	.

```
------------------------------------------ Cluster=1 ------------------------------------

                                           OBS    BIOM

                                            1      10
                                            2      18


------------------------------------------ Cluster=2 ------------------------------------

                                           OBS    BIOM

                                            3      2
                                            4      4
                                            5      14
                                            6      22
                                            7      28


------------------------------------------ Cluster=3 ------------------------------------

                                           OBS    BIOM

                                            8      6
```

```
*******************************************************************
**************  Input data for cluster analysis without hierarchy **************
*******************************************************************
**************  Sampling method: average  ******************************
**************  Sampling date: 30th September 2009  **********************
**************  Analysed variable: biomass  *****************************
*******************************************************************


Title 'ANALYSE DE CLUSTER BIOMASS DES VERS DE TERRE 30 SEPT 09' ;
Title2 'ECHANTILLON MIX SANS HIRARQY';

data versdt;
      input biom$ v1-v2;
cards;
 2            447.139614349776        182.045510952467
 4            356.388690529825        494.650708585373
 6            458.195751281304        293.417117588875
 10           373.009742165807        513.411689961881
 14           493.615853821972        377.730430606691
 18           359.896284376828        442.086659266695
 22           787.508818187998        455.542692359945
 28           262.485725603633        599.064603414701
;


Proc fastclus maxclusters=3 out=resultat maxiter=10;
      var v1-v2;
       title 'Donnes de vers de terre pour 3 cluster' ;
run ;


proc sort data=resultat out=resultat ;
by cluster;
proc print data=resultat;
var biom;
by cluster;
run;
```

****
************** Raw results of cluster analysis ****************************
****
************** Analysed variable: biomass ******************************
************** Sampling method: average *******************************
************** Sampling date: 30th September 2009 **********************
****

Initial Seeds

Cluster	V1	V2
1	447.140	182.046
2	262.486	599.065
3	787.509	455.543

Minimum Distance Between Initial Seeds = 436.637

Iteration	Criterion	Relative Change in Cluster Seeds 1	2	3
1	88.8754	0.2385	0.2633	0
2	50.5987	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 50.599

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	3	71.4970	104.1	2	261.6
2	4	58.4756	115.0	1	261.6
3	1	.	0	1	363.9

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	157.3821	42.248363	0.948527	18.427601
V2	132.3121	80.048813	0.738554	2.824877
OVER-ALL	145.3885	64.002877	0.861576	6.224190

Pseudo F Statistic = 15.56
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	466.317	284.398
2	337.945	512.303
3	787.509	455.543

Cluster Standard Deviations

Cluster	V1	V2
1	24.2791	98.1538
2	50.8123	65.2450
3	.	.

-- Cluster=1 ------------------------------------

OBS	BIOM
1	2
2	6
3	14

-- Cluster=2 ------------------------------------

OBS	BIOM
4	4
5	10
6	18
7	28

-- Cluster=3 ------------------------------------

OBS	BIOM
8	22

```
*******************************************************************
**************  Input data for cluster analysis without hierarchy **************
*******************************************************************
**************  Sampling method: average  ******************************
**************  Sampling date: 7th October 2009  *************************
**************  Analysed variable: biomass  ******************************
*******************************************************************


Title 'ANALYSE DE CLUSTER BIOMASS DES VERS DE TERRE 07 OCT  09' ;
Title2 'ECHANTILLON MIX SANS HIRARQY';

data versdt;
        input biom$ v1-v2;
cards;
 2          192.19902621118      322.448567678443
 4          390.983970368892     375.911964329985
 6          438.85185189573      211.388971651163
 10         595.138403808553     286.359255593709
 14         606.641098858388     597.830696781708
 18         557.981846074352     347.92916128647
 22         482.179196819549     551.18347380258
 28         625.088067348299     486.57825363547
;


Proc fastclus maxclusters=3 out=resultat maxiter=10;
       var v1-v2;
         title 'Donnes de vers de terre pour 3 cluster' ;
run ;


proc sort data=resultat out=resultat ;
by cluster;
proc print data=resultat;
var biom;
by cluster;
run;
```

****

************** Raw results of cluster analysis ***************************

****

************** Analysed variable: biomass ******************************

************** Sampling method: average ******************************

************** Sampling date: 7th October 2009 *************************

****

Initial Seeds

Cluster	V1	V2
1	192.199	322.449
2	606.641	597.831
3	595.138	286.359

Minimum Distance Between Initial Seeds = 311.6838

Iteration	Criterion	Relative Change in Cluster Seeds 1	2	3
1	82.1490	0.3302	0.2034	0.2074
2	62.3489	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 62.349

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	2	102.9	102.9	3	248.4
2	3	67.6886	89.3244	3	266.4
3	3	75.3064	115.8	1	248.4

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	145.6889	95.052699	0.695948	2.288911
V2	134.8181	58.348965	0.866204	6.474091
OVER-ALL	140.3588	78.865764	0.774488	3.434354

Pseudo F Statistic = 8.59
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	291.591	349.180
2	571.303	545.197
3	530.657	281.892

Cluster Standard Deviations

Cluster	V1	V2
1	140.562	37.804
2	77.732	55.867
3	81.648	68.380

```
------------------------------------------ Cluster=1 ------------------------------------

                                           OBS    BIOM

                                            1      2
                                            2      4


------------------------------------------ Cluster=2 ------------------------------------

                                           OBS    BIOM

                                            3      14
                                            4      22
                                            5      28


------------------------------------------ Cluster=3 ------------------------------------

                                           OBS    BIOM

                                            6      6
                                            7      10
                                            8      18
```

```
*******************************************************************
**************  Input data for cluster analysis without hierarchy **************
*******************************************************************
**************  Sampling method: surface  *******************************
**************  Sampling date: 16th September 2009  **********************
**************  Analysed variable: abundance  ****************************
*******************************************************************


Title 'ANALYSE DE CLUSTER DES VERS DE TERRE 16 SEPT 09' ;
Title2 'ECHANTILLON SURFACE SANS HIRARQY';

data versdt;
        input popup$ v1-v2;
cards;
 2          1516.94045273336        1092.19712596802
 4          1759.65092517069        1607.95687989736
 6          2760.83162397471        2002.36139760803
 10         2214.7330609907         1759.65092517069
 14         758.470226366679        1516.94045273336
 18         1152.87474407735        1183.21355313202
 22         1092.19712596802        515.759753929342
 28         1243.89117124135        637.11499014801
;


Proc fastclus maxclusters=3 out=resultat maxiter=10;
       var v1-v2;
         title 'Donnes de vers de terre pour 3 cluster' ;
run ;


proc sort data=resultat out=resultat ;
by cluster;
proc print data=resultat;
var popup;
by cluster;
run;
```

****

************** Raw results of cluster analysis ***************************

****

************** Analysed variable: abundance ***************************

************** Sampling method: surface ******************************

************** Sampling date: 16th September 2009 **********************

****

Initial Seeds

Cluster	V1	V2
1	1092.20	515.76
2	758.47	1516.94
3	2760.83	2002.36

Minimum Distance Between Initial Seeds = 1055.337

Iteration	Criterion	Relative Change in Cluster Seeds 1	2	3
1	369.6	0.2859	0.4474	0.2831
2	258.0	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 257.95

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	3	263.3	415.1	2	690.4
2	3	390.1	562.9	1	690.4
3	2	298.8	298.8	2	1340.1

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	658.9281	387.4192	0.753079	3.049877
V2	528.7266	250.6703	0.839448	5.228516
OVER-ALL	597.3852	326.2892	0.786907	3.692795

Pseudo F Statistic = 9.23
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	1284.34	748.36
2	1223.67	1436.04
3	2487.78	1881.01

Cluster Standard Deviations

Cluster	V1	V2
1	215.242	303.893
2	504.330	223.631
3	386.150	171.622

```
------------------------------------------- Cluster=1 -------------------------------------

                                        OBS    POPUP

                                         1      2
                                         2      22
                                         3      28

------------------------------------------- Cluster=2 -------------------------------------

                                        OBS    POPUP

                                         4      4
                                         5      14
                                         6      18

------------------------------------------- Cluster=3 -------------------------------------

                                        OBS    POPUP

                                         7      6
                                         8      10
```

```
********************************************************************
************** Input data for cluster analysis without hierarchy **************
********************************************************************
************** Sampling method: surface *******************************
************** Sampling date: 30th September 2009 ***********************
************** Analysed variable: abundance ****************************
********************************************************************


Title 'ANALYSE DE CLUSTER DES VERS DE TERRE 30 SEPT 09' ;
Title2 'ECHANTILLON SURFACE SANS HIRARQY';

data versdt;
        input popup$ v1-v2;
cards;
 2          879.825462585347        1759.65092517069
 4          1001.18069880402        1031.51950785868
 6          1122.53593502268        1243.89117124135
 10         667.453799202677        1213.55236218669
 14         1365.24640746002        1213.55236218669
 18         910.164271640014        970.841889749349
 22         1304.56878935069        1213.55236218669
 28         1092.19712596802        788.809035421346

;


Proc fastclus maxclusters=3 out=resultat maxiter=10;
       var v1-v2;
         title 'Donnes de vers de terre pour 3 cluster' ;
run ;


proc sort data=resultat out=resultat ;
by cluster;
proc print data=resultat;
var popup;
by cluster;
run;
```

****

************** Raw results of cluster analysis ***************************

****

************** Analysed variable: abundance ***************************

************** Sampling method: surface ******************************

************** Sampling date: 30th September 2009 **********************

****

Initial Seeds

Cluster	V1	V2
1	879.83	1759.65
2	667.45	1213.55
3	1365.25	1213.55

Minimum Distance Between Initial Seeds = 585.9397

Iteration	Criterion	Relative Change in Cluster Seeds 1	2	3
1	190.5	0	0.4073	0.2980
2	134.2	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 134.15

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	1	.	0	2	688.0
2	3	151.2	238.7	3	364.1
3	4	181.0	350.7	2	364.1

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	229.3040	150.7303	0.691362	2.240041
V2	283.4169	186.7337	0.689926	2.225033
OVER-ALL	257.7843	169.6895	0.690494	2.230954

Pseudo F Statistic = 5.58
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	879.83	1759.65
2	859.60	1071.97
3	1221.14	1114.95

Cluster Standard Deviations

Cluster	V1	V2
1	.	.
2	172.514	126.311
3	134.259	217.898

```
------------------------------------------- Cluster=1 -------------------------------------

                                            OBS    POPUP

                                             1       2


------------------------------------------- Cluster=2 -------------------------------------

                                            OBS    POPUP

                                             2      4
                                             3      10
                                             4      18


------------------------------------------- Cluster=3 -------------------------------------

                                            OBS    POPUP

                                             5      6
                                             6      14
                                             7      22
                                             8      28
```

```
*****************************************************************
**************  Input data for cluster analysis without hierarchy **************
*****************************************************************
**************  Sampling method: surface  ******************************
**************  Sampling date: 7th October 2009  *************************
**************  Analysed variable: abundance  ***************************
*****************************************************************


Title 'ANALYSE DE CLUSTER BIOMASS DES VERS DE TERRE 07 OCT 09' ;
Title2 'ECHANTILLON MIX SANS HIRARQY';

data versdt;
        input biom$ v1-v2;
cards;
 2              357.421509473034        366.644507425652
 4              380.721714827018        351.293070043991
 6              326.354569001055        451.705426372223
 10             320.389959140907        405.89079081877
 14             307.544507387161        286.258798954406
 18             103.0032906215          273.516499151446
 22             121.485693097604        317.292366736425
 28             248.298881065207        227.61691493264
;


Proc fastclus maxclusters=3 out=resultat maxiter=10;
       var v1-v2;
         title 'Donnes de vers de terre pour 3 cluster' ;
run ;


proc sort data=resultat out=resultat ;
by cluster;
proc print data=resultat;
var biom;
by cluster;
run;
```

****

************** Raw results of cluster analysis ***************************

****

************** Analysed variable: abundance ***************************

************** Sampling method: surface ******************************

************** Sampling date: 7th October 2009 *************************

****

Initial Seeds

Cluster	V1	V2
1	3488.96	3246.25
2	1881.01	2639.48
3	606.78	1486.60

Minimum Distance Between Initial Seeds = 1718.366

Iteration	Criterion	Relative Change in Cluster Seeds 1	2	3
1	470.6	0.2207	0.1225	0.1628
2	420.3	0.1856	0.1225	0.1545
3	370.4	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 370.4

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	3	473.7	637.8	2	1148.8
2	1	.	0	1	1148.8
3	4	465.0	796.7	2	1412.7

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	1014.4238	618.1106	0.734805	2.770809
V2	895.5915	238.6632	0.949275	18.714113
OVER-ALL	956.8542	468.5194	0.828748	4.839349

Pseudo F Statistic = 12.10
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	2902.41	3165.35
2	1881.01	2639.48
3	1145.29	1433.51

Cluster Standard Deviations

Cluster	V1	V2
1	576.703	341.002
2	.	.
3	644.238	131.953

```
------------------------------------------ Cluster=1 ------------------------------------

                                          OBS    POPUP

                                           1       2
                                           2       4
                                           3       6


------------------------------------------ Cluster=2 ------------------------------------

                                          OBS    POPUP

                                           4      10


------------------------------------------ Cluster=3 ------------------------------------

                                          OBS    POPUP

                                           5      14
                                           6      18
                                           7      22
                                           8      28
```

```
*******************************************************************
************** Input data for cluster analysis without hierarchy **************
*******************************************************************
************** Sampling method: surface ******************************
************** Sampling date: 16th September 2009 **********************
************** Analysed variable: biomass *****************************
*******************************************************************


Title 'ANALYSE DE CLUSTER BIOMASS DES VERS DE TERRE 16 SEPT 09' ;
Title2 'ECHANTILLON SURFACE SANS HIRARQY';

data versdt;
        input biom$ v1-v2;
cards;
 2      200.660883087568    141.075462104202
 4      183.307084308299    259.791221935115
 6      343.010575172067    250.750256836824
 10     321.621714788526    223.945919037026
 14     85.8557957438025    186.492659259039
 18     154.879620224076    164.924799902076
 22     177.664065824131    54.8525667708382
 28     104.012385290751    30.6889577312958
;


Proc fastclus maxclusters=3 out=resultat maxiter=10;
       var v1-v2;
         title 'Donnes de vers de terre pour 3 cluster' ;
run ;


proc sort data=resultat out=resultat ;
by cluster;
proc print data=resultat;
var biom;
by cluster;
run;
```

****

************** Raw results of cluster analysis ***************************

****

************** Analysed variable: biomass ******************************

************** Sampling method: surface *******************************

************** Sampling date: 16th September 2009 **********************

****

Initial Seeds

Cluster	V1	V2
1	177.664	54.853
2	85.856	186.493
3	343.011	250.750

Minimum Distance Between Initial Seeds = 160.4926

		Relative Change in Cluster Seeds		
Iteration	Criterion	1	2	3
1	46.9352	0.1664	0.3621	0.1068
2	37.4078	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 37.408

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	3	54.3889	76.7176	2	129.7
2	3	49.9221	70.0198	1	129.7
3	2	17.1461	17.1461	2	193.9

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	92.732475	45.497065	0.828061	4.816008
V2	85.366730	49.070457	0.763988	3.237065
OVER-ALL	89.125727	47.317506	0.798670	3.966958

Pseudo F Statistic = 9.92
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	160.779	75.539
2	141.348	203.736
3	332.316	237.348

Cluster Standard Deviations

Cluster	V1	V2
1	50.4882	58.0279
2	50.1151	49.7284
3	15.1242	18.9535

```
------------------------------------------- Cluster=1 -------------------------------------

                                            OBS    BIOM

                                             1      2
                                             2      22
                                             3      28


------------------------------------------- Cluster=2 -------------------------------------

                                            OBS    BIOM

                                             4      4
                                             5      14
                                             6      18


------------------------------------------- Cluster=3 -------------------------------------

                                            OBS    BIOM

                                             7      6
                                             8      10
```

```
*******************************************************************
**************  Input data for cluster analysis without hierarchy **************
*******************************************************************
**************  Sampling method: surface  *******************************
**************  Sampling date: 30th September 2009  ***********************
**************  Analysed variable: biomass  ******************************
*******************************************************************


Title 'ANALYSE DE BIOMASS CLUSTER DES VERS DE TERRE 30 SEPT 09' ;
Title2 'ECHANTILLON SURFACE SANS HIRARQY';

data versdt;
       input biom$ v1-v2;
cards;
 2            82.8552875282959        120.839476464739
 4            89.4691479022134        125.541991868213
 6            140.165297832562        126.543172567017
 10           109.553439496403        189.10179683774
 14           211.764887201577        179.15066746781
 18           140.165297832562        151.238963137516
 22           211.825564819686        212.644712664162
 28           190.831108953856        130.274846080741

;


Proc fastclus maxclusters=3 out=resultat maxiter=10;
      var v1-v2;
        title 'Donnes de vers de terre pour 3 cluster' ;
run ;


proc sort data=resultat out=resultat ;
by cluster;
proc print data=resultat;
var biom;
by cluster;
run;
```

****

************** Raw results of cluster analysis ***************************

****

************** Analysed variable: biomass *****************************

************** Sampling method: surface ******************************

************** Sampling date: 30th September 2009 **********************

****

Initial Seeds

Cluster	V1	V2
1	82.855	120.839
2	190.831	130.275
3	211.826	212.645

Minimum Distance Between Initial Seeds = 85.00331

Iteration	Criterion	Relative Change in Cluster Seeds 1	2	3
1	27.5542	0.3145	0.4031	0.1970
2	19.2363	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 19.236

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	3	28.6961	46.6258	2	63.7535
2	3	22.7254	34.2622	1	63.7535
3	2	16.7471	16.7471	2	81.1300

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	52.424061	20.484087	0.890945	8.169721
V2	34.921066	27.649937	0.552199	1.233134
OVER-ALL	44.540785	24.332251	0.786833	3.691149

Pseudo F Statistic = 9.23
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	93.959	145.161
2	157.054	136.019
3	211.795	195.898

Cluster Standard Deviations

Cluster	V1	V2
1	13.9039	38.1263
2	29.2519	13.3123
3	0.0429	23.6839

```
------------------------------------------ Cluster=1 ------------------------------------

                                          OBS    BIOM

                                           1      2
                                           2      4
                                           3      10


------------------------------------------ Cluster=2 ------------------------------------

                                          OBS    BIOM

                                           4      6
                                           5      18
                                           6      28


------------------------------------------ Cluster=3 ------------------------------------

                                          OBS    BIOM

                                           7      14
                                           8      22
```

```
*******************************************************************
**************  Input data for cluster analysis without hierarchy **************
*******************************************************************
**************  Sampling method: surface  ******************************
**************  Sampling date: 7th October 2009  *************************
**************  Analysed variable: biomass  *****************************
*******************************************************************


Title 'ANALYSE DE CLUSTER BIOMASS DES VERS DE TERRE 07 OCT 09' ;
Title2 'ECHANTILLON MIX SANS HIRARQY';

data versdt;
       input biom$ v1-v2;
cards;
 2              357.421509473034         366.644507425652
 4              380.721714827018         351.293070043991
 6              326.354569001055         451.705426372223
 10             320.389959140907         405.89079081877
 14             307.544507387161         286.258798954406
 18             103.0032906215           273.516499151446
 22             121.485693097604         317.292366736425
 28             248.298881065207         227.61691493264
;


Proc fastclus maxclusters=3 out=resultat maxiter=10;
       var v1-v2;
         title 'Donnes de vers de terre pour 3 cluster' ;
run ;


proc sort data=resultat out=resultat ;
by cluster;
proc print data=resultat;
var biom;
by cluster;
run;
```

****

************** Raw results of cluster analysis ***************************

****

************** Analysed variable: biomass *****************************

************** Sampling method: surface ******************************

************** Sampling date: 7th October 2009 *************************

****

Initial Seeds

Cluster	V1	V2
1	307.545	286.259
2	103.003	273.516
3	326.355	451.705

Minimum Distance Between Initial Seeds = 166.5125

Iteration	Criterion	Relative Change in Cluster Seeds 1	2	3
1	42.6661	0.0307	0.1427	0.2668
2	34.1747	0.2793	0	0.1097
3	28.5313	0	0	0

Convergence criterion is satisfied.

Criterion Based on Final Seeds = 28.531

Cluster Summary

Cluster	Frequency	RMS Std Deviation	Maximum Distance from Seed to Observation	Nearest Cluster	Distance Between Cluster Centroids
1	2	41.6801	41.6801	3	153.0
2	2	23.7588	23.7588	1	170.1
3	4	37.4636	61.1399	1	153.0

Statistics for Variables

Variable	Total STD	Within STD	R-Squared	RSQ/(1-RSQ)
V1	105.1790	29.337656	0.944427	16.994308
V2	73.594552	41.763755	0.769973	3.347307
OVER-ALL	90.771075	36.089536	0.887088	7.856465

Pseudo F Statistic = 19.64
Approximate Expected Over-All R-Squared = .
Cubic Clustering Criterion = .
WARNING: The two above values are invalid for correlated variables.

Cluster Means

Cluster	V1	V2
1	277.922	256.938
2	112.244	295.404
3	346.222	393.883

Cluster Standard Deviations

Cluster	V1	V2
1	41.8930	41.4661
2	13.0690	30.9542
3	28.1524	44.8830

```
------------------------------------------- Cluster=1 -------------------------------------

                                          OBS    BIOM

                                           1      14
                                           2      28


------------------------------------------- Cluster=2 -------------------------------------

                                          OBS    BIOM

                                           3      18
                                           4      22


------------------------------------------- Cluster=3 -------------------------------------

                                          OBS    BIOM

                                           5      2
                                           6      4
                                           7      6
                                           8      10
```

Appendix 2: communication presented during the workshop “Ecological engineering, from concepts to applications”, Paris 2-4 december 2009

My work was included in this synthesis which presents the main results and illustrates the diversity of collaborations which were associated within this project.

Biomass production and water purification from fresh liquid manure by vermiculture, macrophytes ponds and constructed wetlands to recover nutrients and recycle water for flushing in pig housing

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[4]CEMAGREF, UR GERE, 17 avenue de Cucillé, 35044 Rennes, France
[5]Shanghai Jiao Tong University, Department of Environmental Science and Technology, School of Agriculture and Biology, 800#, Dongchuan Road, Shanghai 201101, P.R.China
[6]Chambres d'Agriculture de Bretagne, Station Expérimentale de Guernevez, 29520 Saint-Goazec, France

Abstract. A treatment system has been designed for pig housing on slatted-floor, with the aim together to recycle the water for excretion washing, and to produce, from the nutrients contained in the effluent, organic matter and plants that can be reused on the farm. Several experiments were carried out in order to: (i) well settling the system, (ii) determining the parameters of abatement and by-product production for technical and economical optimizations. From 2003 to 2007, studies were performed on a vermifilter and a macrophyte lagooning. From these results, a complete demonstration plant including a screen, a vermifilter, a macrophyte lagooning, and a complementary water storage pond, was connected to a 30 pregnant sows piggery, using the recycled water for flushing, and collecting rainfall to compensate for evapotranspiration. After two years of functioning, it was showed that, during the warm season, the whole plant produced an effluent suitable for flushing, where over 70% of the phosphorus and potassium, 95% of the COD and nitrogen, 99.8% of endocrine disruptors, and 99.99% of pathogenic micro-organisms were removed.

Keywords: lagooning; macrophytes ponds; pig manure; pollution abatement; reuse; vermifiltration.
PACS: 89. 60. -k

INTRODUCTION

Water consumption by animal farms is very high, and in many regions their wastewater pollutes the ecosystem, whereas water treatment is expensive compared to the income generated by animal production. Besides, flushing of fresh liquid manure, avoiding storage below the animals in livestock buildings, has been developed for a better control of pathogens, odour and polluting gas emissions. Treatment of the diluted manure is then necessary to recycle the water and avoid excessive water consumption during flushing, and to extract the nutrients for further agronomic uses and recycling.

The system of flushing is usually used in Australia, with nevertheless an absence of effective treatment of the diluted fresh liquid manure, which leads to the odorous gas emission, from anaerobic lagoons [1]. In addition, various systems of treatment of water and production of biomass combined were described in the literature, vermiculture [2], macrophytes ponds [3] or helophytes filters [4]. At Guernévez (Finistère, France), such systems were tested in pilot units, and a demonstration plant was built, including a screen, a vermifilter, a settling tank, macrophytes ponds, and constructed wetlands [5]. Interesting results were obtained during the first months of functioning [6].

In this paper, thanks to two years of experiments, we precise the advantages of the system, and define the improvements to bring to it, from the analysis of the results obtained at the pilot and demonstration scales.

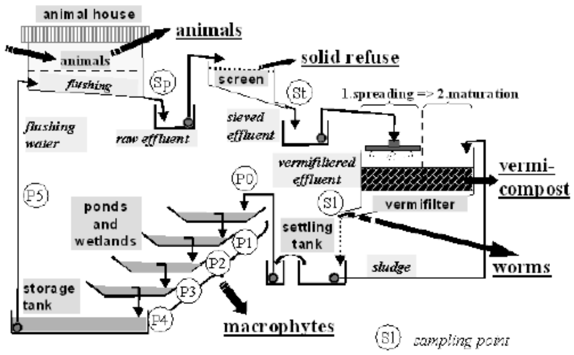

FIGURE 1. General scheme of the prototype of clean piggery built at Guernévez, with indication of the sampling points (in the circles).

MATERIAL AND METHODS

Pilot scale plants

The experimental vermifilter was composed of four elementary sub-units 5 m long × 2.5 m wide × 1 m high, placed in a room with natural ventilation. They were filled with a mixture of wood chips (one-third of total mass) and earthworm (*Eisenia andrei*) litter (two-thirds of total mass), the latter made of bark, wood chips, peat, straw, and vermicompost resulting from previous experiments and containing the earthworms. The material was about 0.7 m high [7]. After sieving, fresh liquid manure was sprinkled onto the vermifilter, at different loads and frequencies, according to the experiments.

The pilot of pollution abatement by macrophytes is composed of three lines a, b and c of 4 basins 1, 2, 3 and 4 in series, alternatively lagoons and constructed wetlands with horizontal subsurface flow, except for basin 2c subdivided into two and which can be managed with horizontal or vertical flow. The depth of the basins goes from 1.1 m for the first lagoons to 0.35 m (planted beds for completion) and the time of retention is 5 d for the first lagoons and 4 d for the other basins (except basin 2c, when it functions with vertical flow, one week in water, one week in air). The support of the constructed wetlands is made up mainly of gravel, particle size being 6 to 10 mm. Areas of the basins are of 0.65 m^2 at level 1,2.5 m^2 at level 2 (2 m^2 for 2c), 1.1 m^2 at level 3 and 5 m^2 at level 4 [5].

Demonstration plant

The whole prototype comprises a livestock building, a screen, a vermifilter, a settling tank and a macrophyte treatment combined system. All equipment was controlled automatically with float level switches and a clock. The complete description is given in Morand et al. [6], and summarized in figure 1.

Sampling and analyses

For the demonstration plant, the effluent samplings, about twice a month, and chemical analyses, following the standard methods, were made as described in Morand and al. [6]. On September 19th 2008, the sampling was completed in order to perform microbial and endocrine disruptors' analyses.

Microbial analyses were performed as described in Morand and al. [6].

The endocrine disruptors were searched by the estrogenic activity, because of the presence of pregnant sows in the piggery. Sampling of 1 L has been done after each step of treatment. Samples were split into pre-cleaned 500 mL glass bottles and then freezed at -20°C. Samples were thawed 24 h before extraction. Water (1 L) was filtered with a Whatman GF/C filter. The filtrate was concentrated by solid phase extraction (SPE) on mini-columns. After rinsing, drying under vacuum, and storing at –20°C, the SPE columns were thawed and extraction was made by 10 ml of ethyl acetate:methanol (5:1 v/v) mixture. The eluates were dried, then solved again with 1 mL of ethanol for bioassay. Water extracts were applied to cells lines at concentrations varying from 0.001% to 0.3% (vol/vol) of the test culture medium.

Cell cultures and bioassay procedures were based on previously described methods [8]. Summarily described, the medium used for MELN cell line culture was Dulbeco's Modified Eagle Medium (DMEM) with red phenol and supplemented with 5% foetal calf serum (FCS). For the bioassay, 5 days prior to the experiments the medium was replaced by DMEM, without red phenol, supplemented with 5% stripped serum (DCC-FCS medium). Water extracts to be tested were prepared 4× concentrated in the same medium and were tested at different dilutions in order to achieve a dose–response curve in the bioassay. MELN cells were plated in 96-well plates and water extracts were added 1 day after seeding. Cells were incubated with the samples for 16 h at 37°C. Then the medium was replaced by a culture medium containing 3×104 M of luciferin. Luminescence was measured in intact cells using a Microbeta Wallac Luminometer for 2 s and expressed as relative luminescence units (RLU). RLU of MELN cells with samples was expressed relative to the value obtained with E2 (10 nM). MELN cell basal activity was around 20% of the maximum activity.

Biological activities were expressed in estrogenic equivalents (E_2-EQ) for 1 L of water. Equivalents were calculated as the concentration of E_2 resulting in the same activation of luciferase expression in MELN cells as tested sample. Calculation was based on the 50% effective concentration (EC_{50}) from the estrogen-responsive gene transactivation dose-response curve, with E_2 as positive control (EC_{50} of estradiol: 17.6 pM).

The bioassays were performed in quadruplicate and repeated three times.

RESULTS AND DISCUSSION

First experiments concerning the vermifilter and the macrophytes lagooning plant at the pilot scale, obtained from 2003 to 2007, permitted to define the design of the demonstration plant. The experiments at this pilot scale have continued until now, with the aim to improve the demonstration plant. Some results, already published, are summarized here, and are completed by the data necessary for determining the change to be brought to the demonstration plant.

Pilot vermifilter

Trials on the volumetric load of the raw effluent (5600 mg C L^{-1}, 820 mg N L^{-1}, 210 mg P L^{-1}, and 790 mg K L^{-1}) permitted to reach a removal efficiency around 60% for ammoniacal nitrogen, 40% for dry matter, COD and total nitrogen, and 20% for phosphorus and potassium [7].

Macrophytes lagooning pilot

For the macrophyte lagooning system combining 2 lagoons and 2 constructed wetlands, the efficiency was 70% in yearly average for COD, N and P, with a hydraulic retention time between 4 and 5 days (N load of 1.2 g m^{-2} d^{-1}). The plant productivity, the capacity of the plants to withstand climatic variations and various effluent loads, the complementarity of the treatment stages were also observed. Four species of floating plants (*Azolla caroliniana*, *Eichhornia crassipes*, *Hydrocotyle vulgaris*, *Pistia stratiotes*) and two of rooted plants (*Phragmites australis* and *Glyceria aquatica*) were found to be best suited, either in series or in alternation with time, to maximise nitrogen abatement and exportation of potassium and phosphorus [5].

The productivity of the basins in biomass was found to be around 20 t MS ha^{-1} y^{-1}, a little less for some floating plants, like *Hydrocotyle* or when put too belatedly in the lagoons [5]. The nutrients' content has also been determined (Table 1).

Floating plants are more efficient to remove nutrients and heavy metals than rooted plants (factors of 2 for N and K, 4 for P, Cu and Zn, with, however, individual features, like the few quantity of copper removed by *Azolla*). This can be due to the fact that the whole plants are harvested for the first ones, and only the aerial part for the second ones.

TABLE 1. Contents of the plants in different elements. Contents are given as means obtained from three harvests for the floating plants except *Lemna* spp., and from one harvest for the rooted plants and *Lemna* spp. (July 7, 2007).

Plants	dry weight (DW) (% wet weight)	N (% DW)	P (% DW)	K (% DW)	Cu (mg/kg DW)	Zn (mg/kg DW)
Eicchornia crassipes	4,15	5,62	1,40	4,56	26,1	170
Pistia stratiotes	4,88	4,34	0,74	4,67	83,7	327
Azolla caroliniana	4,35	5,04	1,16	3,72	8,63	116
Hydrocotyle vulgaris	4,80	4,76	0,96	5,01	14,2	114
Lemna spp.	5,29	6,39	1,75	2,64	54,3	286
Phragmites australis	25,0	2,88	0,30	2,49	9,80	73,2
Typha latifolia	14,3	2,37	0,39	2,25	11,9	37,2
Glyceria aquatica	23,8	1,56	0,27	1,58	3,08	24,1
Juncus inflexus	30,8	1,65	0,15	1,54	4,11	30,3

Demonstration plant

The demonstration plant began to function in 2007, and functions with the design described here since July 2008, a few fittings-up having been made at this date. Different problems occurred, some mechanical: bad functioning of the sieve or of the vermifilter moving, leading to a stopping of the automatism; others biological : clogging of the vermifilter or absence of growth of the floating macrophytes. All thoses problems were solved by different ways: manual management of the system for a period, emptying of lagoon 1, diminishing of the flushing number, … Nevertheless the prototype functioned continuously, except the few days necessary for the interventions. It is then particularly interesting to review the capacity of the system to absorb the management changes and the improvements to avoid the met problems.

Nutrients abatement in the effluent

Since the set-up of the demonstration plant with the design shown in figure 1, it has been possible to follow the evolution of the pollution abatement in the recycled effluent since the piggery output until the piggery input (lagoon 5 output) between the summers 2008 and 2009 (Figure 2). If the concentration of the nitrogen doesn't increase in one year (16.0, 20.4, 25.6, and 16.3 in lagoon 5 output respectively in summer 2008, autumn 2008, spring 2009 and summer 2009), it is not truth for phosphorus (34.1, 46.4, 35.3, and 45.9) and potassium (166, 292, 268, and 312), knowing that the decrease recorded for spring 2009 was after an emptying of lagoon 1.

Micro-organisms removal

Three types of micro-organism were tested during a sampling campaign, on September, the 19th, 2008 [6]. The results obtained are summarized in Table 2. If the first levels of the demonstration plant have relatively few effects on the decrease in bacteria number, the succession of the basins permit an abatement of four logarithmic units, to be compared with other biological manure treatments, resulting in a 100-fold decrease [9].

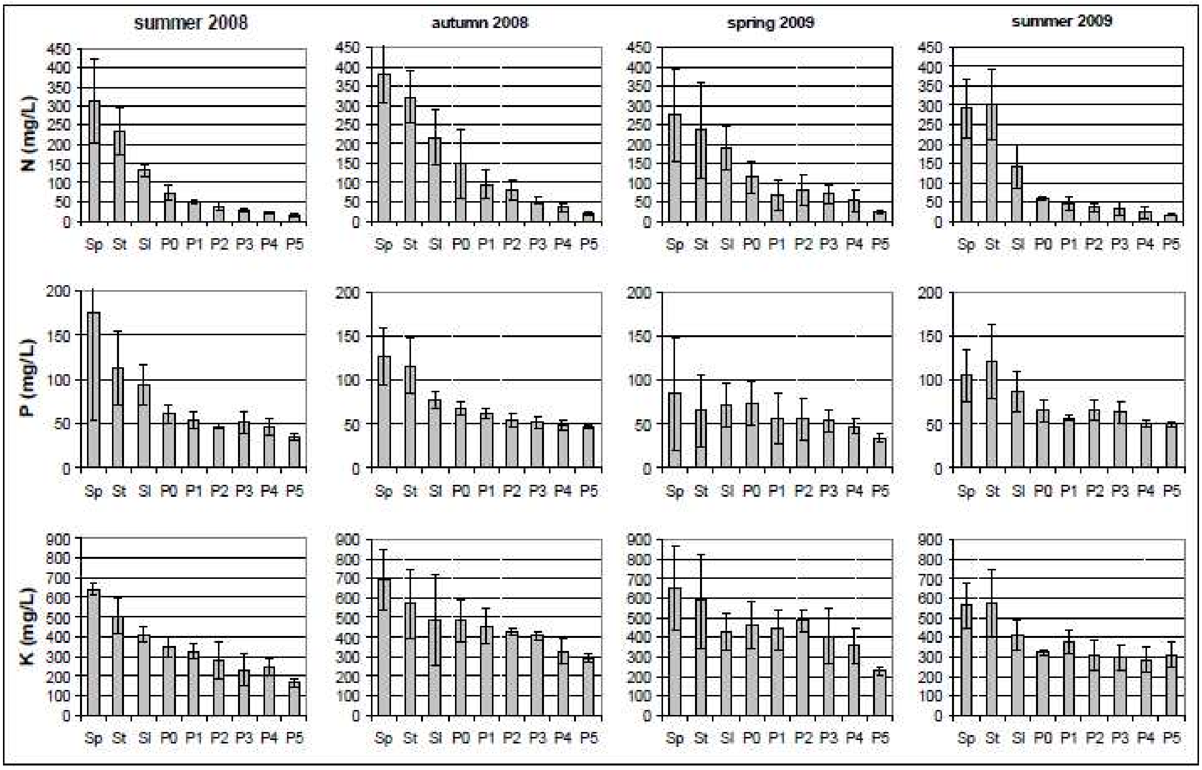

FIGURE 1. Nutrients abatement along the circulation of the effluent for recycling. Sampling points refer to figure 1. The values are the averages of samplings put at three or four different dates during the seasons indicated

TABLE 2. Concentrations of *E. coli*, enterococci and *C. perfringens* along the treatment plant. Sampling points refer to figure 1; the feces replace the output of the piggery (Sp). Values are averages of three determinations. Removal efficiency is calculated from *E. coli*.

Sampling	enterococci (number/mL)	*C. perfringens* (number/mL)	*E.coli* (number/mL)	removal efficiency (%) by step	total
feces	600000	5400	4400000	-	-
St	28000	5450	850000	84	84
Sl	19000	5950	476667	44	91
P0	7000	360	213333	55	96
P1	1933	79	44667	79	99,1
P2	97	2	1933	96	99,96
P3	40	<0,01	868	55	99,98
P4	<0,01	<0,01	27	97	99,999
P5	<0,01	<0,01	4	85	99,9999

Endocrine disruptors abatement in the effluent

Estrogenic activity was assessed in samples by measuring luciferase activation in MELN cells. All samples showed estrogenic activity when present at 0.3% concentration in test culture medium (Figure 3). The first tested level effluent, St, was the most active and for the other samples, there is a linear decrease of the activities depending of treatment. The last sample, P5 exhibited only very little activity. Taking into account the EC_{50} of the reference ligand E2, we evaluated the concentration of estrogenic compounds in the samples. Concentration ranged between 1011 ng/L to 2 ng/L (Table 3). At the effluent "P0" the estrogenicity removal efficiency was 77% and the treatment P1 increased the removal to 96%. Estrogenic activity removal from "P2" to "P5" weakly increased from 93% to 99.8%, but at each step, the individual abatement is very similar. The experimental treatment system removal efficiency results in underlining the important role of the vermifilter (Sl) and of the settling tank (P0) for estrogenic compounds removal in pig housing. It is interesting to note that macrophyte lagooning and water storage pond (P5) improve estrogenic activity removal.

Relationships between removal efficiency and growth of the plant

A calculation taking into account their harvest and their content in nutrients give exportation by the plants out of the system of about 25 kg for nitrogen, 6 for phosphorus and 20 for potassium - first estimation from the figures given above.

Besides, the nutrients brought to the system by the pigs feeding can be estimated to 320 kg for nitrogen, 80 for phosphorus and 210 for potassium.

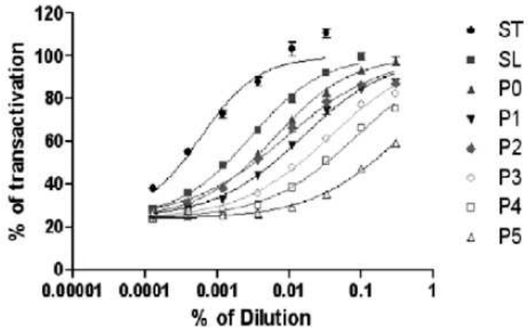

FIGURE 3. Luciferase activity induced on MELN cell line by the sample. Curves are percent of transactivation (mean of four measures and standard deviation) of luciferase gene according to the concentration of tested compound. Percent of transactivation is calculated compared to maximal activity obtained with estradiol at 10^{-8}M.

TABLE 3. Estradiol equivalents and removal efficiency. Equivalents were calculated as the concentration of E_2 resulting in the same activation of luciferase expression in MELN cells as tested sample. Calculation was based on the EC_{50} (50% effective concentration) from the ER transactivation dose-response curve of sample with E_2 as positive control and took into consideration volume of extraction.

Sampling	E_2-EQ (ng/L)	estrogenic removal efficiency (%) by step	total
St	1011	-	-
Sl	231	77	77
P0	107	54	89
P1	41	62	95,9
P2	64	-56	93,7
P3	17	59	98,3
P4	8	53	99,2
P5	2	75	99,8

It is well known that many other mechanisms than the absorption and removal by harvest of the vegetables are efficient for removing nutrients, for instance nitrification/denitrification for nitrogen and sedimentation for phosphorus. It appears that this is also truth for potassium, the adsorption on the organic matter being undoubtedly the explanation.

Nevertheless, the comparison of the phosphorus and potassium removal between 2008 and 2009 shows that their content in the recycled effluent increased from the first to the second year. As potassium seems to be the main problem, modifications to be brought to the plant should be thought principally for this parameter.

It is probable that it is also trapped in the sediment, due to the mass of dead loaded plants. The first thing to be done is then to drag the lagoons each year.

A second remark concerns the sieve. It is inefficient on potassium. Moreover, its efficiency on nitrogen and potassium was lower than expected, certainly because of the significant dilution of the liquid manure (1/15 for the demonstration plant). As it is costly and as its management has been a source of difficulties for the whole system, its keeping is not useful.

It becomes then necessary to increase the size of the other compartments, rather the vermifilter and lagoons. It is in fact easier to remove the vermicompost and sludge, than the sediments formed in the planted filters. The transformation of lagoons in vertical filters, sometimes suggested, would be unsuitable because i) the rooted plants stems, which render the sludge removal very difficult, while their deposit is significant, due to the fact that liquid manure is much richer than municipal wastewaters; ii) the efficiency of the lagoons for removing potassium per surface unit is bigger; iii) the vermifilter and the lagoons assume also a nitrification function, sufficient here, because nitrogen abatement is not the challenge. On the contrary, the horizontal filters are very useful, having a buffer function, and assuming the soundness of the system.

CONCLUSION

Recycling water for using it in a semi-close system (external supply by rain and eventual emptying of excess) needs to be sure that a progressive enrichment in nutrients and pollutants could not occur. The challenge is then greater than for a simple wastewater treatment.

The demonstration plant experienced in Guernévez necessitate again a few improvements to reach this aim, but it is already established that, as far the micro-organisms and the endocrine disruptors are concerned, they are sufficiently eliminated, to giving a water convenient for flushing and not leading to a progressive enrichment.

It is also sufficiently robust in order that the piggery could function continuously with the flushing, even if, sometimes, it was necessary to release a small part of the effluent.

ACKNOWLEDGMENTS

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REFERENCES

1. FSA Environmental, "Alternative systems for piggery treatment", Report, Environment Protection Agency / Rural City of Murray Bridge. FSA Environmental, Queensland, South Australia, 2000, 137 pp.
2. M. B. Bouché and P. Soto, "An industrial use of soil animals for environment: the treatment of organically polluted water by lombrifiltration" in *XIVth International Colloquium On Soil Zoology And Ecology, Mont Saint Aignan, August 30 to September 3*, University of Rouen France, pp.1-13.
3. K.R. Reddy and W.H.Smith, *Aquatic plants for water treatment and resource recovery. Conference on research and application, 20-24 July 1986, Orlando*, Magnolia Publ. Inc., Orlando, Florida, U.S.A., 1987, XXIV - 1032 pp.
4. A.Ennabili, M. Ater and M. Radoux *Aquatic Botany* **62**, 45-56 (1998).
5. P. Morand, P. Robin, J. P. Qiu, Y. S. Li, Luth, D. Cluzeau, G. Hamon, C. Amblard, S. Fievet, D. Oudart, A.-M. Pourcher, B. Picot and B. Landrain, "Test of an Extensive System Combining Screen, Vermifilter, Macrophyte Lagoons, and Constructed Wetlands to Recover Nutrients from Fresh Manure and Recycle Water for Flushing in Pig Housing" in *Progress in Environmental Science and Technology, vol. II*, edited by S. C. Li, Y. J. Wang, F. X. Cao, P. Huang and Y. Zhang, Beijing Institute of Technology, Beijing, 2009, pp. 1122-1132.
6. P. Morand, P. Robin, A.-M. Pourcher, D. Oudart, S. Fievet, Luth, D. Cluzeau, B. Picot and B. Landrain, "Design of an integrated piggery system with recycled water, biomass production and water purification by vermiculture, macrophyte ponds and constructed wetlands". 8th IWA Specialist Group Conference on Waste Stabilization Ponds, Belo Horizonte, Brazil 26-30 April 2009, in press.
7. Y.S. Li, P. Robin, D. Cluzeau, M. Bouché, J.P. Qui, A. Laplanche, M. Hassouna, P. Morand, C. Dappelo and J. Callarec, *Ecological Engineering* **32**, 301-309 (2008).
8. P. Balaguer, H. Fenet, V. Georget, F. Comunale, B. Térouanne, R. Gilbin, E. Gomez, A.M. Boussioux, C. Sultan, M. Pons, J.C. Nicolas and C. Casellas, *Ecotoxicology* **9** (1), 105-114 (2000).
9. H. N. Chinivasagam, R. J. Thomas, K. Casey, E. McGahan, E. A. Gardner, M. Rafiee and P. J. Blackall, *Journal of applied Microbiology* **97**, 883-891(2004).

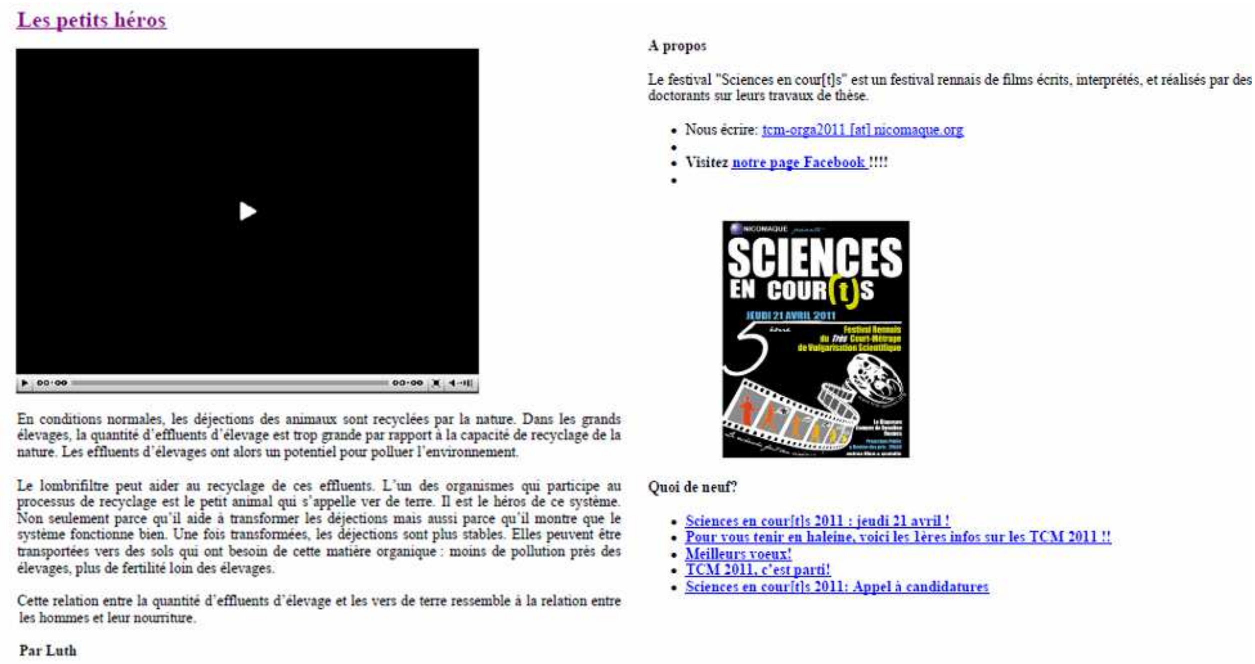
Appendix 3: knowledge use for socio-economical purposes

1. film realisation

The vermifiltration experiment served as a basis for the realisation of a short film for young public.

It was realised within the help of Nicomaque association and presented during the “Science en cour[t]s” festival in Rennes, april 2010.

The film can be seen on http://tcm-rennes.org/?page_id=547



Les petits héros

En conditions normales, les déjections des animaux sont recyclées par la nature. Dans les grands élevages, la quantité d'effluents d'élevage est trop grande par rapport à la capacité de recyclage de la nature. Les effluents d'élevages ont alors un potentiel pour polluer l'environnement.

Le lombrifiltre peut aider au recyclage de ces effluents. L'un des organismes qui participe au processus de recyclage est le petit animal qui s'appelle ver de terre. Il est le héros de ce système. Non seulement parce qu'il aide à transformer les déjections mais aussi parce qu'il montre que le système fonctionne bien. Une fois transformées, les déjections sont plus stables. Elles peuvent être transportées vers des sols qui ont besoin de cette matière organique : moins de pollution près des élevages, plus de fertilité loin des élevages.

Cette relation entre la quantité d'effluents d'élevage et les vers de terre ressemble à la relation entre les hommes et leur nourriture.

Par Luth

A propos

Le festival "Sciences en cour[t]s" est un festival rennais de films écrits, interprétés, et réalisés par des doctorants sur leurs travaux de thèse.

- Nous écrire: tcm-orga2011 [at] nicomaque.org
- Visitez notre page Facebook !!!!

Quoi de neuf?

- Sciences en cour[t]s 2011 : jeudi 21 avril !
- Pour vous tenir en haleine, voici les 1ères infos sur les TCM 2011 !!
- Meilleurs voeux!
- TCM 2011, c'est parti!
- Sciences en cour[t]s 2011: Appel à candidatures

Figure 67. Web page of the film “Les Petits Héros”, by Luth

2. modelling the biological filtration with macrophyte lagooning

The experiments carried on in the "station expérimentale" showed that the treating efficiency depends on the plant and on the level. The effect of all combinations of plants in the different levels is long and expensive to measure. Therefore a model was developped in 2008 using Matlab software for the comparison of various combinations. The objective of the model is to use existing knowledge to choose the best combination that should be evaluated, depending on the composition of the input.

The decrease of concentration in lagoon is influenced by sedimentation, volatilization (nitrification/denitrification in the case of nitrogen) and plant uptake. The plant uptake is considered as less efficient (Zemanova et al., 2010) but it is a very simple factor which we can get from analyses. In our experiment, in last lagoon or the lagoon that has lower concentration level, the plant uptake factor was higher than sedimentation.

Because every lagoon in «station expérimentale» has only one plant, it is easier to determine the factors that influence the decrease of chemical elements.

In the following equations, the capital letters E, I, O, correspond respectively to efficiency (percentage without unit), input concentration (in mg/L), output concentration (in mg/L), the subscript SP means “without plant”, the subscript “P” means “with plant” , the subscript “S” means “due to sedimentation” , the subscript “D” means “due to volatilisation (denitrification for N)” , the subscript “A” means “due to plant uptake”.

The following equations were used in the model:

$$E_{SP} = (I_{SP} - O_{SP}) / I_{SP}$$

$$= (I_{SP} - (O_{SP\ S} + O_{SP\ D})) / I_{SP}$$

$$E_{P} = (I_{P} - O_{P}) / I_{P}$$

$$= (I_{P} - (O_{P\ S} + O_{P\ D} + \mathbf{O_{P\ A}})) / I_{P}$$

Hypothesis:

O_A is significant

$O_{SP\ S} = O_{P\ S} = O_S$

$O_{SP\ D} = O_{P\ D} = O_D$

$I_{SP} = I_P = I$

From previous equations we can deduce:

$$E_P - E_{SP} = ((I - (O_S + O_D + \mathbf{O_{P\ A}})) / I) - (I - (O_S + O_D)) / I)$$

$$E_P - E_{SP} = - \mathbf{O_{P\ A}} / I$$

$$E_P = E_{SP} + \mathbf{O_{P\ A}} / I \text{ ....(1)}$$

Otherwise efficiency is calculated from:

$E_P = (I_P - O_P) / I_P$

Therefore:

$E_P \times I = (I - O_P)$

$O_P = I - (E_P \times I)$

$O_P = I \times (1-E_P)$ ……………………..(2)

$O = I \times (1- (E_{SP} + \mathbf{O_{P\,A}} / I))$

$O = I - (I \times E_{SP}) + \mathbf{O_{P\,A}}$ .... (3)

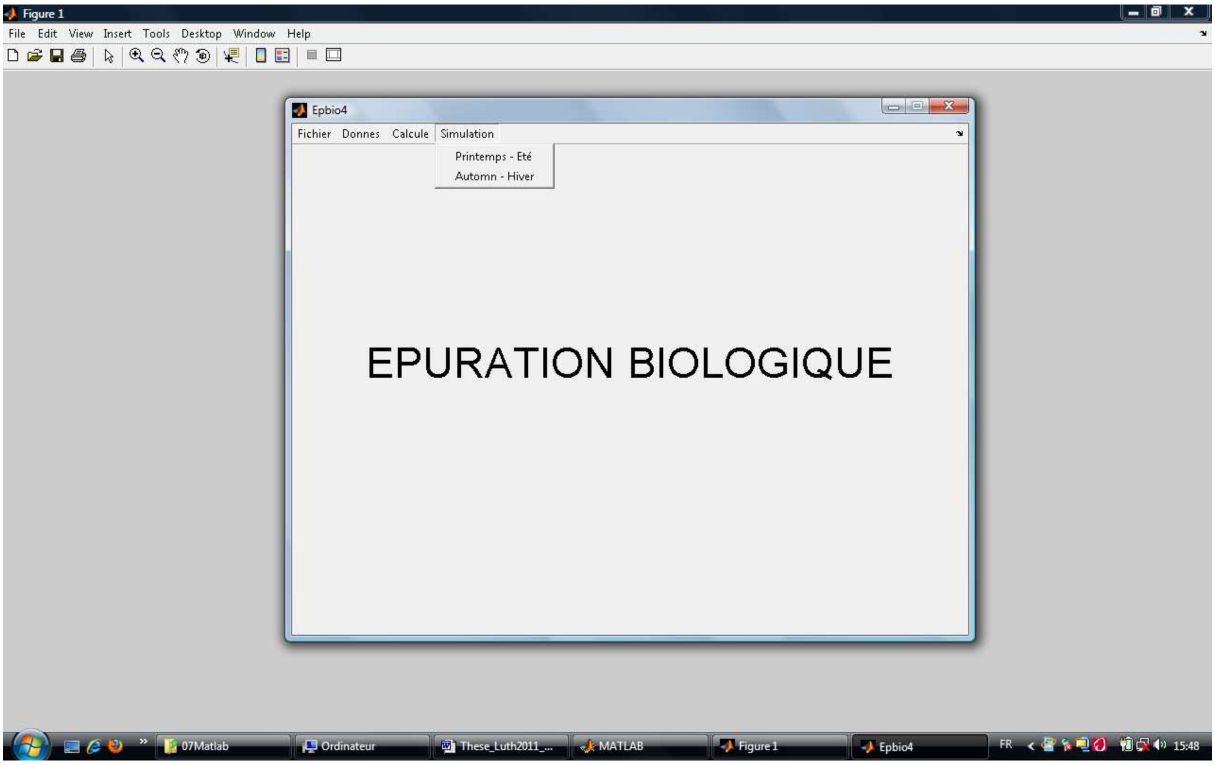


Figure 68. Starting Page program

The program is started in Figure 68. In the menu program, there are several choices. ***Fichiers, Donnes, Calcule*** and ***Simulation***. ***Fichiers*** menu is used for general operations. ***Donnes*** menu can be used for data input and for modification of the data. ***Calcule*** can be used to calculate a single parameter. ***Simulation*** menu has function to present the simulations process that are found in vermifilter and lagoon. Because several parameters are different between Spring-Summer and Autumn-Winter, there will two types of simulation. They are Spring-Summer simulation and Autumn-

Winter ones. The equations that are used for both simulations are be same but the parameters are different.

In the first version of computer program, the aim is to apply the equation on Matlab program. The decrease in concentration indicates that the program works well. If equation (3) is applied to Matlab program, the form of the program is illustrated in Figure 69. When the program is started, the value of chemical element concentration must be entered in the box of piggery outlet. Then when ***Simulation*** button is clicked, the program will be calculated the result itself.

In the equation (3), the calculation result of output will always be changed follow the change of input and output plant/ plant uptake value. The value of efficiency without plant will be got by the experiment data.

Input value is always changed because several factors such as feed condition of animal or the concentration of storage lagoon. The value of output plants will have several values because the uptake capability is different for several plants. This value can be used to choose the best combination of plants from first to last lagoon.

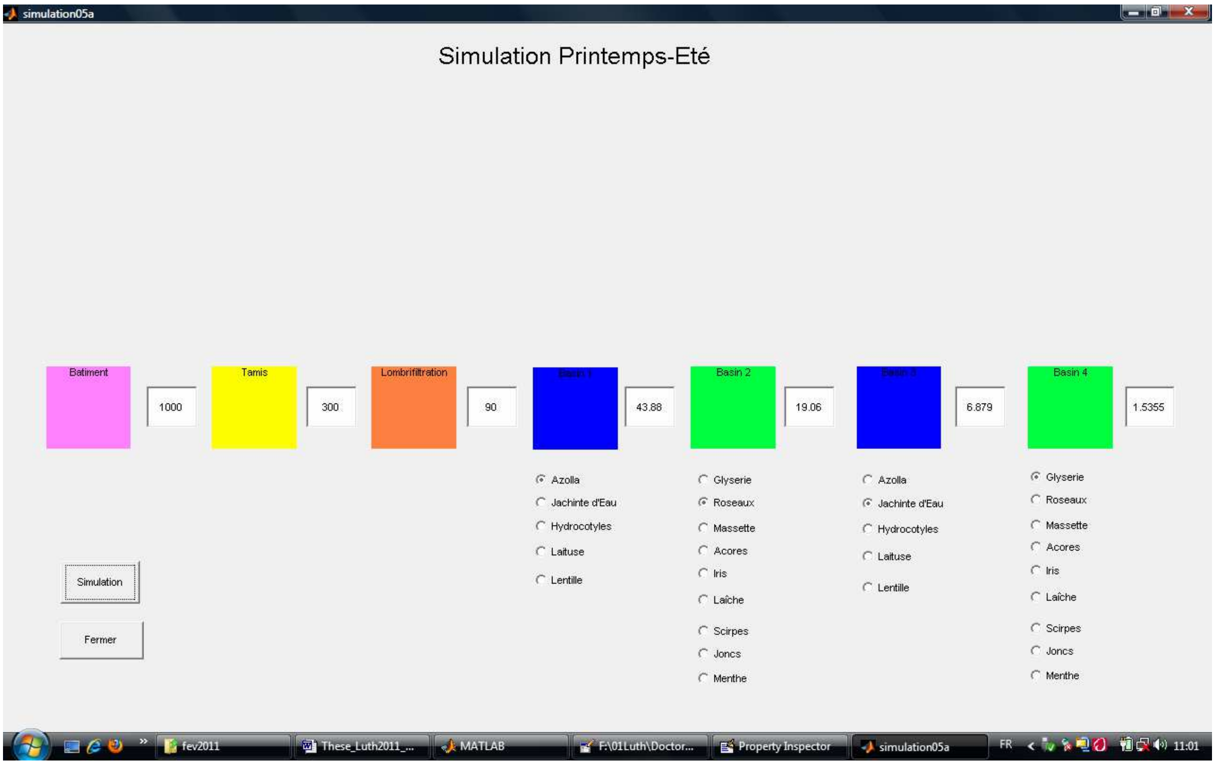


Figure 69. Simulation Spring-Summer page

On the Figure 70, lagoon system uses the combination of Azolla, Reeds, Hyacinth and Glyceria. On the Figure 71, lagoon system uses the combination of Hyacinth, Glyceria, water lettuce and Carex. The concentration value of sieve and vermifiltre outlets are the same. The concentrations in the outlets of plants boxes are different.

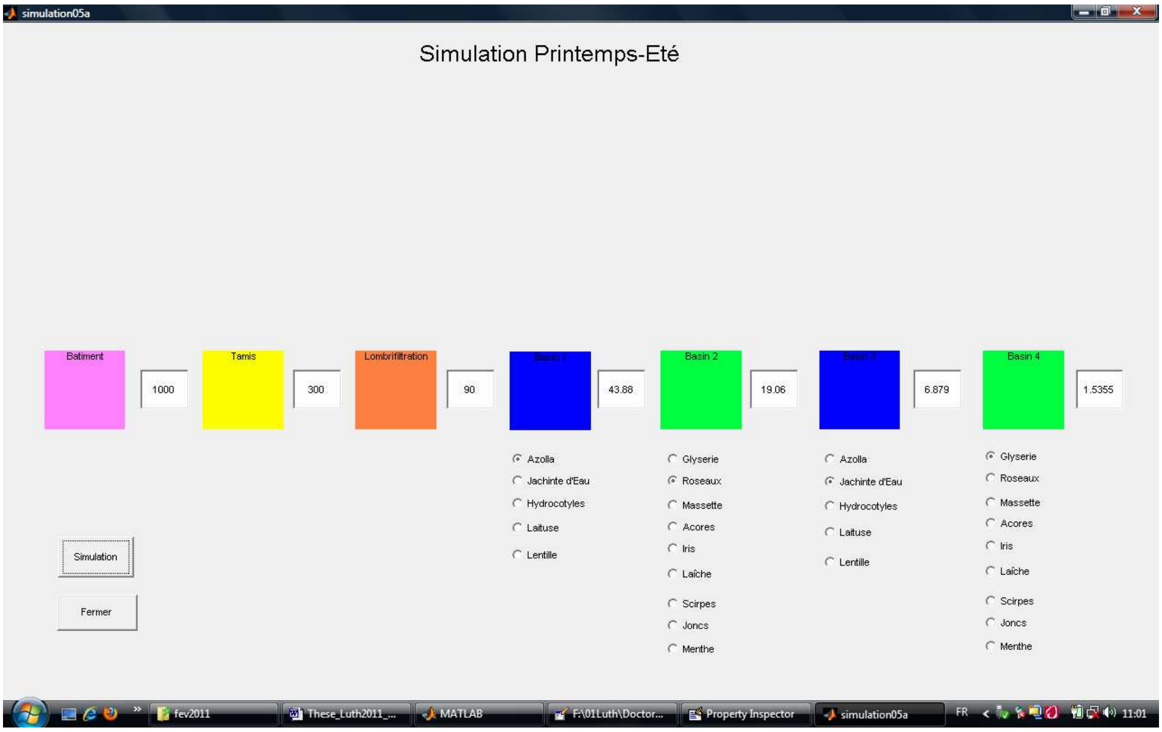


Figure 70. Simulation Spring-Summer page

This result illustrates the effect of choosing different parameters for different plants. The application of various plant combinations will influence the evolution of concentrations.

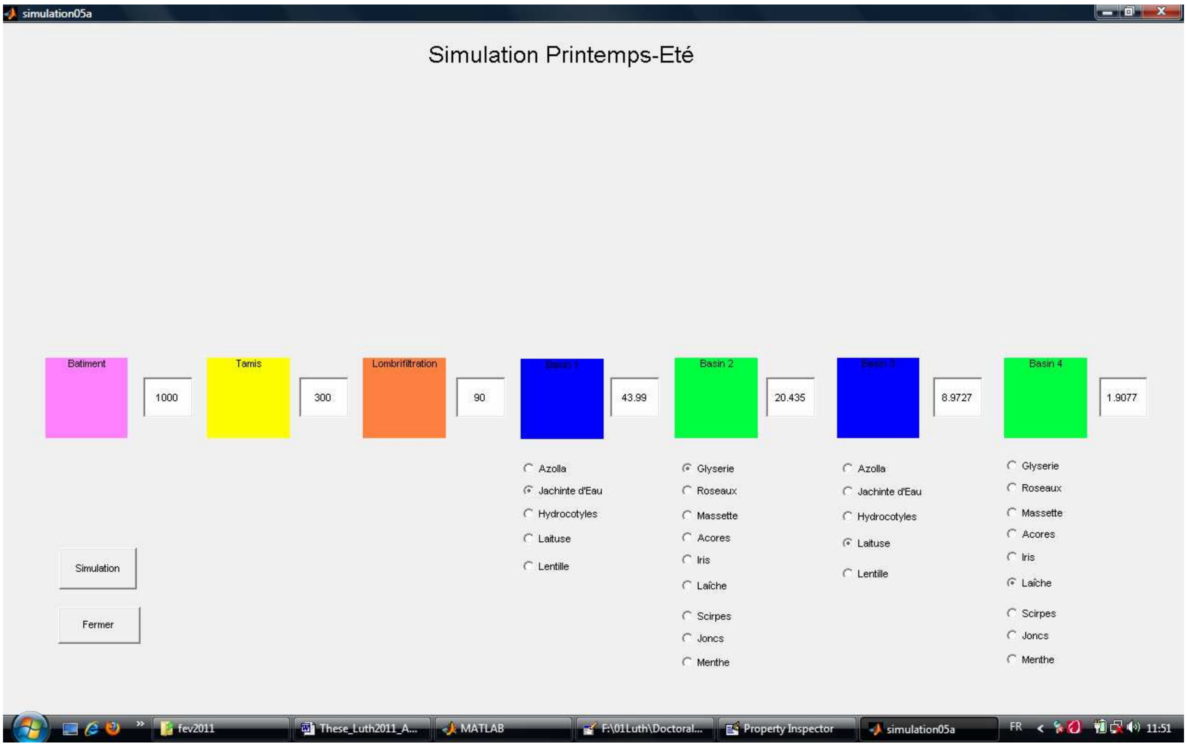


Figure 71. Simulation Spring-summer page (other combination)

The combinations of plants in Figure 70 and Figure 72 are similar. But the first input concentrations of the piggery outlet are different. The results of simulations that are found in boxes are different. This results illustrates the application of the model to various input concentrations using the same plant data.

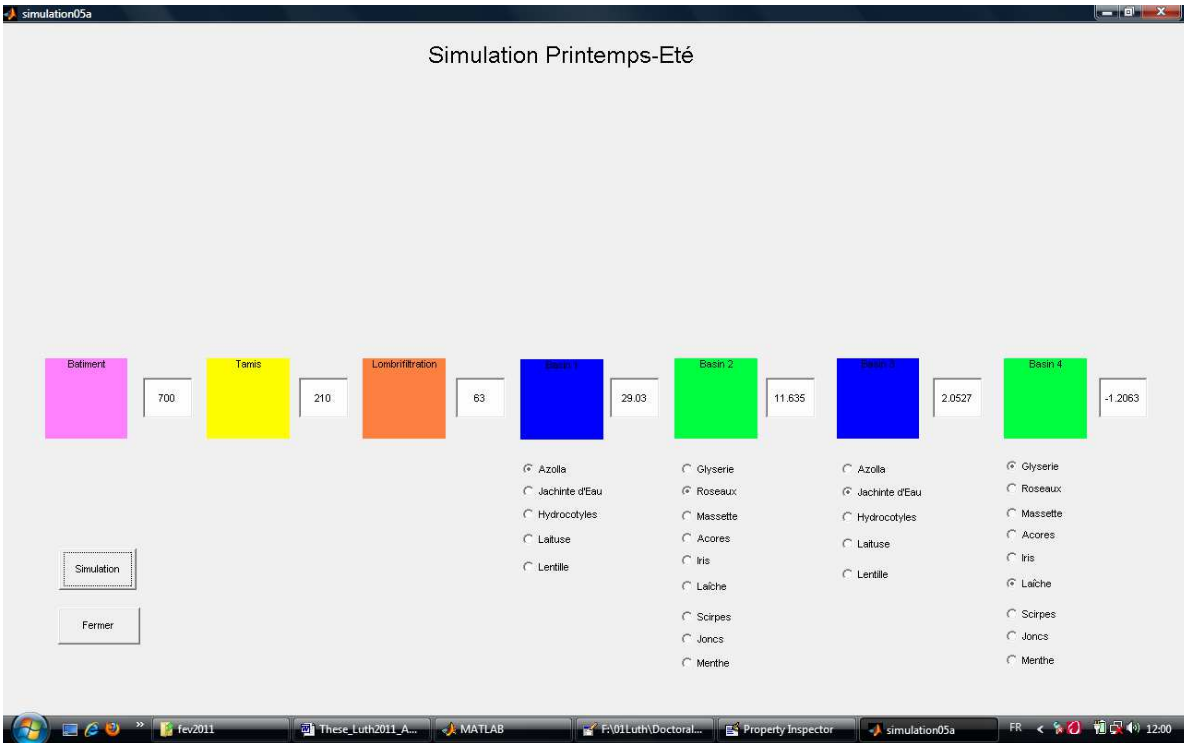


Figure 72. Different input concentration

In the next version program, several chemical elements will be calculated together (Figure 73). This version needs a more complex database to support their variables. There will be the input variation.

Figure 73. Next version

3. Adding value to the byproducts of the treatment system

A project was developped to use the vermicompost and the water within horticultural production. It was developped within the framework of the Entrepreneuriales Challenge (Rennes, 2009).

The business project would use several products from the research installation. The main idea of business was to use recycle product for reduce several costs of productions. The business would avoid the use of chemical product because they will make several pollutions to environment and their price is not stable or depends to price of petroleum.

The products that would be developed were orchids and poinsettia. The plantation media for the flowers was wood chips of vermifilter. The water of lagoon will be used as organic liquid fertilizer. Vermicompost of vermifilter can be used as solid fertilizer. The plants from lagoon such as azolla or others plants can be composted and also used as solid organic fertilizer.

The price of product was cheaper than in the market. The selling method that was used was B to B (Buyer to Buyer) because the business will produce high quantities of flowers. After sixth months of production, poinsettia could be sold. The orchids will be sold after 1,5 years of production.

RESUME

La production animale augmente continuellement à l'échelle mondiale depuis quelques décennies, dans les pays développés d'abord et maintenant dans les pays en développement. Des systèmes industriels ont donc été développés pour améliorer la productivité des élevages, pour augmenter rapidement la production animale et pour fournir la nourriture consommée par les villes. Ils sont efficaces en termes de biosécurité et d'efficacité de conversion des aliments du bétail mais ils ont des incidences sur l'environnement telles que les émissions d'odeur, les émissions d'ammoniac ou de gaz à effet de serre, ou la pollution de l'eau. La durabilité de ces systèmes dépend de leur capacité à limiter leurs impacts sur la raréfaction des ressources naturelles et à limiter leurs fuites de sorte que l'environnement naturel et la biodiversité puissent être préservés près des élevages. Des systèmes de traitement onéreux ne pouvant pas être employés pour des raisons économiques, l'ingénierie écologique fournit les concepts qui peuvent aider à trouver des solutions plus efficientes économiquement et écologiquement.

Notre travail a commencé avec la mise en route d'un système associant un bâtiment d'élevage de porcs, une séparation de phase liquide/solide de l'effluent du bâtiment, un lombrifiltre et un ensemble de zones humides artificielles. Destiné à augmenter l'efficacité de recyclage de l'eau et à produire des biomasses utilisables pour la nutrition animale, la fertilisation, la production d'énergie, etc., ce système combine donc la dilution élevée des effluents, permettant la diminution des émissions, à la réutilisation de l'eau et des nutriments. L'eau utilisée pour l'évacuation fréquente des déjections est ainsi recyclée. Les nutriments sont réutilisés sur l'exploitation agricole ou exportés. L'emprise au sol du système est environ 50 fois inférieure à celle requise pour l'épandage des effluents.

L'objectif fondamental de la thèse était d'améliorer la compréhension du système pour en préciser les avantages et les limites. L'objectif finalisé était d'étudier si les connaissances produites permettaient d'améliorer la conception et la gestion du système.

Des méthodes spécifiques ont été développées pour étudier, sous l'angle des processus et sous l'angle systémique, un dispositif dont les dimensions ne permettaient pas une reproduction dans un laboratoire. Elles ont été appliquées aux émissions gazeuses du lombrifiltre et à l'efficacité de traitement des zones humides artificielles.

Nos résultats permettent de définir une « quantité optimale » d'effluent qui maximise la population de vers de terre (preferendum). Au-dessus de ce seuil, les vers de terre meurent en raison de conditions anoxiques. Quand la population de vers de terre est maximale, les émissions d'ammoniac et de gaz à effet de serre sont limitées en regard du flux d'intrant. Par conséquent, l'abondance de vers de terre peut être employée comme bioindicateur de faibles émissions dans les systèmes de transformation d'effluent. L'effet des lombriciens sur les émissions gazeuses est surtout indirect, par leur influence sur la structure de la couche organique, sa porosité, les transferts de matière et sa population microbienne.

La « quantité optimale » transférée entre deux niveaux successifs peut être définie pour la production de végétation des zones humides artificielles. Par rapport à un système ouvert, le recyclage de l'eau induit un changement de la stoechiométrie des nutriments, en raison d'efficacités de traitement différentes de ces nutriments : par exemple, le taux de réduction du potassium est inférieur à celui de l'azote ; cette différence induit une augmentation de concentration en potassium dans l'eau par rapport à l'azote. La concentration en potassium se stabilise lorsque la rétention par tous les compartiments correspond à une diminution de masse équivalente au flux de potassium excrété par les animaux. Cela montre que la stoechiométrie des nutriments devrait changer dans les milieux agricoles et probablement dans les productions où l'efficacité du recyclage est augmentée. L'estimation du bilan de matière du système, montre que les émissions d'ammoniac et de gaz à effet de serre sont réduites par rapport aux flux d'azote, et que les produits organiques (lombricomposts et boues des lagunes) contribuent majoritairement à l'abattement des nutriments.

Des recommandations pour la conception et la gestion des systèmes qui améliorent le recyclage des effluents sont proposées à partir de ces connaissances. Nos résultats ont été et pourront être mobilisés pour des buts socio-économiques.

Luth, 2011, Ph.D., abstract:

EFFECT OF THE ASSOCIATION OF VERMIFILTRATION AND MACROPHYTE LAGOONING ON MANURE RECYCLING ON THE ANIMAL FARM

Animal production increased regularly since some decades, in developed countries at first, and now in developing countries. Industrial systems have been developed to increase rapidly the productivity of animal farms and to supply the food consumed by the towns. They are efficient in terms of biosecurity and of feed conversion efficiency but they have severe environmental impacts such as the odor emissions, the ammonia or greenhouse gas emissions, or the water pollution. The sustainability of these systems depends on their ability to limit their impact on resource depletion and to limit their leakages so that the wild environment and the biodiversity can be preserved beside the producing areas. Expensive treatment systems can not be used because of economical reasons. Ecological engineering provides concepts that can help finding solutions more efficient economically and ecologically.

Our work began with the starting up of a new system of animal production that associates a pig house with manure flushing and screening, a vermifilter, lagooning, and constructed wetlands. This system was designed to increases the recycling efficiency of water and to produce biomass for animal feed, fertilization, biogas, etc. The system combines high manure dilution, which allows a decrease in polluting emissions, to the reuse of water and nutrients. Water is reused for excretion flushing. The nutrients are either reused within the farm or exported. The needed surface is around 50 times less than for manure spreading.

The fundamental objective of the present work was to improve the understanding of the system to define more precisely its advantages and its limits. The applied objective was to study if this new knowledge was useful to improve the design and the management of this system.

Specific methods were developed to study from the process or from a systemic point of view a recycling system that was too large to be reproduced in a laboratory. They were applied to the gaseous emissions of the vermifilter and to the treatment efficiency of the combination of lagoons and constructed wetlands.

The results show that an “optimal transfer” of liquid can be defined that will maximize the earthworm population (preferendum). Above this input the earthworms die because of anoxic conditions. When earthworm population is maximal, the ammonia and the greenhouse gases are minimized as related to the input flux. Therefore, the earthworm abundance can be used as a bioindicator of low energy and low emissions in manure transforming systems. The effect on gaseous emissions is mostly indirect, through the influence of earthworms on the structure of the organic layer, its free air space, transfer of organic particles and its microbial population.

This “optimal transfer” between two successive levels also exists for the vegetation production of lagoons and constructed wetlands. If we compare “recycling” to “open” system, the water recycling will induces a change in the stoechiometry of nutrients, because of the various treatment efficiencies of elements: for example, potassium abatement rate is less than nitrogen abatement rate; this case induces an increase in potassium concentration in the water compared to nitrogen. Potassium concentration reaches a stable level when the retention by all subsystems corresponds to a mass decrease equivalent to the potassium excreted by the animals. This case shows that the stoechiometry of nutrients should change in agricultural systems with increased recycling efficiency. Calculating the mass balance of the system shows that ammonia and greenhouse gas emissions were low, regarding the nitrogen fluxes, and that the organic products (worm casts and sludge from lagoons) were the major contributors to the removal of nutrients.

Recommendations for the design and management of systems that improve manure recycling are proposed, based on this knowledge. Our results were and can be further used for socio-economical purposes.