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Management of nitrogen from underseeded clover and manures in spring wheat

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment
of the requirements for the degree of Doctor of Philosophy

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FOREWORD

The present thesis was part of a multidisciplinary project taking place as a component of the Green Plan of the Canada-Quebec Auxiliary Agreement for a Sustainable Environment in Agriculture. This study was entitled *Determination of the Fertilizer Value of Farm Manures in Grain Corn and Spring Cereals Cropping Systems*. The focus of the thesis was on the N fertilizer value of animal manures in spring wheat production with red clover present or not as an underseeded companion crop.

The thesis comprises six sections. The general introduction and the literature review constitute respectively the first and the second section of the thesis. Section 3, 4 and 5 constitute the body of the thesis and each represents a complete manuscript. Section 7 is a general conclusion. This format conforms to regulations of the Faculty of Graduate Studies and Research which are reported in appendix A. The three manuscripts are still to be submitted to the *Canadian Journal of Soil Science*.

Although all the work was under the responsibility of the candidate, the project was supervised by her advisors, Dr. R.R. Simard of Agriculture and Agri-Food Canada and by Prof. A. F. MacKenzie of Macdonald College of McGill University. Both will appear as co-authors on the papers.

ABSTRACT

Manure and underseeded clover are sustainable N sources for spring wheat on gleysolic soils of the St. Lawrence lowlands. Farmers rely on little information to manage adequately these alternatives to fertilizer N. This study documents in spring wheat (*Triticum aestivum* L. cv Algot) i) the agronomic value of underseeded red clover (*Trifolium pratense* L. cv Arlington) ii) the impacts of application time and underseeded clover on manure N recovery iii) the residual NO_3^- in the soil profile that constitutes a potential risk of N transfer from soil to air and water associated to clover alone or combined with manures and, iv) the use of a plant N availability index. A four year field experiment was established on a St. Urbain clay (Orthic Humic Gleysol) at St. Bruno de Montarville (45°33'N; 73°21'W) in 1993. Ammonium nitrate at 0 to 160 kg N ha⁻¹, swine liquid manure (SLM) and dairy solid manure (DSM) were used either alone or in combination with clover ploughed down in fall as green manure. Manures were applied at pre-seeding, in post-emergence or after harvest. The impact of clover on wheat yield was related solely to an improved N nutrition. Clover supplied fertilizer N equivalents of approximately 80 kg ha⁻¹ to the succeeding wheat crop. Clover combined with manures increased available N in the soil profile so that estimated recovery of manures N was strongly reduced in 1995. Apparent N recovery of SLM was higher than for DSM with values of 5% and 17% in 1994 and 1995 compared to 2 and 4% for DSM. Application time did not significantly affect manure N recovery. Limited risk of N transfer to water and air was associated with post-harvest manure application and underseeded red clover because those practices increased soil profile NO_3^- in fall and the end of April. Fluxes of

N estimated by NH_4^+ and NO_3^- sorbed *in situ* on ionic exchange membranes (IEMs) provided better monitoring of N released by added organic N sources than N extracted by 2M KCl and also were better related to wheat N uptake. IEMs appear to be a promising alternative to 2M KCl and water extractions in poorly drained fine-textured soils receiving N from organic sources and subjected to cool and wet spring conditions.

RÉSUMÉ

Peu d'information est disponible aux agriculteurs pour gérer adéquatement l'azote provenant des fumiers dans la production de blé de printemps en compagnonnage avec le trèfle produit sur les sols gleysoliques des Basses-Terres du St.-Laurent. Pourtant il s'agit d'une alternative intéressante aux engrais de synthèse dans le cadre d'une agriculture durable. Cette étude documente pour le blé de printemps (*Triticum aestivum* L. cv Algot) i) la valeur agronomique du trèfle rouge (*Trifolium pratense* L. cv Arlington) en culture compagne, ii) l'effet du temps d'application et du trèfle sur le recouvrement du N des fumiers, iii) les risques de transfert de N des sols dans l'eau et dans l'atmosphère associés au trèfle rouge en culture compagne seul ou en combinaison avec les fumiers et iv) l'utilisation d'un indice de la disponibilité de N au blé. Une expérience au champ de quatre ans a été établie en 1993 sur une argile St.-Urbain (gleysol humique orthique) à St.-Bruno de Montarville (45°33'N; 73°21'W) en 1993. Du nitrate d'ammonium à des taux de 0 à 160 kg N ha⁻¹, du lisier de porc (LP) et du fumier solide de bovins laitiers (FB) ont été utilisés seuls ou avec le trèfle enfoui à l'automne comme engrais vert. Les fumiers étaient appliqués soit au semis, soit en post-émergence ou après la récolte. L'impact du trèfle sur le rendement du blé était attribuable uniquement à l'amélioration de la nutrition azotée. Le trèfle rouge a fourni à la culture suivante de blé l'équivalent de 80 kg N ha⁻¹ d'engrais minéral azoté. Combiné aux fumiers, le trèfle a augmenté la quantité de N disponible aux plantes de telle sorte que le recouvrement du N des fumiers a été fortement réduit en 1995. Le recouvrement apparent de N du LP était supérieur à celui du FB avec des valeurs de 5 et 17% en 1994 et 1995 comparées à 2 et 4% pour le

FB. Des risques limités de transfert de N vers l'eau ou l'atmosphère ont été associés à l'application post-récolte des fumiers et à la présence de trèfle parce que ces pratiques ont augmenté la quantité de NO_3^- dans le profil de sol à l'automne et au début du printemps. Les flux de NO_3^- et de NH_4^+ estimés *via* leur sorption *in situ* sur des membranes d'échange ionique (MEIs) ont permis un meilleur suivi du N relâché par les sources organiques. Les flux de N étaient aussi davantage reliés au prélèvement en N du blé que le N minéral extrait au KCl 2M. Les MEIs semblent être une alternative prometteuse aux extractions au KCl 2M et à l'eau pour les sols recevant du N de sources organiques et soumis à des printemps frais et pluvieux.

“Si jamais - et pareille occurrence est furieusement possible - si jamais l'on allait accuser l'auteur de ce bref mais instructif poème d'écrire des inepties, cette accusation serait fondée sur le vers:

“Et puis l'on confondait gouvernail et beaupré.”

En prévision de cette pénible éventualité, je n'en appellerai pas (comme je le pourrais) d'un air indigné à mes autres écrits pour prouver que je suis incapable d'un tel comportement; je n'insisterai pas (comme je le pourrais) sur le puissant dessein moral du poème lui-même, sur les principes arithmétiques que l'on y inculque avec tant de prudence, ni sur ses nobles enseignements en matière d'Histoire Naturelle; - je prendrai le parti plus prosaïque d'expliquer tout bonnement comment les choses advinrent.

Lewis Carroll La Chasse au Snark

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The author feels as a privilege to have this opportunity to express her gratitude toward her advisors, Prof. Angus F. MacKenzie and Dr. Régis R. Simard. Their knowledge and their confidence were most appreciated. The author was fortunate enough to benefit the best of two worlds: the energy and the enthusiasm of Dr. Simard and the wisdom that Prof. MacKenzie acquired through a long and fruitful career.

It should be mentioned that Dr. Simard committed a real leap of faith six years ago by taking under his supervision for her master degree a very inexperienced student. He provided a firm but human guidance in this fascinating but sometimes arid and complex world that is soil science. After all those years of formation, I now feel as a very inexperienced soil scientist. But I am confident that Dr. Simard gave me the survival kit that will indispensable to face new and unexpected questions.

Involved on a less formal way, are those people with who I had great scientific exchanges and whom I should thank for all the inspiration that came from those discussions: Dr. Thi Sen Tran of the Quebec Minister of Agriculture, Food and Fishery, Dr. Suzanne Beauchemin, Ms. Noura Ziadi, and Ms. Sylvie Côté of Agriculture and Agri-Food Canada. This thesis would not look the same without their contribution.

I am grateful to the direction of the St. Foy Soils and Crops Research and Development Centre of Agriculture and Agri-Food Canada for the paper work and computer facilities. They should also be thank for the laboratory facilities and the financial support.

I thank also people of the Québec Minister of Agriculture, Food and Fishery working at the St. Bruno de Montarville experimental farm who took care of all the field work. I especially acknowledge is Prof. Chantal Hamel (McGill University) and Dr. Gilles Tremblay who not only supervised and co-ordinated all the field work but who answered very patiently to all my information requests about the project.

Finally, this thesis is dedicated to the memory of my most unusual grandmother, Ms. Antoinette Pagé, who never taught me cooking, sewing or gardening but who insisted repeatedly that a woman should go to the university and achieve a strong formation. I hope she would be happy that for once, I tried to follow her advice...

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GENERAL INTRODUCTION

The shift toward sustainable agriculture and the need to dispose of wastes generated by animal production (Sims 1995) has renewed the interest for investigating efficient management of sources of N as alternatives to mineral fertilizers in various cropping systems and environments. Wani *et al.* (1994) found that spring cereal yields can be sustained through legume and livestock manure N inputs. In Quebec, spring wheat maximum grain yield was reached on heavy texture soils of the St. Lawrence lowlands when 90 to 130 kg N ha⁻¹ was applied as ammonium nitrate (Ayoub *et al.* 1994; Simard *et al.* 1997). Eventually, those N requirements could be fulfilled through legume and/or manures.

The integration of a legume crop in spring cereal farming systems is beneficial to the soil N availability to crops and thereby enabling some reduction in the amount of fertilizer N required for optimal yield (Ledgard and Giller 1995). Many studies document cropping sequences involving cereals and legumes. But another system that received little attention is cereal in companionship with an underseeded legume buried as green manure in late fall. Contrary to rotations, this avoids losing years of cereal production (cash crops) and still brings biologically fixed N to the soil that may be subsequently used by the cereal. Even if it is recognised that N is largely involved in the observed response of crop yields after a preceding legume crop (Rowland *et al.* 1988), many other non-N nutritional effects such as competition (Williams and Hayes 1991; Jones and Clements 1993) and leaf-disease reduction (Stevenson and van Kessel 1996) can be invoked for the observed changes in cereal yields and N uptake. Although the use of legumes as an

alternative to N fertilizers is promising, a better knowledge of their overall agronomic value (N and non-N effects) is required for their efficient management. There is no currently published estimation for the N furnished to wheat by underseeded clover and incorporated to soil as green manure in fall in cool and humid climates of the northern hemisphere.

In Quebec, it is an interesting option to recycle the large quantities of N coming from the dairy and hog productions in spring cereals. It is well recognized that manures show lower N availability than mineral N fertilizers (Sims 1995). Therefore, on a total N basis, manures have to be applied at higher rates than mineral fertilizer to meet wheat N demand. Amongst important factors affecting the N availability of manure, time of application was found to be critical but the small body of literature on this subject presents conflicting evidence (Culley *et al.* 1981; Beauchamp 1983; Jokela 1992; Zebarth *et al.* 1996) and therefore needs to be further documented. Manures could also be used in conjunction with legume green manure. To our knowledge, no scientific literature documents management of N from manures and interseeded legumes in spring cereal monoculture.

Sustainable alternatives to conventional practices must conciliate agronomic and environmental considerations. The presence of NO_3^- after harvest and in early spring was related to NO_3^- leaching (Chichester 1977; Malhi and Nyborg 1986; Liang *et al.* 1991) and denitrification (Goodroad and Keeney 1984; Liang *et al.* 1991; Liang et MacKenzie 1997). Therefore, reduction of contamination risk implies that NO_3^- present in the soil profile in fall and early spring prior to seeding should be kept to a minimum. To avoid excessive residual N in the soil profile, N application should match crop needs and highly

labile N from buried green residues has to be released in synchronisation with plants needs the following season.

Literature is not unanimous on effects of multiple cropping systems combining cereal and legume on N losses reduction (Izaurrealde *et al.* 1995; Owens *et al.* 1995; Weed and Kanwar 1996; HØgh-Jensen and Schjoerring 1997; Kuo *et al.* 1997). The impacts of the complex interactions between the type of manures, their time of application and the presence of a legume companion crop on the residual NO_3^- left in the soil profile in fall and early spring remains to be documented.

The amount of N from different sources added to match crop N requirement as precisely as possible would be better estimated if the available N already present in the soil was considered. This could mitigate potential effects due to over-fertilization as well as under-fertilization and therefore reconcile agronomic to environmental imperatives. This is of a particular importance in agro-ecosystems receiving N inputs mostly from organic sources. The successive additions of organic N could result in the build-up of a soil organic pool which will release significant amounts of N through mineralization. In southern Quebec, there is not such an index of soil N availability applied in the current soil testing practice. Moreover world-wide, the quest of a N index robust to varying pedoclimatic conditions is still intensely pursued (Geypens and Vandendriessche 1996). Nitrogen indices based on chemical extraction had limited successes under specific soil, climate and agro-ecosystem. For soils and climatic conditions comparable to those prevailing in Quebec, Magdoff *et al.* (1984) found that the NO_3^- content measured in the soil plough layer 4 to 6 weeks after seeding was related to the corn N uptake and thus was considered as an effective index of N availability. A promising alternative to chemical

extractions is the N sorbed on ion exchange resins when they are placed *in situ*. They were used in environmental studies (Skogley and Dobermann 1996) and tested as fertility index for numerous nutrients (Abrams and Jarrell 1992; Qian *et al.* 1996; Schoenau and Huang 1991). Ion exchange resins in the form of membranes appear to be the most suitable form for *in situ* use. The capacity of the technique to provide a meaningful index of N availability under field conditions is still to be demonstrated.

This thesis focuses on the management of N from underseeded red clover residues and manures in spring wheat in order to fulfil wheat N requirements while minimizing potential N transfer from the soil to the atmosphere and surface and ground waters. Its objectives were drawn from the above considerations. The objectives of the first paper were i) to estimate the N and non-N effects of red clover on spring wheat in a clover mixed intercropping system ii) to assess the effect of the clover on NO_3^- presence at critical times for N leaching and denitrification. The objectives of the second paper were to i) to assess the impact of manure application time and of red clover interseeding on N recovery of swine liquid manure (SLM) and dairy solid manure (DSM) by spring wheat, ii) to estimate the effect of the different types of manure, application time and, of red clover companion crop on the NO_3^- in the soil profile in early spring and after harvest and, iii) to test NO_3^- measured in the plough layer 30 days after seeding as an index of N availability to spring wheat. Finally, the objectives of the third paper were i) to test the capacity of N sorbed on ionic exchange membranes (IEMs) to discriminate between treatments comprising N from different sources during spring wheat growing season compared to conventional chemical indices and ii) to establish a relationship between wheat N uptake and N fluxes measured on IEMs.

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REVIEW OF LITERATURE

NITROGEN MANAGEMENT

The sustained removal of soil nitrogen (N) due to the crop export from the field and various natural losses regularly cause short supply of this nutrient in agroecosystems. Farmers relied for centuries on legumes and manure to replace N transported of their fields. But, at the beginning of the 20th century, the invention of the ammonia synthesis through the Haber-Bosch process allowed the production of mineral N fertilizers. This resulted in the use of N-rich fertilizers which are readily available and homogeneous sources of N rather than heterogeneous and slow-releaser sources of N such as manure and legumes (Smil 1997). The wide availability and simplicity of use of N mineral fertilizers contributed to their massive application in many agroecosystems. Nowadays, there is increasing interest in disposing soundly of animal production wastes while providing an adequate N supply to crops and in reducing fertilization costs by using alternatives to fertilizers such as manure or legume. This is reflected by the rapid growth of the body of literature concerning organic N sources.

As the global N overload problem grows really critical (Vitousek *et al.* 1997; Simon Moffat 1998), diffuse pollution of water and atmosphere from agricultural activities are increasingly identified as major actors in problems ranging from local health to global changes. Intensive agriculture causes massive introduction of reactive N into soils and water. Stevenson (1986) stated that no more than two thirds of the N added as fertilizer can be accounted for by crop removal or in the soil at the end of the growing

season; losses of as much as one-half are not uncommon. The N lost in the environment alters the chemistry of the atmosphere and of aquatic ecosystems, contributes to eutrophication of the biosphere, has substantial regional effects on biological diversity in the most affected areas (Vitousek 1994) and contributes to the acidification of many arable soils. The province of Quebec contributes its share of this world-wide load. As an example, many studies have showed that water from agricultural watersheds had greater N concentrations than that from forested watersheds (Simoneau 1991, 1990).

Adequate N management in agroecosystems should meet environmental and agronomic issues. Nitrogen management can be defined as the way by which farmers will fulfil the N needs of their crop. When added to the soil, the N enters in the complex biogeochemical N cycle. The various components of the N cycle and their interaction may result in insufficient level of soil N to insure optimal crop yields or to an excessive one and so pose a threat to the environment. As a specific example from Quebec, for wheat, C.P.V.Q. (1996) recommended 90 kg N ha^{-1} to obtain optimum crop yields and grain quality. But according to many studies (Tran 1996; Chalifour *et al.* 1994), in soils with previous legume crops or with high organic matter contents, optimal rates of N can be as low as 0 to 50 kg N ha^{-1} . On the other hand, in soil with low available N, the rates necessary to reach optimum wheat yields can be up to $100\text{-}120 \text{ kg N ha}^{-1}$.

In regard to the overall N cycle, the challenge of sustainable agriculture is to avoid excessive N inputs and to synchronize the maximum soil N availability in the root zone with maximal plant uptake rate (Swift *et al.* 1980). This allows the crop N requirements to be fulfilled while the residual N left in the soil profile is reduced in fall and early spring when risk of N transfer to water and air is important (Chichester 1977; Goodroad and

Keeney 1984; Malhi and Nyborg 1986; Keeney and Follett 1991; Liang *et al.* 1991; Liang et MacKenzie 1997).

Nitrogen management is currently based on the N budget approach also named the balance sheet method (Remy and Viaux 1982; Meisinger and Randall 1991; Tran 1996). Nitrogen budgets rely on the concept of conservation of mass that simply states that the N inputs into a particular ecosystem less the N outputs must equal the change of N stored within the ecosystem (Meisinger and Randall 1991). This budget considers anticipated need of the crop, the available N of the soil and of the external sources (plant residues, fertilizer and manures). The systems outputs are crop uptake, losses through denitrification, NH_3 volatilisation and NO_3^- leaching. The systems inputs are mineral N from fertilizer and manure and N from the mineralization of organic N from soil organic matter, manures and plant residues. The required amount of N to be applied as fertilizer or manure can be estimated by this approach. An equation of a simplified N budget in a system where a cereal is underseeded with a legume could be:

$$N_{\text{uptake}} = \sum a N_{\text{source}} + N_{\text{legume}}$$

where N_{uptake} represents the total N uptake required to obtain maximum or optimum yield, N_{source} the total N added from the different sources and N_{legume} the N supplied by the legume to the cereal (N credits). The constant a is the specific efficiency coefficient or percentage of recovery of the organic or mineral sources (Remy and Viaux 1982; Sims 1995; Meisinger and Randall 1991).

Fertilizer N additions should be the easiest and most accurately known inputs into a defined agricultural system (Meisinger and Randall 1991). Only a fraction of the N applied is effectively taken up by crops. Applied fertilizer efficiency usually decreases as

the amount of total plant available N increases, diminishing the economic return per unit of N fertilizer applied. In Quebec, ^{15}N studies showed that efficiency coefficients of mineral fertilizers for cereals varied between 35 to 50% with a mean of 40% (Tran 1996; Chalifour *et al.* 1994). Therefore a considerable amount of N is lost, accumulated or redistributed in different forms in the soil profile. Yearly losses from mineral fertilizers can be as high as 20 to 40% of the amount applied (Tran 1996). Losses increase if the crop yield is lower than expected. Coarse-textured soils are particularly prone to losses by leaching. Nitrogen losses introduced considerable variance in the efficiency coefficient estimation because they are quite specific to a given set of climatic and pedologic conditions (Geypens and Vandendriessche 1996). Yield responses of crops to mineral N addition also vary in relation to the amount of soil plant-available inorganic N ($\text{NO}_3^- + \text{NH}_4^+$) at the beginning of the growing season, and N supplied throughout the season by mineralization (Bock and Hergert 1991).

The organic N fraction of manures and legumes, which can represent a considerable proportion of their total N content, is made available to plants *via* mineralization by soil microorganisms. This process is highly dependent on the soil chemical and physical properties (Tabatabai and Al-Khafaji 1980; Chae and Tabatabai 1986) and on climatic conditions (Sabey 1969; Tabatabai and Al-Khafaji 1980; Flowers and Arnold 1983; MacDonald *et al.* 1995). Therefore, all estimates for N supplied by manure and legumes are very imprecise because they are means of specific field trials that do not necessarily reflect local climatic and pedologic conditions. Available N from legume residues and manures will be discussed in further details in the next sections.

In many regions of the world, N fertilizer recommendations rely on the use of a soil test of N (Rice and Havlin 1994; Geypens and Vandendriessche 1996). This allows an adjustment of N supplied by fertilizers or manure to that already present in the soil and potentially available to plants. In Quebec, the actual system of recommendations that is applied in the current practice does not consider the status of soil N (C.P.V.Q. 1996). The use of a soil test of N as an index of N bioavailability will be discussed in the last section of the review of literature.

RED CLOVER AS A COMPANION CROP

Red clover (*Trifolium pratense* L.) is a short-lived perennial species, highly productive for two and, sometimes, three years. Its success as herbage legume is due to its adaptation to a wide range of soil and environmental conditions (Frame *et al.* 1997). Only excessively wet or acidic soils are unsuitable for its establishment. The optimum temperature for red clover growth is 20-25°C but it grows between 7°C and up to 40°C. Red clover is more tolerant of low light intensity than other forage legumes (Taylor and Smith 1995). It was found that red clover establishment can be satisfactory when clover is sown under a grain-cereal cover, and this is practised in several regions of the world, e.g. Scandinavia and Atlantic Canada (Frame *et al.* 1997). There is also a renewed interest in its role as green manure due to its low seed cost (Christie *et al.* 1992). Previous considerations make red clover an adequate companion crop to spring wheat for most climatic and pedologic conditions prevailing in eastern Canada.

In the Netherlands, Elgersma and Hassink (1997) estimated that clover furnished from 55 to 113 kg N ha⁻¹ yr⁻¹ to grass in a forage trial with perennial ryegrass-clover mixture. Pasture legumes have produced from 50 up to 250 kg N ha⁻¹ y⁻¹ from atmospheric N₂ fixation, (Ledgard and Steele 1992). At 15 sites in the cereal belt of Australia, wheat after lupine or pea produced more biomass and had a greater N content than wheat after wheat or barley; on average these crops assimilated 36 kg N ha⁻¹ more after a legume than after a cereal. The improved wheat yield after legumes averaged 0.8 Mg ha⁻¹, an increase of 38% over the other cropping sequences. Of the two possible sources of additional N for wheat after legumes, namely mineral N conserved in soil (up to 60 kg N ha⁻¹) and the total N added in the residues of these legumes (up to 152 kg N ha⁻¹), both were considered significant to the growth of the following wheat crop. It can be assumed that, in companionship with a cereal, clover plough down in fall could be a very significant source of N for the crop growing the following year.

Two main sources contribute to increase soil N available to a succeeding crop: a net N input *via* the symbiotic fixation and the conservation of N previously applied by the legume crop (Evans *et al.* 1991). There is a natural feedback mechanism between N₂ fixation and soil N. Decreases in N₂ fixation were observed with increasing rate of fertilizer N application (Ledgard and Giller 1995). The relative amounts of the legume N coming from fixed N or derived from soil is hard to estimate and to predict.

Legumes can either reduce residual N left in the soil by the cereal by scavenging it or increase residual N in the soil profile through the mineralization of biologically-fixed N at a time which may not be synchronized with plant N uptake. Tilman *et al.* (1996) proved that soil mineral N was utilised more completely when there was a greater

diversity of species in a given ecosystem, leading to lower N losses. HØgh-Jensen and Schjoerring (1997) confirmed those results in clover-ryegrass mixture which ensured a more efficient absorption of fertilizer N and soil derived N than pure stands of the same species. In addition, the soil without any plant cover is subjected to more leaching (Webster and Goulding 1995). Nevertheless, the literature shows conflicting evidence of the effects of rotation systems combining cereals and legumes on N losses reduction (Izaurrealde *et al.* 1995; Owens *et al.* 1995; Weed and Kanwar 1996).

There are numerous mechanisms by which N of legumes can become available to the companion non-legumes or to subsequent crops (Ledgard and Giller 1995). These mechanisms are collectively called N transfer. Among them, in non-grazing ecosystems, mineralization from above ground (senesced shoot) or below ground (senesced root and nodule) is the most important mechanism of N transfer. The amount of N below ground at harvest is often less than 10% of that in the above-ground parts, and the extent to which any benefit is observed depends on whether the above-ground residues are returned to the soil or removed from the fields. When the residues are buried, the N fixed by legumes enters in the detrital food chain and is subsequently mineralized by the soil microbial biomass and made available to a following crop. Field and laboratory studies have clearly shown increased N transfer in close proximity of roots between legumes and non-legumes in both pastoral and inter-cropping systems (Jensen 1996; Ledgard and Giller 1995). Although the importance of N₂ fixation and succeeding N mineralization have been known since the ancient times, there are very few quantitative estimates of this overall process under natural conditions (Meisinger and Randall 1991). In an incubation study at 30°C, the N mineralization of alfalfa (*Medicago sativa* L.) in different soils followed a

fast initial rate up to 12 weeks and a steady N release between 12 and 26 weeks (Chae and Tabatabai 1986).

In addition to N nutritional effects, many authors reported, for legumes in rotation with cereals, effects on cereals that go beyond the N supply. They are termed non-N nutritional effects or rotation effects (Smith *et al.* 1987; Lory *et al.* 1995). Among those effects, the following were documented: interruption of pest cycle (Cook 1988), positive or negative soil moisture effects (Smith *et al.* 1987; Roder *et al.* 1989), negative allelopathic effects (Raimbault *et al.* 1990) and improved soil physical properties (McVay *et al.* 1989; Raimbault and Vyn 1992). As a companion crop, legumes can also compete with cereals for available resources such as water, light and nutrients (Ofori and Stern 1987; Jones and Clements 1993). For rotations, it was found that the non-N effects can introduce serious bias in the estimation of legume N credits (Smith *et al.* 1987; Lory *et al.* 1995). It was concluded that non-N effects should be considered when legume N credits are estimated.

MANURE AS AN ALTERNATIVE TO MINERAL N FERTILIZERS

Manure N plant availability is difficult to estimate because of the variability in N composition, the spatial variability of manure applications, and the N losses that manures undergo in storage and after application (Meisinger and Randall 1991; Paul and Beauchamp 1995; Sims 1995). This complicates the task of meeting plant N requirements with manures but also of protecting air and water resources. Nitrate leaching and

denitrification were observed in various agroecosystems where soils received N inputs through manure (Stout *et al.* 1997; Paul and Zebarth 1997; Carpenter *et al.* 1998).

Manure contains inorganic N and organic N in variable proportions. The best way to overcome the uncertainties in the amount and relative composition of the N applied is to analyze manures before application (Meisinger and Randall 1991). On a total N basis, crop availability of N from manures is generally lower than N from inorganic fertilizers because of the slow release of the organically bound N and the volatilization of NH_3 from the surface applied manures when they are not incorporated immediately after application (Beauchamp 1983; Sims 1995).

The estimated amount of inorganic N in dairy solid manure and in swine liquid manure is 35% and 70% of the total, mainly in ammoniacal form (G. Barnett, personal communication). All inorganic N is often assumed to be as plant-available as that of mineral fertilizer (C.P.V.Q. 1995; Pratt *et al.* 1973) but Paul and Beauchamp (1995) found lower availability for NH_4^+ from manures than of the mineral fertilizer in corn production. This was attributed to immobilization in the soil microbial biomass. Previous research with manure (Beauchamp 1986) estimated the amount of organic N available to plants the first year of application as 10% of total organic N and this was observed with different application rates. Consecutive additions of manure contribute to the buildup of an organic N pool which increases the amount of N made available *via* mineralization to the crop (Xie and MacKenzie 1986). After a few years of manure application to the same land, substantial amounts of crop N can be supplied by the accumulation of organic N coming from manure (Sims 1995).

Although most N in the manure is in organic form and not immediately available to plants, N contained in such materials can be mineralized until its C:N ratio is similar to the C:N ratio typical for soil which is about 10:1 (Scheppers and Mosier, 1991). Chae and Tabatabai (1986) observed patterns of mineralization for animal manures (cow, chicken, hog and horse) in an incubation study at 30°C; there was little or no mineralization during the initial period of incubation (up to 4 weeks), then a marked increase in the rates of mineralization between 4 and 12 weeks, and a steady, constant release of mineral N from 12 to 26 weeks of incubation. Flowers and Arnold (1983) observed a net initial period of immobilization as pig slurry was added to soil and a subsequent rate of mineralization not significantly greater than in the untreated soil although organic N represented 43% of total N in the manure.

Ideally producers would prefer to have flexibility for the moment at which they apply manures during the growing season. Very little published information is available on the effect of the time of application on plant N availability of manures. Jokela (1992) found that dairy manure applied on corn at two different times (planting or six-leaf stage) had little or no effect on corn yield. MacKenzie *et al.* (1989) observed that spring application of swine liquid manure and dairy liquid manure increased corn yields compared to fall application (12% for dairy liquid manure and, 9 to 21% for swine liquid manure).

IONIC EXCHANGE MEMBRANES AS TOOLS TO MEASURE NITROGEN BIOAVAILABILITY INDICES

Fate of N is usually assessed by measuring variations in the different organic and inorganic soil N pools through a given period of time. Inorganic forms of N extracted by neutral salt solutions are the most common measurements used to observe indirectly biogeochemical processes and to assess bioavailable N (Magdoff *et al.* 1984; Sims *et al.* 1995). Under field conditions, soil extractable NH_4^+ and NO_3^- contents can change rapidly and widely in response to variation in water content and addition of labile organic matter (Sarrantonio and Scott 1988; Lessard *et al.* 1996). Therefore, an intensive sampling strategy over an extended period of time is required to adequately follow the release of manure N in areas subjected to cool spring temperatures and sporadic episodes of heavy rainfall such as found in Quebec. The task is very tedious and nearly impossible to achieve in current practices. This explains the failure to predict plant available N based on spring inorganic N testing in regions which show patterns of high and unevenly distributed rainfall (Belanger *et al.* 1998; Bittman and Kowalenko, 1998). Among other drawbacks of soil N supply power assessment by chemical extraction is the inability to integrate factors that affect it, such as diffusion (Barber 1995) and soil spatial organisation (van Vuuren *et al.* 1996; Strong *et al.* 1998) over a defined period of time.

Ions exchange resins may provide a very interesting alternative to chemical extractants. Dorfner (1991) defined ions exchangers as insoluble polymeric or macromolecular substances with fixed ions. Those permanent positive or negative charges confer to the ion exchange resin the capacity to sorb ions and to exchange them

with those in the soil solution. Ion exchange resin bags have been used for many years for the evaluation of N availability under field conditions in forests and showed differences among ecosystems and treatments (Binkley and Hart 1989). This technique has also been applied successfully by Hübner *et al.* 1991 in an agronomic study to determine N mineralized in field incubated soil cores.

As placed *in situ*, resins are sensitive to diffusion processes (Helfferich 1962). Diffusion controls the movement of many ions (notably NH_4^+) in the soil and so, affects plant nutrient uptake (Barber 1995). Ions sorbed on the resin will reflect the bioavailability of nutrients in a more accurate way than chemical extraction methods. Laboratory studies have shown that the accumulation of ions in resin bags depends strongly on mineralization rate, ion form (NO_3^- moves to the bags with less resistance than does NH_4^+), water movement to the bags, and competition for nutrients with microbes and plants (Binkley and Hart 1989). Many disadvantages are related to the use of resin bags: soil disturbance, dependence upon placement methods and effort required to bury the bags which restrict their extensive use.

Recently, Cooperband and Logan (1994) used anionic exchange membranes (AEM) to measure *in situ* changes in labile P because it alleviates diffusion problems cause by the three dimensional spherical structure of resin bags and permits the measurement of P fluxes with minimal soil disturbance. Subler *et al.* (1995) and Qian and Schoenau (1995) adapted the AEM method for use in soil laboratory incubation and established that it can be a reliable estimate of soil available N and of nitrification and mineralization processes. Qian and Schoenau (1995) also showed that the NO_3^- released

from soil organic matter and accumulated on the AEM reflected the expected effect of three different tillage systems and two landscape positions on mineralizable N.

Briefly, the major advantages of the membranes over chemical extractions is that they trap ions over a wide range of redox conditions (van Raij 1998), they can be inserted into the soil with minimum soil disturbance (Cooperband and Logan 1994; Abrams and Jarrell 1992) and therefore N sorbed may be more representative of field conditions over a period of time, and finally, the ions sorbed are influenced by diffusion (Vaidyanathan and Nye 1966). Therefore IEMs could provide information that chemical extractions do not in terms of nutrient bioavailability. To our knowledge, only one published study reported a relationship between NO_3^- sorbed on anionic exchange membrane and plant N uptake and this, for an experiment done under the controlled conditions of a growth chamber (Qian and Schoenau 1995).

The first mineral by-product of mineralization is ammonia (Nannipieri *et al.* 1994) but the particular form of mineral N mostly found in the soil is function of the redox conditions. Nitrate is stable only under strongly oxidizing conditions while ammonia is stable under strongly reducing conditions (Sposito 1989). In many studies, both forms were measured with exchange resin bags to assess the overall mineralized N (Binkley and Hart 1989). Until now, no use of *in situ* cationic exchange membranes to measure available NH_4^+ is reported in the literature, although they were proved useful in assessing plant-available potassium (Qian *et al.* 1996).

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**PAPER 1. UNDERSEEDED CLOVER AS A NITROGEN SOURCE FOR SPRING
WHEAT ON A GLEYSOL**

ABSTRACT

Although there is a potential to substantially reduce N fertilizer inputs by cropping spring cereals with an interseeded legume, agronomic value and potential risks of N losses associated with this practice are not documented under the conditions of eastern Canada. This study estimated non-N nutritional effects and N credits for interseeded clover (*Trifolium pratense* L. cv. Arlington) in spring wheat production (*Triticum aestivum* L. cv. Algot) and assessed fall and spring residual nitrate. The soil was a St. Urbain clay (Orthic Humic Gleysol) located in the St. Lawrence lowlands. The N mineral fertilizer was applied as NH_4NO_3 at 0, 40, 80, 120 and 160 kg N ha⁻¹. All treatments were combined or not with red clover as a companion crop which was incorporated as green manure in late fall. Clover significantly influenced wheat yield response to N fertilization in 1994 and 1995. Clover did not elicit non-N nutritional effects on wheat yield through plant competition. No apparent current-N transfer from clover to associated wheat occurred during the 1993 growing season. Higher yields observed with clover were therefore attributed entirely to N supplied by clover through mineralization of residues incorporated in the soil the previous fall. N fertilizer replacement value of clover was approximately 80 kg N ha⁻¹ for 1994 and 1995. Clover occasionally residual N- NO_3^- measured in the soil profile in late fall and early spring. Interseeded red clover may provide most of N needs of a companion spring wheat crop in fine-textured gleysolic soils with limited risk of air and water N contamination.

Key words: N credits; non-N nutritional effect; N potential losses

INTRODUCTION

Intercropping of legumes with cereal crops for other purposes than forage production was developed mainly in tropical areas (Ofori and Stern 1987). This management alternative is receiving an increasing interest in some temperate (Williams and Hayes 1991; Jones 1992; Jones and Clements 1993, Stute and Posner 1995) and arid regions (Guldan *et al.* 1997). But to our knowledge, information about the agronomic and environmental aspects of those systems for cold and humid climates such as in eastern Canada are lacking, despite possible advantages over cereals cultivated in monoculture.

Legume-derived N is reported as N credits. Their estimation is usually done by the approach of Shrader *et al.* (1966): the crop yield obtained after the legume phase of a rotation is compared to yields obtained with fertilizer N alone. The value obtained is the legume fertilizer N replacement value. These N credits were found to be overestimated (Lory *et al.* 1995) because legumes in rotation not only supply N (Ladd and Amato 1986; Janzen *et al.* 1990; Harris *et al.* 1994 and Hossain *et al.* 1996) but can also result in non-nutritional effects on the main crop (Janzen and Schaalje 1992; Stevenson and van Kessel 1996). Evidence suggests that underseeded legume can affect cereal yields otherwise than by simple contribution through improved N nutrition. Competition for light, water and nutrients, reduction of weeds and diseases *etc.* can influence yields (Francis 1989), but previous studies did not quantitatively separate N effects and non-N effects on cereal yield.

Use of alternative N sources should also consider possible environmental impacts. In Quebec, Painchaud (1997) reported deterioration of water quality due to excess of

nitrate (NO_3^-), especially in watersheds of the St. Lawrence lowlands. Also release of N_2O via denitrification, which is a greenhouse gas that depletes ozone layer, was reported for soils receiving excess fertilizer N (Liang and MacKenzie 1997). The NO_3^- presence in soils after harvest and in early spring was associated with risk of both leaching (Chichester 1977; Malhi and Nyborg 1986; Liang *et al.* 1991) and denitrification (Goodroad and Keeney 1984; Liang *et al.* 1991; Liang et MacKenzie 1997). Reduction of air and water pollution risk from agricultural soils implies that residual NO_3^- content in fall and early spring should be kept to a minimum.

The impact of a legume companion crop on soil profile NO_3^- during strategic periods of possible transfer from soil to air and water is not documented for specific conditions in eastern Canada. Furthermore, conflicting evidence exists on the effects of multiple cropping systems combining cereal and legume on N loss reduction (Izaurrealde *et al.* 1995; Owens *et al.* 1995; Weed and Kanwar 1996; Høgh-Jensen and Schjoerring 1997; Kuo *et al.* 1997). While legumes can increase efficiency of the cereal N use by scavenging the N applied in excess of the cereal needs, the mineralization of biologically-fixed N can increase the plant-available soil N pool for subsequent transfer if it is released at time that is not synchronized with plant uptake.

The objectives of this study were i) to estimate the N and non-N effects of red clover on spring wheat yields in a clover-spring wheat mixed intercropping system in order to accurately estimate legume N credits ii) to assess the effect of the presence of clover on soil profile NO_3^- levels in fall and early spring.

MATERIALS AND METHODS

Site

The site was located at the experimental farm of the Quebec Ministry of Agriculture, Fisheries and Food, at St. Bruno de Montarville (45°33'N; 73°21'W). Monthly total precipitation and average air temperature data were obtained from the nearby St. Hubert meteorological station (45°31'N; 73°25'W) of Environment Canada (Table 1.1). The soil was a St. Urbain clay, an Orthic Humic Gleysol (Table 1.2). Two years prior to the experiment, sorghum (*Sorghum vulgare* Pers.) was ploughed-under to improve soil structure. Site was not tile-drained and there were some cropping limitations due to excess water during the course of this study.

Treatments

Spring wheat (*Triticum aestivum* L. cv Algot) was seeded each year in mid-May at 400 plants m⁻² with a 18-cm row spacing. The N fertilizer was applied as NH₄NO₃ at 0, 40, 80, 120 and 160 kg N ha⁻¹ before seeding. All plots also received 20 kg P₂O₅ ha⁻¹ as triple super-phosphate (0-46-0). Fertilizers were immediately incorporated in the soil with a disk harrow. Fertilizer treatments were applied with or without red clover (*Trifolium pratense* L. cv. Arlington), underseeded as a companion crop at 7 kg ha⁻¹. Wheat straw was removed from the plots at harvest at the end of August. Clover was incorporated in mid-November as green manure by chisel ploughing. The experimental design was a randomized complete block in four replicates (plot size: 13.5 m²).

Wheat Yields and N Uptake

Wheat was harvested at maturity (Zadoks 87 to 91 growth stages) with a Wintersteiger plot harvester. Harvested surfaces were measured for each plot and varied from 6 m² to 9 m². Straw dry matter (DM) yield was determined from randomly located quadrats with a 0.18 m² surface. Grain and straw were dried in a forced-air oven at 60°C. Plant sub-samples were ground to pass a 1-mm sieve. Total N in 0.1 g grain and straw sub-samples was determined after digestion with a sulphuric acid-hydrogen peroxide mixture (Richards 1993). The NH₄⁺ in the digests was determined colorimetrically by the salicylate method (Nkonge and Ballance 1982) on a Hitachi U-1000 spectrophotometer (Hitachi Ltd., Tokyo, Japan). Wheat N uptake was calculated by the following equation:

$$Nup = (\%Nst * DMYst/100) + (\%Ng * DMYg/100)$$

where:

Nup= total N uptake in the plant (kg N ha⁻¹),

%Nst= percentage of total N in the straw,

DMYst= straw dry matter yield (kg ha⁻¹),

%Ng= percentage of total N in the grain, and

DMYg= grain dry matter yield (kg ha⁻¹).

Soil NO₃⁻

Soil samples were collected 4 times a year: at the end of April (except in 1993), 30 days after seeding, two or three days after cereal harvest and at mid-November (prior to soil tillage). One core of 7 cm in diameter was taken for each plot to a 90 cm depth. The cores were separated into 0 to 20, 20 to 50 and 50 to 90 cm layers. Soil samples were stored at

4°C until analysis. Sub-samples of fresh soil were oven-dried at 105°C for 24 h to determine gravimetric water content. Water extraction was performed on 2.5 g fresh sub-samples to determine soil NO_3^- content. Soil-water ratio (w/v) was 1:10. The soil suspensions were agitated for 30 minutes on a reciprocating shaker, then centrifugated at 15 000 rpm and filtered through Whatman No. 42 filter paper. The NO_3^- content of the water extracts was measured by ion exchange with high-performance liquid chromatography (Dionex DX300, Dionex Corporation, Sunnyvale, California) using an anion exchange column (Dionex IonPac CS5A) eluted with KCl 35 mM at 1 mL min^{-1} . Nitrate was detected by UV photometric detection (Dionex Variable Wavelength Detector-II) at the wavelength of 214 nm. Soil NO_3^- concentrations (mg N kg^{-1} soil dried-weight) were converted to N mass (kg ha^{-1}) from a specific layer for an area of 1 ha by multiplying NO_3^- -N concentration by the layer depth (cm), bulk density (Table 1.1) and a conversion factor of 0.1. Nitrate content for the soil profile was the sum of the three layers.

Non-N Nutritional Effects and N Credits: Theory and Estimation

The overall effect of clover on wheat yield is usually assessed by the traditional method (Shrader *et al.* 1966). The wheat yield obtained in presence of a legume is reported on the best-fit regression line of the wheat yield-fertilizer N rates relationship without clover. The N credits of a legume are calculated as the amount of fertilizer N that must be applied to a crop to attain a crop yield equal to that grown in rotation or interseeded with a legume and no N fertilizer. The classic assumption of the traditional method is that the clover effect on the wheat yield is solely due to the fertilizer N replacement value of the

legume. Therefore, the traditional approach cannot be used unless it has been demonstrated that non-N effects are not present (Smith *et al.* 1987; Lory *et al.* 1995). In the present study, it was assumed that verification of the presence or not of non-N effects would facilitate a more accurate estimation of legume N credits.

Janzen and Schaage (1992) established, with ^{15}N in an assay under growth chamber conditions, that, when legumes cause non-N effects, the relationship between dry matter yield and cereal N uptake is modified compared to that without legume. The difference between the yield-N uptake relationship with and without legume was used to partition the response of cereal to legume into N and non-N components. As proposed by Janzen and Schaage (1992), the relationship between grain dry matter yield and N uptake was used in the present study to distinguish nutritional and non-nutritional yield response. The rationale behind their approach is that, if the relationship between yield and N uptake is unaffected by the presence of the legume, then any response to legume residues is exclusively attributable to enhanced soil N fertility. In contrast, if the relationship between yield and N uptake is modified by the presence of clover, then the yield increase per unit of N uptake can be interpreted as a non-N nutritional response. On that basis, differences between regression curves can be used to quantify non-N nutritional effects. The possibility that clover influenced wheat yield through non-N effects was discarded when regression lines had identical parameters. Once non-N effects on wheat yield are estimated, they can be subtracted from the overall effect of clover on wheat yield previously estimated by the traditional method to obtain N credits.

Statistical Analysis

Analysis of variance (ANOVA) was performed on grain yield and soil NO_3^- -N data (SAS Institute 1990) to test treatment effects. Regression analysis was performed to study the relationships between wheat yield and the fertilizer N rate using either PROC REG or PROC NLIN (SAS Institute 1990).

Regression analysis was also performed to describe the relationship between grain yield and N uptake in presence or not of red clover for each year (SAS Institute 1990). Significant differences between regression curve parameters (intercept, slope and/or curvature coefficients) were detected by comparing general model (*i.e.* each regression line having different specific parameters) to more restrictive models (*i.e.* regression lines having identical parameters) with an F-test, as in Cook and Weisberg (1990). Restrictions to the general model were rejected if they significantly increased the error sums of squares at $\alpha=0.05$.

RESULTS

Non-N Nutritional Effects and N Credit Partition

In 1993, quadratic models best described the relationship between wheat yield and N fertilizer rate in the presence or absence of clover (Fig. 1.1a). No significant effect of clover on wheat yield response curves to N fertilizer was found and the standard deviations of wheat yield in the two cropping systems were strongly overlapping (Table 1.3). Therefore the first year of the association, red clover did not significantly modify

wheat N response. In the absence of clover, maximum grain yield of 2928 kg DM ha⁻¹ was obtained at 126 kg N ha⁻¹. Nitrogen credits from clover were zero.

A quadratic model also best described the grain yield response to N uptake in 1993 (Fig. 1.2a). The curves obtained with and without clover were overlapping. Comparison of restrictive to more general models confirmed statistically (results not shown) that the wheat yield to N uptake relationship was not modified by clover. One equation fit the relationships. In 1993, the estimated maximum yield reached 2963 kg DM ha⁻¹ for N uptake of 134 kg ha⁻¹. Because of the similarity between the wheat yield-N uptake responses of wheat yield to the N uptake, it was concluded that non-N nutritional effects did not affect wheat yields.

In 1994, wheat yields responded linearly to increasing fertilizer N rates (Fig. 1.1b). Clover (Table 1.3) produced a positive shift of the yield curve as demonstrated by an intercept of 2099 kg grain DM ha⁻¹ compared to 1740 kg ha⁻¹ in the absence of red clover. The impact of clover on the shift decreased as the rate of fertilizer N increased. This resulted of a lower slope (2.58 *versus* 4.38) of the N yield curve in the presence compared to the absence of clover. Because the N yield response was linear, no maximum yield could be determined.

In 1994, relationships of grain yield to N uptake were quadratic (Fig. 1.2b). Comparison of restrictive to more general models confirmed statistically that clover did not change the response of wheat yield to N uptake. One equation sufficed to describe the relationship. The grain yield reached 2572 kg DM ha⁻¹ for an N uptake of 81 kg ha⁻¹. As no modification by the legume of the yield response in relation to N uptake was found (Fig. 1.2b), non-N nutritional effects of clover were considered as nil. The N credits

obtained by the traditional method were 81 kg ha^{-1} and were attributed solely to N supplied by clover residues (nutritional effects).

In 1995, the form of the crop response to NH_4NO_3 differed depending on the companionship or not with clover, as expressed by the significant interaction effect with fertilizer and clover (Table 1.3). Without clover, the yield relationship was a linear-plateau (Fig. 1.1c). The maximum wheat grain yield of $2427 \text{ kg DM ha}^{-1}$ was reached at 85 kg N ha^{-1} . In presence of clover, wheat yields did not respond to fertilizer N. The mean yield obtained was $2408 \text{ kg DM grain ha}^{-1}$.

In 1995 (Fig. 1.2c), grain yield increased linearly with N uptake in wheat alone. Grain yield was independent of N uptake when grown in association to clover. It can be assumed that, once N uptake reached 70 to 80 kg ha^{-1} , available soil N was sufficient to meet wheat needs and additional N uptake did not increase wheat yield. Yields reached a plateau ($2408 \text{ kg DM ha}^{-1}$) with N uptake of 78 kg ha^{-1} . Therefore, the response of grain yield shifted along the x axis rather than being modified by clover. As clover did not affected the wheat yield response in relation to N uptake, non-N effects of clover were again estimated to be nil. The N credits calculated by the traditional method equalled 85 kg N ha^{-1} .

Post-Harvest, Fall and Spring NO_3^- -N in the Soil Profile

Clover did not significantly affect post-harvest profile (0-90 cm) NO_3^- -N contents (Table 1.4). With or without clover, NO_3^- -N contents in the soil profile for a given rate of mineral N fertilizer were comparable (Fig. 1.3). Only in 1993 was a trend for red clover to lower nitrate in the soil profile. This was expressed by the reduced content of NO_3^- -N

observed when clover was present for the rate of 160 kg N ha^{-1} of NH_4NO_3 (Fig. 1.3a). Averaged over all treatments, the profile NO_3^- -N was 46 kg N ha^{-1} in 1993, 66 kg N ha^{-1} in 1994 and 62 kg N ha^{-1} in 1995 (Fig. 1.3).

Interseeding clover in wheat did not result in higher mid-November NO_3^- -N profile contents in 1993 (Fig. 1.4a). Clover affected NO_3^- -N significantly (Table 1.4) either alone in 1994, or in interaction with the fertilization in 1995. In 1994, red clover caused an overall increase of 5 kg N ha^{-1} in the soil profile NO_3^- (Fig. 1.4b) compared to wheat alone. Finally, in 1995, the interaction of clover and the added N led to higher soil NO_3^- -N content as the fertilizer N applied increased. Profile NO_3^- -N averaged over all treatments was 40, 17 and 41 kg N ha^{-1} in 1993, 1994 and 1995, respectively.

There was no consistent pattern of treatment effects on NO_3^- -N measured in the soil profile at the end of April of 1994, 1995 and 1996 (Fig. 1.5). While clover had no significant effect in spring 1994 and 1996 (Table 1.4), it increased significantly the overall amount of NO_3^- by 15 kg N ha^{-1} over wheat alone in 1995 (Fig. 1.5b). On average, spring NO_3^- -N soil profile contents were respectively of 39, 57 and 30 kg ha^{-1} in 1994, 1995 and 1996.

DISCUSSION

Grain yield response pattern to mineral N fertilizer varied from one year to another in models (quadratic, linear and linear-plateau) and in maxima estimated with the selected model (Fig. 1.1). May rainfall conditions may have affected soil and fertilizer N availability to wheat (Table 1.1). Highest May rainfall was measured in 1994 with 112

mm compared to 86 mm in 1993 and 67 mm in 1995. High spring rainfall could have triggered losses of fertilizer N applied in spring by denitrification and/or leaching and, resulted in less plant-available N. Malhi and Nyborg (1986) reported loss of 44 kg N ha⁻¹ under conditions prevailing in early spring for soils of north-central Alberta. Liang and MacKenzie (1997) found that, for a sandy clay loam in the St. Lawrence Lowlands, losses of nitrate through denitrification were related to soil moisture in April and May. This could explain why, in 1994, the yield-rate relationship obeyed a linear form with no maximum: if soil and fertilizer N were lost, the available N left in the soil could have been insufficient for the cereal to reach maximum grain yield.

Lory *et al.* (1995) demonstrated that N credits could be accurately assessed in the presence of non-N effects with the difference method (Smith *et al.* 1987) in field experiments. Nevertheless this approach requires a characterization of the entire response curve to identify the maximum N rate. Therefore the approach suggested is applicable only when the relationship between cereal yield and N fertilizer rate reaches a maximum. This is impossible for a linear response as observed in 1994 (Fig. 1.1b). In the present study, the method proposed by Janzen and Schaage (1992) overcame problems associated to this limitation of the difference method. It allowed for a reliable estimation of N credits because it was possible to eliminate the possibility that yields were influenced by non-N effects. Observations from the wheat yield-N uptake relationship (Fig. 1.2) clearly showed that wheat yield had not been modified by non-N nutritional effects induced by clover. To our knowledge, it is the first time that this method, designed from laboratory experimentation, was proven to be useful in interpreting data obtained from field trials.

The possible negative effect of the clover competition on wheat was not observed in the present study as the legume never induced a down shift of the wheat yield curve (Fig. 1.1). This contrast with Jones and Clements (1993) and Williams and Hayes (1991) who observed competition between spring cereals and white clover (*Trifolium repens* L). The lack of competition in our study was probably due to the fact that the cereal was not established in a perennial stand of clover but rather seeded simultaneously with red clover. As the cereal component has a higher early growth rate, height advantage and a more extensive root system than red clover, it competed effectively with the legume for light and nutrients.

As no non-N nutritional effects were measurable under conditions prevailing for this study, it was assumed that clover affected wheat yield strictly through an increased soil N availability to wheat. Literature suggests two possible pathways by which the N_2 fixed by intercropped legume may be available to the associated cereal: current N transfer and residual N transfer. Some authors (Eaglesham *et al.* 1981; Patra *et al.* 1986; HØgh-Jensen and Schjoerring 1997) reported evidence of a mechanism by which legumes growing simultaneously with a cereal can supply some N to the intercropped cereal in the current season (current N transfer). The second mechanism, the residual N transfer, is well documented and refers to N made available to a succeeding cereal crop by the mineralization of clover residues. This study provided some indirect evidence that residual N transfer is the only significant mechanism by which N is supplied to the wheat under the conditions of poorly drained clay soils of the St. Lawrence lowlands.

The presence of clover did not affect the shape of yield-N rate relationship in 1993 (Fig. 1.1a). Since no significant modification of yield response by clover had been

observed in the first year of cropping (Fig. 1.1a), it was assumed that no significant current N transfer occurred. Our field observations expand to field situations a laboratory study of Elabaddi *et al.* (1996) who did not detect current N transfer between wheat (*Triticum turgidum*) and medic (*Medicago trunculata*).

The red clover provided fertilizer-N equivalents to spring wheat of 81 kg ha⁻¹ in 1994 and 85 kg ha⁻¹ in 1995. Those values were comparable to those obtained in other systems where legumes were interseeded to a main crop. Under dryland conditions in the Northern Great Plains of United States, Guldan *et al.* (1997) found fertilizer replacement value ranging from 78 to 140 kg N ha⁻¹ of alfalfa (*Medicago sativa*) and hairy vetch (*Vicia villosa*) green-manure interseeded with corn for a subsequent forage sorghum (*Sorghum bicolor*) crop. In the Netherlands, Elgersma and Hassink (1997) found an apparent N transfer from clover to grass which varied from 55 to 113 kg N ha⁻¹ yr⁻¹ in a forage trial with perennial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) mixture.

Our observations seem to discard the hypothesis that clover uptake of soil NO₃⁻ decreased the residual NO₃⁻ pool and then reduced NO₃⁻ leaching and denitrification losses. Clover intercropping increased the risk of transfer to surface and groundwater or to the atmosphere by raising the amount of NO₃⁻ found in the soil profile in the fall and at the end of the following April. Those increases could most probably be attributed to net mineralization of clover. Dou *et al.* (1995) also reported higher fall soil profile NO₃⁻ for corn combined with a green manure cover crop than for soil under corn alone and fertilized at the economic optimum rate. Nevertheless, Weed and Kanwar (1996) and Izaurrealde *et al.* (1995) reported lower nitrate below the root zone and in the drainage water for two types of rotations including both cereals and legumes than for cereal under

monoculture. Higher NO_3^- in the soil profile is only indicative of a risk but does not necessarily translate into significant N losses through denitrification or NO_3^- leaching.

CONCLUSIONS

The impact of red clover in an intercropped wheat-clover system under the St. Lawrence lowlands conditions and conventional tillage in fine-textured gleysolic soils appears to be mostly limited to the supply of N to succeeding spring cereals. Our results suggest that recommended mineral N fertilizer application of 90 kg ha^{-1} for wheat (C.P.V.Q. 1996) could be reduced by up to 90% because red clover credits were estimated to be 81 and 85 kg N ha^{-1} for 1994 and 1995, respectively. Therefore, the present study indicates that red clover companion cropping is an alternative to mineral fertilizer N in sustainable spring wheat production systems for heavy clay soils. Conversely, it appears that clover is an inappropriate cover crop to trap residual N that is not taken up by the main crop and therefore susceptible of being transferred to the atmosphere or to surface and ground waters. However the addition of this labile organic C source to soil may have helped to maintain the biological activity and a good soil structure which are often negatively affected by monoculture systems on these fine-textured soils. A longer period of experiment may have permitted observations of non-nutritional benefits of the red clover companion crop through improved soil structure and microbial activity.

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Table 1. 1. Monthly precipitation and temperature at the St. Hubert Environment Canada Weather Station (45°31'N; 73°25'W)

| Month | Total precipitation (mm) | | | | Average temperature (°C) | | | |
|-----------|--------------------------|------|------|---------------------|--------------------------|------|------|---------------------|
| | 1993 | 1994 | 1995 | Normal ¹ | 1993 | 1994 | 1995 | Normal ¹ |
| March | 64 | 65 | 42 | 74 | -4.4 | -3.1 | -0.8 | -2.3 |
| April | 175 | 94 | 57 | 78 | 5.6 | 5.6 | 3.6 | 5.6 |
| May | 86 | 112 | 68 | 75 | 13 | 12 | 12.8 | 12.7 |
| June | 103 | 79 | 51 | 87 | 17.3 | 19.1 | 19.4 | 17.9 |
| July | 88 | 18 | 172 | 90 | 21.3 | 21.4 | 21.7 | 20.6 |
| August | 42 | 58 | 76 | 104 | 20.6 | 17.8 | 19.8 | 19 |
| September | 102 | 286 | 68 | 90 | 13.8 | 14.4 | 12.6 | 14.2 |
| October | 131 | 17 | 181 | 79 | 6.3 | 9.4 | 11 | 8 |
| November | 78 | 120 | 132 | 101 | 1 | 3.6 | -0.9 | 1.4 |

¹normals are estimated over the period of 1928 to 1990

Table 1. 2. Physico-chemical characteristics of the St. Urbain soil

| Layer | Particle size distribution | | | Organic C | pH | | CEC pH7 | Bulk density |
|-------|----------------------------|------|------|-----------|-------------------|-------|-------------------------|--------------------|
| | sand | silt | clay | | CaCl ₂ | water | | |
| cm | % | % | % | % | | | meq 100 g ⁻¹ | g cm ⁻³ |
| 0-20 | 18.0 | 28.0 | 54.0 | 1.9 | 6.8 | 7.5 | 31.25 | 1.4 |
| 20-50 | 8.0 | 25.5 | 66.5 | - | 6.9 | 7.6 | 29.12 | 1.4 |
| 50-90 | 1.8 | 21.4 | 76.8 | - | 7.2 | 7.9 | 28.14 | 1.4 |

Table 1. 3. Mean squares of the analysis of variance of red clover (*Trifolium pratense* L. cv. Arlington) and N mineral fertilizer effects on spring wheat (*Triticum aestivum* L. cv. Algot) yields from 1993 to 1995

| Source | d.f. | 1993 | 1994 | 1995 |
|-----------------|------|-----------------------|----------------------|----------------------|
| Block | 3 | 316516 ^{**} | 89754 ^{ns} | 233473 ^{**} |
| Clover | 1 | 53280 ^{ns} | 464631 [*] | 267052 [*] |
| Mineral N rate | 4 | 1556482 ^{**} | 413757 ^{**} | 125395 [*] |
| Clover * N rate | 4 | 118499 ^{td} | 42683 ^{ns} | 148146 [*] |
| Error | 27 | 61662 | 78148 | 44901 |

^{**}significant at $\alpha=0.01$ ^{*}significant at $\alpha=0.05$ ^{td}trend at $\alpha=0.20$ ^{ns}non significant

Table 1. 4. Mean squares of the analysis of variance of red clover (*Trifolium pratense* L. cv. Arlington) and N mineral fertilizer effects on amounts of N-NO_3^- measured in the soil profile (0-90cm) at post-harvest, mid-November and the end of April from 1993 to 1995

| Source | d.f. | 1993 | 1994 | 1995 |
|--------------------------------------------------------------|------|--------------------|--------------------|-------------------|
| <i>Post-Harvest NO_3^--N</i> | | | | |
| Block | 3 | 2756 ^{**} | 677 ^{**} | 874 ^{td} |
| Clover | 1 | 620 ^{td} | 2 ^{ns} | 546 ^{ns} |
| Mineral N rate | 4 | 525 ^{ns} | 234 ^{ns} | 2160 [*] |
| Clover * N rate | 4 | 510 ^{ns} | 208 ^{ns} | 693 ^{ns} |
| Error | 27 | 357 | 166 | 446 |
| <i>Late Fall NO_3^--N</i> | | | | |
| Block | 3 | 3680 ^{**} | 60 [*] | 186 ^{ns} |
| Clover | 1 | 89 ^{ns} | 283 ^{**} | 357 ^{td} |
| Mineral N rate | 4 | 647 ^{td} | 19 ^{ns} | 870 ^{**} |
| Clover * N rate | 4 | 455 ^{td} | 4 ^{ns} | 522 [*] |
| Error | 27 | 281 | 18 | 165 |
| <i>End of April NO_3^--N^z</i> | | | | |
| Block | 3 | 902 ^{**} | 617 ^{td} | 269 [*] |
| Clover | 1 | 129 ^{ns} | 3063 ^{**} | 101 ^{ns} |
| Mineral N rate | 4 | 316 [*] | 122 ^{ns} | 75 ^{ns} |
| Clover * N rate | 4 | 243 ^{td} | 126 ^{ns} | 11 ^{ns} |
| Error | 27 | 100 | 241 | 83 |

^{**} significant at $\alpha=0.01$ ^{*} significant at $\alpha=0.05$ ^{td} trend at $\alpha=0.20$ ^{ns} non significant

^z consider one year later then those figuring at the top of each column

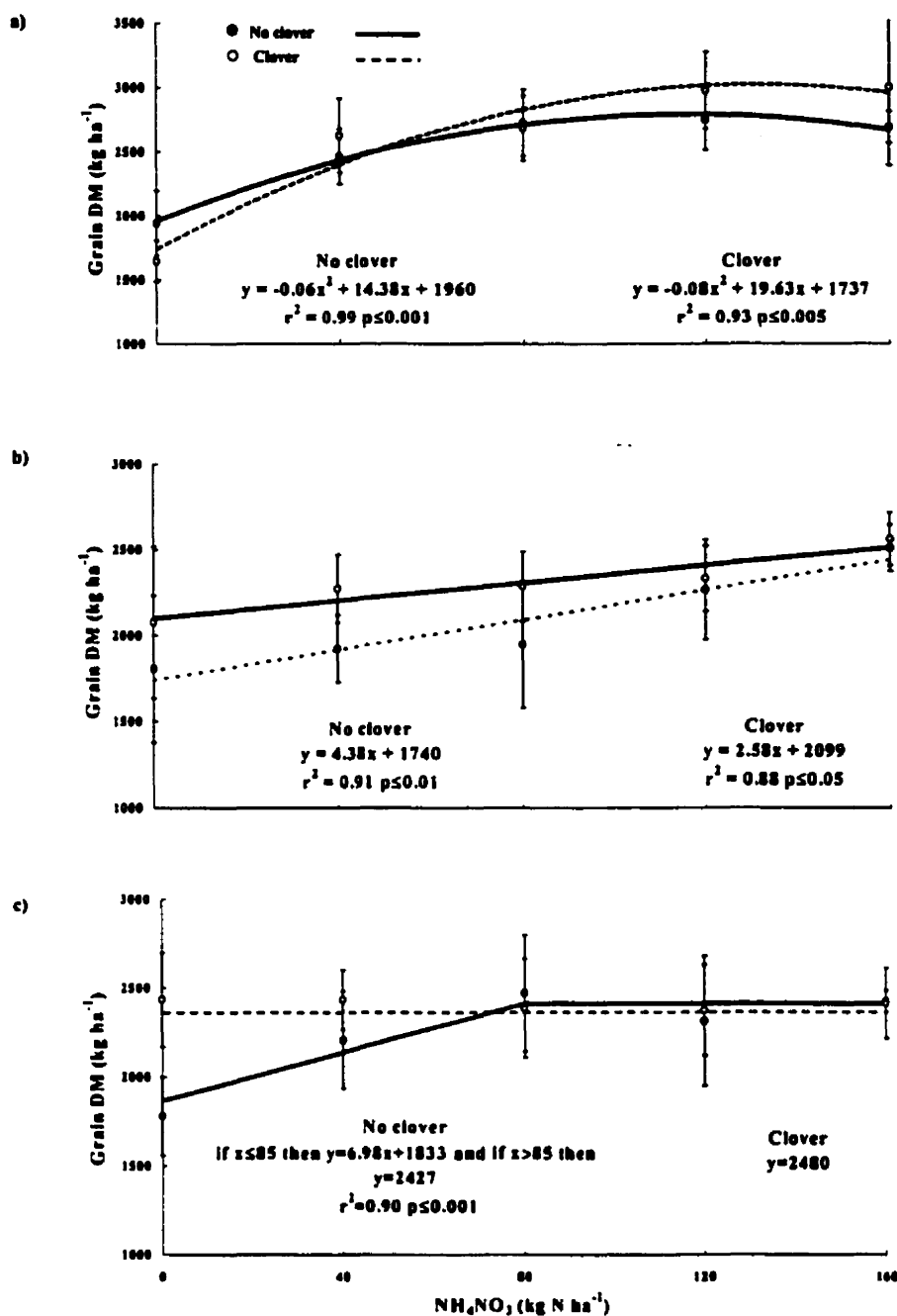


Fig. 1. 1. Wheat (*Triticum aestivum* L. cv. Algot) yields obtained in a) 1993, b) 1994 and c) 1995 on a St. Urbain clay as a function of mineral N rates applied as NH₄NO₃ with and without red clover (*Trifolium pratense* L. cv. Arlington). Each datum point is the mean of four replicates. Error bars shown are standard errors of the mean.

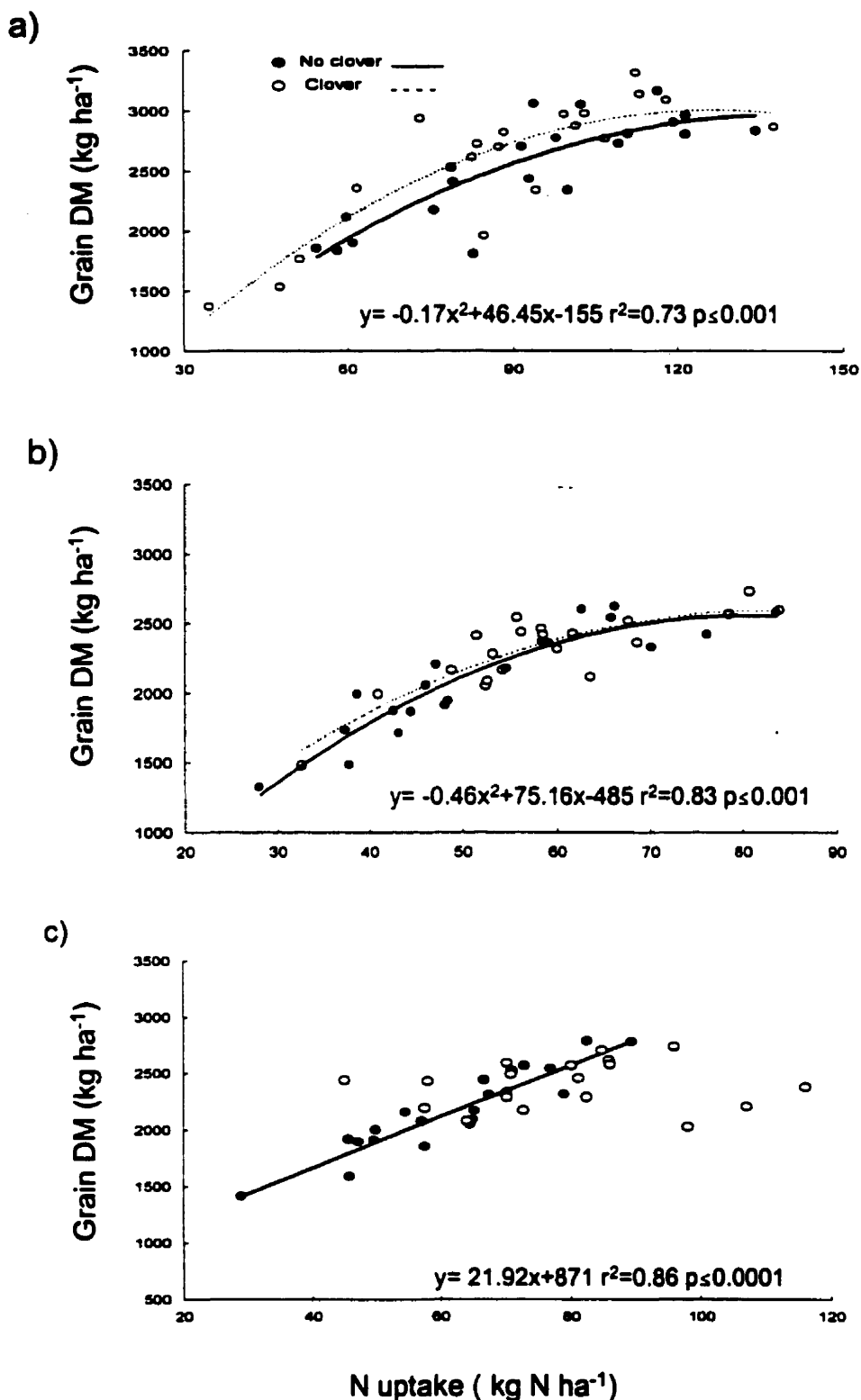


Fig. 1. 2. Wheat (*Triticum aestivum* L. cv. Algot) yields obtained in a) 1993, b) 1994 and c) 1995 on a St. Urbain clay as a function of wheat N uptake with and without red clover (*Trifolium pratense* L. cv. Arlington). Each datum point represents one measurement.

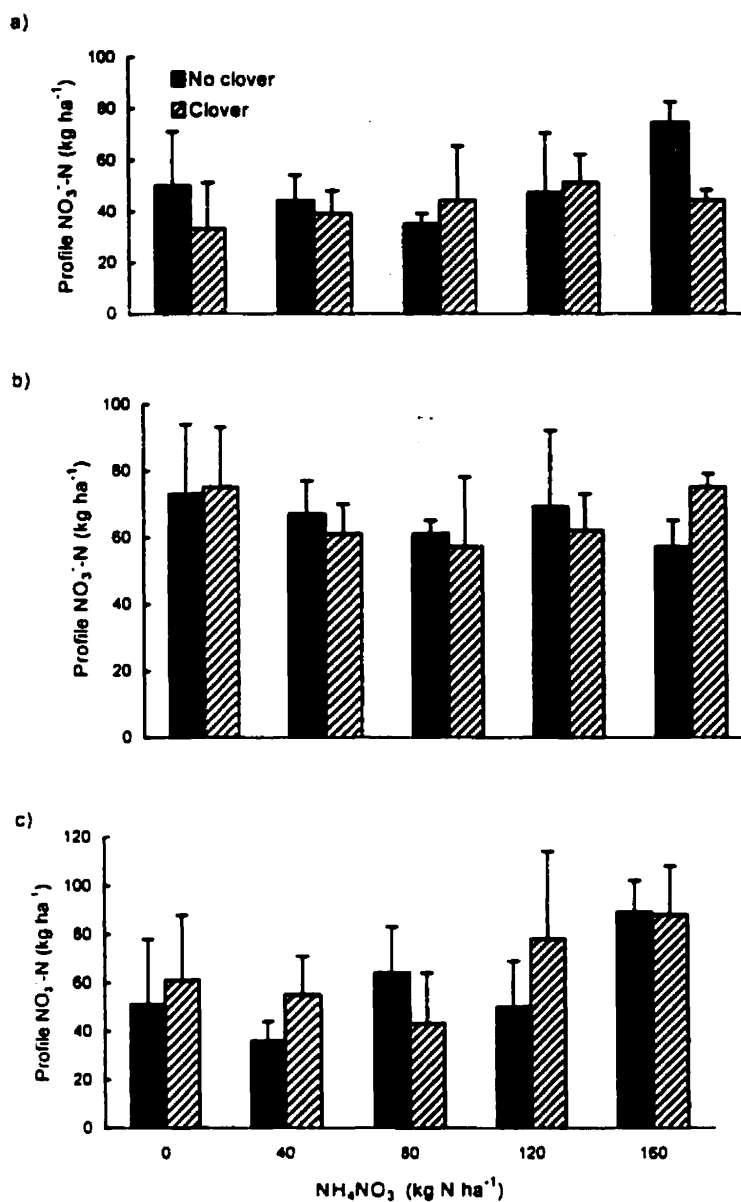


Fig. 1. 3. Nitrate measured after harvest of spring wheat (*Triticum aestivum* L. cv. Algot) in the profile (0-90 cm) of a St. Urbain soil in a) 1993, b) 1994 and c) 1995. Treatments combined rates of NH_4NO_3 and presence or not of red clover (*Trifolium pratense* L. cv. Arlington) as companion crop. Each datum bar is the mean of four replicates. Error bars shown are standard errors of the mean.

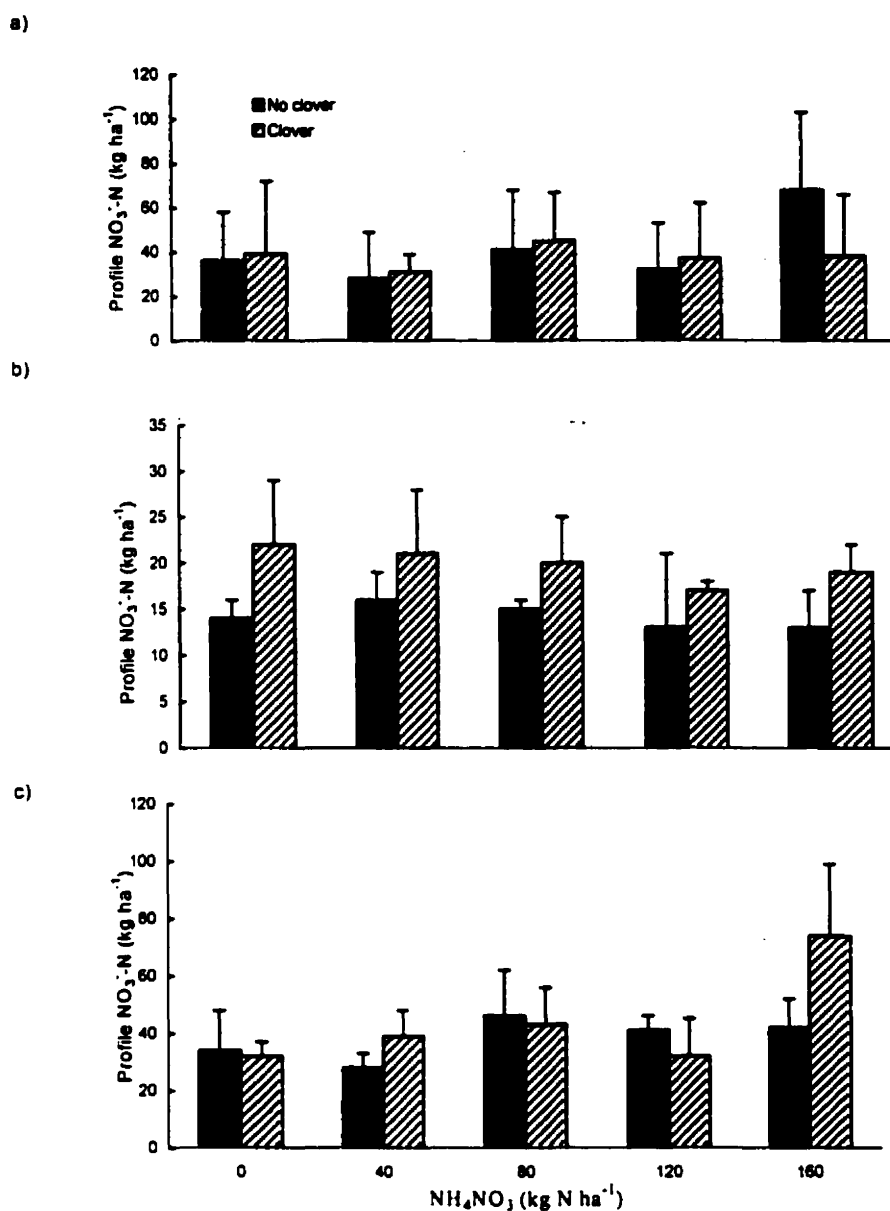


Fig. 1. 4. Nitrate measured at mid-November in the profile (0-90 cm) of a St. Urbain soil. Treatments applied on spring wheat (*Triticum aestivum* L. cv. Algot) combined rates of NH₄NO₃ and presence or not of red clover (*Trifolium pratense* L. cv. Arlington) as companion crop in a) 1993, b) 1994 and c) 1995. Each datum bar is the mean of four replicates. Error bars shown are standard errors of the mean.

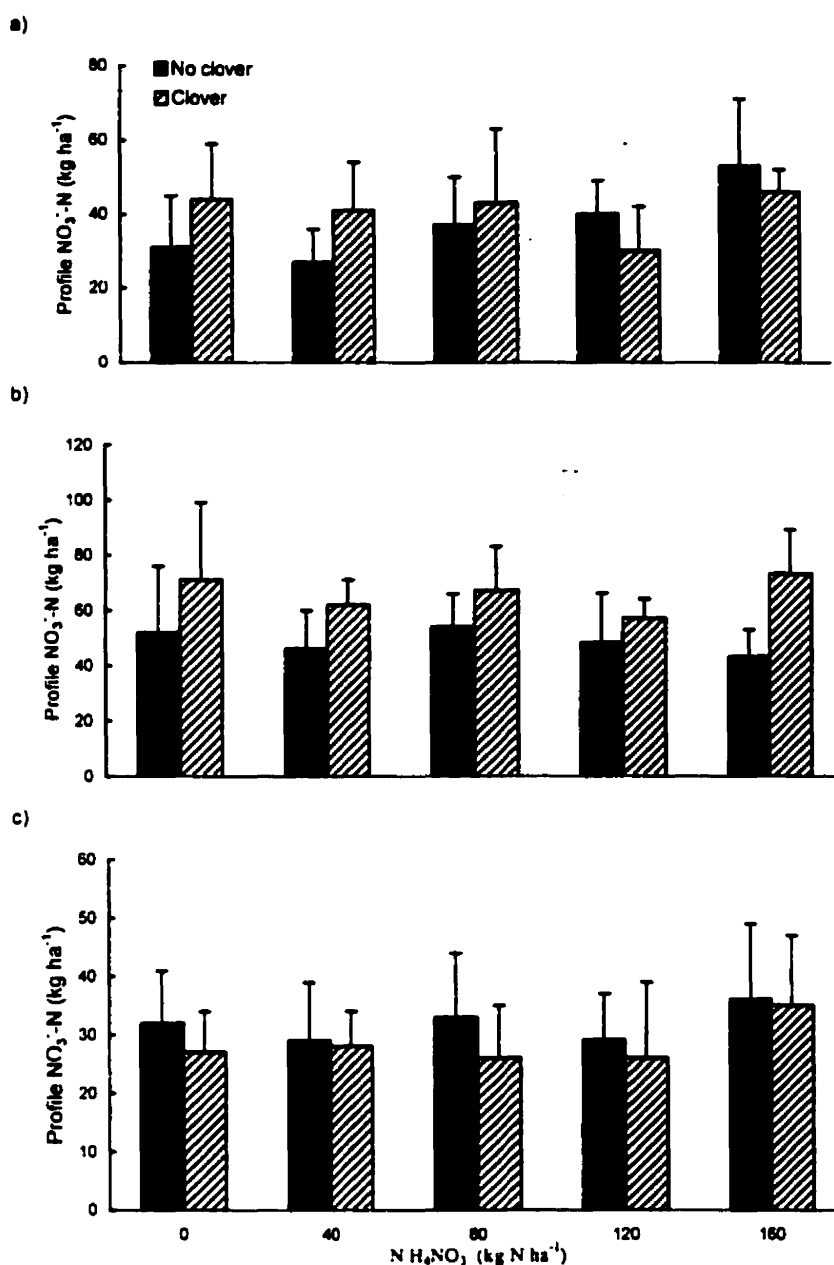


Fig. 1. 5. Nitrate measured at the end of April in the profile (0-90 cm) of a St. Urbain soil. Treatments applied on spring wheat (*Triticum aestivum* L. cv. Algot) combined rates of NH₄NO₃ and presence or not of red clover (*Trifolium pratense* L. cv. Arlington) as companion crop in a) 1994, b) 1995 and c) 1996. Each datum bar is the mean of four replicates. Error bars shown are standard errors of the mean.

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FOREWORD THAT PROVIDES A LOGICAL BRIDGE BETWEEN PAPER 1 AND PAPER 2

In the previous paper, the value of underseeded red clover as a N source to spring wheat was estimated in a system where additional N was provided as NH_4NO_3 . But in eastern Canada, the large amount of wastes generated by animal production encourages producers to value manure in crops as a source of nutrients, notably N. Nitrogen supplied by manure could be, in large part, of organic form, and thus manure addition could also bring to soils a significant input of C. As the N cycle is mostly driven by biological processes, it is obvious that N input from manures will behave differently in the soil than N from mineral fertilizer. Therefore, it is not possible to infer from the previous paper how the interactions of N from interseeded clover and from manure will affect the N available to plants and the residual N susceptible to be lost in fall and early spring. A thorough survey of the literature brought no information on the combined use of manures and legume as sources of N and therefore this point remains to be documented.

Among management factors that could affect availability of manure N is application time. The very small body of literature on this subject presents conflicting evidence. Since farmers wish flexibility in regard to the time of manure application, this point was also documented in the second paper.

In addition to the previous considerations, to our knowledge, there is no scientific literature documenting the value of manures as a N source for spring wheat production on a site representative of climatic and pedologic conditions of the St. Lawrence Lowlands. Agronomic recommendations, derived from specific field studies, are most accurate if

applied under similar pedologic and climatic conditions. Therefore, the value of manures as N source, estimated from a field trial located in the St. Lawrence lowlands, should be more reliable than the actual provincial recommendations.

**PAPER 2: NITROGEN MANAGEMENT OF MANURE FOR SPRING WHEAT
INTERSEEDED WITH RED CLOVER**

ABSTRACT

In eastern Canada, spring cereal N requirements could be met by recycling the large amount of manure generated by animal production and by the interseeding of legumes. Objectives of this study were to document i) the interaction of clover and manure on wheat N recovery and ii) the potential risk of N transfer out the crop environment, and iii) to test the soil NO_3^- contents to 20 cm at 30 days after seeding as an index of N availability to wheat. The experiment was established on a St. Urbain clay (Orthic Humic Gleysol) located in the St. Lawrence lowlands at St. Bruno de Montarville. Spring wheat (*Triticum aestivum* L. cv. Algot) was interseeded or not with red clover (*Trifolium pratense* L. cv Arlington) and was ploughed down in late fall as green manure. Wheat alone or with clover was fertilized either with NH_4NO_3 applied at pre-seeding at rates of 0 to 160 kg N ha^{-1} or with swine liquid manure (SLM) or dairy solid manure (DSM). Manures were applied at pre-seeding, at Zadoks 12 wheat growth stage or after harvest. Apparent N recovery of SLM was higher than for DSM with SLM values averaging 5 and 17% in 1994 and 1995 and DSM values of 2 and 4%. There was no effect of application time on apparent recovery of manure N by wheat. Post-harvest application of manure increased fall and spring soil profile NO_3^- contents. In 1995, the presence of clover decreased N recovery from SLM and from DSM and increased the soil profile residual NO_3^- measured in fall and spring of both years. The absence of relationship between soil NO_3^- to 20 cm observed in post-emergence and wheat N uptake outlines the need to turn toward other indices of N availability.

Key words: N index, apparent N recovery, residual NO_3^-

INTRODUCTION

Nitrogen availability of manures is generally lower than that of mineral fertilizers (Sims 1995). Therefore, on a total N basis, manures are applied at higher rates than commercial fertilizers to fulfil wheat N demand. Farmers could improve plant recovery of manures N through more efficient management practices such as timely application and the use of a cover crop that can trap the residual N in fall and release it the following year to the benefit of the succeeding crop.

Studies of the impact of the time of application of manure on N availability to crops report conflicting evidence. Some authors concluded that time of application has no impact on crop yield (Beauchamp 1983; Jokela 1992) or N uptake (Jokela 1992). Zebarth *et al.* (1996) found in the mild and wet climate of south coastal British Columbia that N applied as liquid manure in fall was lost and they observed no increase in corn yield or N uptake the following year.

Spring cereal production can be sustained through legume and livestock manure N inputs (Wani *et al.* 1994). It was found that for crop rotations involving corn and legumes, the amounts of N available from manure and legumes were additive and therefore, yields were less responsive to manure N additions following a legume crop (Baldock and Musgrave 1980; Lory *et al.* 1995). To our knowledge, no literature documents the effect of a legume underseeded as a companion crop to a spring cereal on the recovery of N from manure.

Transfers of soil N to atmosphere and aquatic ecosystems were observed where soils received N inputs from manure applications (Stout *et al.* 1997; Paul and Zebarth

1997). In the northern hemisphere, climatic conditions ideal for N losses occur mostly in fall and spring, when soil moisture content is high and N uptake by plants is limited (Malhi and Nyborg 1989; Liang and MacKenzie 1997; Paul and Zebarth 1997). Nitrogen losses are associated with high soil NO_3^- contents because NO_3^- is susceptible to denitrification and leaching. Therefore management of N inputs from manure should promote minimal NO_3^- contents in the soil profile in spring when the plants are not present and in the fall, after harvest.

Spring wheat maximum grain yield was reached when 90 to 130 kg N ha⁻¹ was applied as ammonium nitrate for fine-textured soils of the St. Lawrence lowlands (Ayoub *et al.* 1994; Simard *et al.* 1997). Sub-optimal soil N contents could be corrected by post-emergence fertilizer application. An estimate of soil N availability to plants at that time is therefore critical. Gagnon *et al.* (1998) found a positive relationship between soil profile NO_3^- and wheat N uptake. Others have shown that the 60 to 120 cm layer was important to estimate wheat available N (Halvorson *et al.* 1987). Nevertheless, sampling soils to such depth is labor intensive and difficult in wet heavy clays. Previous studies have shown that the content of mineral N measured in the soil plough layer (Magdoff *et al.* 1984; Paul and Beauchamp 1993) 4 to 6 weeks after seeding was related to the corn N uptake. Therefore it was considered as an effective index of N availability to corn integrating the effects of soil and weather (Magdoff *et al.* 1984). The effectiveness of this index remains to be tested in wheat production receiving N through manures or legumes.

The objectives of this study were i) to assess the impact of manure application time and red clover interseeding on N recovery of SLM and DSM by spring wheat, ii) to estimate the effect of the different types of manure, application time and of red clover

companion crop on the NO_3^- in the soil profile in spring and after harvest and, iii) to test NO_3^- to 20 cm measured 30 days after seeding as an index of N availability to spring wheat.

MATERIALS AND METHODS

Site

Details of the experimental site located at St. Bruno de Montarville are given in Paper 1 of this thesis. The soil was a St. Urbain clay (Orthic Humic Gleysol). Meteorological data and physico-chemical soil characteristics appear in Tables 1.1 and 1.2 of Paper 1. Measurements were taken from four replicates of twenty-two selected treatments which were arranged in a randomized complete block design in 1994 and 1995.

Treatments

Mineral N (NH_4NO_3) was applied at 0, 40, 80, 120 and 160 kg N ha⁻¹ before spring wheat (*Triticum aestivum* L. cv Algot) seeding. Dairy solid manure and SLM were applied either before seeding, at the wheat Zadoks 12 growth stage, or after harvest. All plots also received 20 P₂O₅ kg ha⁻¹ as triple super-phosphate (0-46-0). Mineral fertilizers and manures applied before seeding and manures applied after harvest were incorporated in the soil with a disk harrow. All treatments (manures and fertilizer) were compared with or without red clover (*Trifolium pratense* L. cv. Arlington) interseeded as a companion crop at a rate of 7 kg ha⁻¹.

Manure Analysis

Manures were obtained from farmers located near the experimental farm. Manure sampling and analyses were performed approximately 1 to 2 weeks prior to application. Samples were stored at 4°C until analyses were done. Gravimetric water content was measured by oven drying at 105°C according to Gardner (1965). Total organic C was determined by loss on ignition (Ball 1964) at 450°C and total N content by dry combustion on an automatic analyzer (CNS-1000, Leco Corporation, St. Joseph, MI). The NH_4^+ -N and NO_3^- -N contents were determined after extraction with 2M KCl according to the Maynard and Kalra (1993) procedure for soils. Ammonium was measured by the salicylate method (Nkonge and Ballance 1982). Nitrate was determined by high performance liquid chromatography as described earlier in Paper 1. Organic N was obtained by the difference between total N and ($\text{NO}_3^- + \text{NH}_4^+$)-N. Total C added and manure characteristics are reported in Table 2.1.

Apparent N Recovery by Wheat

Apparent N recoveries of NH_4NO_3 and manure total N were expressed as percentage and were calculated as following:

$$\%N_{\text{rec}} = (\text{}^u\text{N}_{\text{up}} - \text{}^{\text{cl}}\text{N}_{\text{up}}) * 100 / \text{}^u\text{N}_{\text{ap}}$$

where:

$\%N_{\text{rec}}$ = percentage of apparent N recovery of mineral fertilizer or manure,

$\text{}^u\text{N}_{\text{up}}$ = total N uptake of wheat (kg N ha^{-1}) for a given treatment,

$\text{}^{\text{cl}}\text{N}_{\text{up}}$ = total N uptake of wheat (kg N ha^{-1}) for the unfertilized control with or without underseeded red clover, and

${}^{\text{u}}\text{N}_{\text{ap}}$ = total N added (kg N ha^{-1}) as manure or NH_4NO_3 .

Details concerning estimation of total N uptake by the spring wheat were reported earlier (Paper 1).

Soil NO_3^- Sampling and Analysis

Soil samples were collected and analyzed for NO_3^- -N content as previously described (Paper 1). Water extractions were performed to determine NO_3^- -N in the 0 to 20 cm layer 30 days after seeding and residual NO_3^- -N in the soil profile (0-90 cm) at the end of April, after harvest and in mid-November. The NO_3^- content of the extracts was measured as described above.

Statistical Analysis

Analysis of variance (ANOVA) with *a priori* orthogonal contrasts were carried out (SAS Institute 1990) to test treatment factor effects on apparent N recovery of the manure and residual NO_3^- contents in the soil profile. Regression analyses were performed in order to find the best-fit curve of the relationship between apparent N recovery and the rate of N applied as NH_4NO_3 . The procedure used was PROC REG or PROC NLIN depending if linear or quadratic curves best described the relationship (SAS Institute 1990). Regression analyses (SAS Institute 1990) were also performed with N wheat uptake as a dependent variable and NO_3^- -N in the 0-20 cm layer 30 days after seeding as the independent variable.

RESULTS AND DISCUSSION

Apparent Recovery of Manure N by wheat

The N apparent recovery was affected by manure type. This is expressed by a trend ($\alpha=0.20$) in 1994 and a significant effect ($\alpha=0.01$) in 1995 (Table 2.2). Swine liquid manure resulted in higher apparent N recovery with 5 and 17% in 1994 and 1995 than for DSM with respective values of 2 and 4% (Fig. 2.1). With DSM, 3800 kg C ha⁻¹ was added to the soil in 1994 and 1900 kg C ha⁻¹ in 1995; for SLM, amounts were of 170 and 140 kg C ha⁻¹. Therefore, the differences in apparent N recovery between SLM and DSM may be due to greater N immobilization by DSM to decompose added C than in the case of SLM. Paul and Beauchamp (1994) observed, under laboratory conditions, that net immobilization of inorganic N occurred after fresh solid beef cattle manure application to soil and it was after 3 wk that net mineralization began. Delogu *et al.* (1998) found that for wheat, more than 60% of total N uptake occurred during tillering (Zadoks 20 to 29 growth stage). Under our experimental conditions, tillering began approximately 4 wk after manure application. Therefore, the amount of N mineralized from DSM at that time was probably insufficient to fulfil crop demand. On the contrary, for SLM, it is most improbable that any manure C triggered N immobilization as the C:N ratios were of 2 in 1994 and 1 in 1995. Miller and MacKenzie (1978) estimated apparent N recovery in corn of 10% for SLM and 13% for DSM for a fine-textured soil and a climatic conditions comparable to those of the present study. The differences between their results and those of the present study could be related to the C:N ratio of the manures and/or the synchronization of the manure N release to the plant N uptake.

Time of application had limited effects on apparent N recovery of SLM and none on DSM (Table 2.2; Fig. 2.1). Pre-seeding application of SLM tended to increase N recovery compared to applications occurring later in the growing season ($\alpha=0.20$). It could be related to the fact that an important fraction of N of SLM was in NH_4^+ form (Table 2.1). Ammonium is highly susceptible of being lost through NH_3 volatilization, especially if the manure is not incorporated into the soil immediately after being applied (Beauchamp *et al.* 1982). Lower volatilization losses could explain the higher N recovery of SLM applied at pre-seeding which is incorporated into the soil while it is not the case when manure was applied on the soil surface in post-emergence. When applied after harvest, the NH_4^+ , which is rapidly nitrified in the aerobic soil zone (Rice *et al.* 1988), can be lost through denitrification following episodes of heavy precipitation (Christensen *et al.* 1990) occurring during fall or by leaching through the soil profile. This could explain why post-harvest application of SLM tended ($\alpha=0.20$) to lower N recovery compared to pre-seeding and post-emergence applications in 1994 (Table 2.2).

The red clover companion crop significantly decreased the apparent N recovery of SLM and DSM in 1995 (Table 2.2; Fig. 2.1b). The effect of red clover on SLM was significant at $\alpha=0.01$. While the apparent N recovery of SLM in 1995 was 17% when wheat was cropped alone, in the presence of clover, it was estimated at -4%. Impact of clover on DSM was expressed by a trend at $\alpha=0.20$. Apparent N recovery of DSM combined with clover was -1% compared to 4% when wheat was not interseeded with clover. In the presence of clover, manure application time had no significant effect on apparent N recovery (Table 2.2).

The additional N taken up by wheat in the control (0N) treatments with clover compared to without clover was 9 kg N ha⁻¹ in 1994 and 26 kg N ha⁻¹ in 1995. Lower apparent N recovery with manures in the presence of clover in 1995 could be attributed to the additive effects of N from both sources (Baldock and Musgrave 1980; Lory *et al.* 1995) which decreased the relative efficiency of N supplied by manures. The average amounts of N measured at the end of the fall in the aerial parts of the clover were 97 kg N ha⁻¹ in 1993 and 62.4 kg N ha⁻¹ in 1994. Recent results obtained by Hu *et al.* (1997) in incubation experiments and in the field showed that more than 30% N in cover crop residues was released by day 35 after incorporation. In the present study red clover was ploughed down at mid-November. At this time the soil rapidly freezes. Most of the mineralization was probably delayed until soil temperature increased in the spring. In 1994, adverse climatic conditions could have caused losses of mineralized N derived from clover residues N (Fig. 2.1). This would explain part of the discrepancies between N supplied to the wheat by clover in 1994 and in 1995. To support this hypothesis, May and June precipitation of 1994 may have resulted in greater N losses through denitrification and NO₃⁻ leaching than in 1995 (191 mm in 1994 compared to 119 mm in 1995). The higher amount of N provided by the clover in 1995 could have also resulted from the successive addition of clover residues which progressively caused the build-up of a larger labile organic soil N pool than in previous years (Biederbeck *et al.* 1998).

In 1994, the apparent N recovery from mineral N varied between 6 and 18% with an average of 11%. In contrast, in 1995, the apparent N recovery was inversely related to NH₄NO₃ application (Fig. 2.2) and ranged between 16 and 35%. The relationship was best described by a quadratic model. The apparent N recovery values found in 1994 were

lower than previously reported N recovery values of mineral N fertilizer by wheat which are typically between 28 and 90% (Novoa and Loomis, 1981; Delogu *et al.* 1998). Some authors observed a greater recovery of N as the rate increased while others reported the reverse (Novoa and Loomis 1981; Ladd and Amato 1986). The high rainfall of spring 1994 would explain lower N recovery which was observed not only for NH_4NO_3 but also, as previously mentioned, for manure and red clover N. This was reflected in the yield-N fertilizer relationship which obeyed a linear form with no maximum in 1994 (Paper 1). In 1995, transfers of N added by clover and manures outside the root zone were limited due to lower precipitation. This resulted in larger soil N contents than in 1994. So, the wheat N uptake from the combined sources (manure and clover) was such that the relative recovery of manure N was limited. This explains the lower N recovery observed as NH_4NO_3 rate increased and those of manures estimated in the presence of clover in 1995.

Residual NO_3^- in the Soil Profile

The amounts of residual NO_3^- -N left in the soil profile (0-90 cm) were not significantly affected by manure type (Table 2.3; Fig. 2.3) in most samplings of 1994 and 1995. Residual NO_3^- -N varied between 13 to 95 kg N ha^{-1} . Fall 1994 was an exception with NO_3^- -N contents from SLM plots being significantly higher than those measured under DSM treatments. This was mainly attributed to post-harvest SLM application which resulted in a soil profile content of 42 kg N ha^{-1} . This is more than twice the average of 18 kg N ha^{-1} measured under the other manure treatments (Table 2.3; Fig. 2.3e). These findings suggest that the high 1994 September rainfall (286 mm) promoted large losses of NO_3^- through leaching or denitrification. The difference between the post-harvest SLM

treatment and the others may be related to the dominance of the N in SLM by NH_4^+ (Table 2.1). Sowden (1976) reported that 40 to 50 % of the NH_4^+ added as manures or mineral fertilizer to a soil with a high ammonium fixing capacity was immediately bound by the clay minerals. There is increasing evidence that under certain conditions, this pool can replenish very significantly the soil solution in NH_4^+ (Steffens and Sparks 1997). The NH_4^+ brought by SLM applied at post-harvest in 1994 (which represented 73.3% of the 90 kg N ha^{-1} applied) and was probably rapidly fixed and protected from excessive losses due to high soil water content. It was then released progressively in the soil solution where it was nitrified the following spring. This explains its higher spring soil N content than in other treatments.

In 1994, post-harvest applications of SLM and DSM resulted in significantly higher ($\alpha=0.01$) soil profile N-NO_3^- contents at mid-November and at the end of April when compared to pre-seeding and post-emergence applications (Table 2.3; Fig. 2.3a, e). In 1995, the same effect was observed but only in spring for SLM (Fig. 2.3b). Therefore, manures applied in fall released a significant quantity of NH_4^+ either as soluble NH_4^+ (Table 2.1) or *via* mineralization of their organic fraction. It was assumed that the NH_4^+ was nitrified and that a significant proportion of the N added as manure persisted in the soil profile regardless of possible denitrification (Petersen and Anderson 1996).

The presence of clover significantly affected the residual NO_3^- -N measured under SLM and DSM treatments in 1994 and 1995 (Table 2.3). Except for spring 1994, the clover increased NO_3^- -N contents in the soil profile (Fig. 2.3). At the end of April 1994, there was a significant difference in the profile NO_3^- -N contents between spring and fall DSM and SLM applications in wheat alone. This difference was not observed in the

presence of clover (Table 2.3). This suggests that clover absorbed the supplemental N (Fig. 2.3a). Clover also decreased residual NO_3^- -N measured at mid-November for DSM applied after harvest in 1994 (Fig. 2.3e), but this was not observed in 1995. The soil without any plant cover, is subjected to more N transfer from the soil to surface and ground water and to the atmosphere (Webster and Goulding 1995; Sharpley *et al.* 1998). It was found that high concentrations of inorganic N in soil can inhibit the process of N_2 symbiotic fixation (Bandyopadhyay *et al.* 1996) and therefore, N uptake from soil can substitute directly for N_2 fixation (Ledgard and Giller 1995). The present results suggest that even though clover significantly reduced the soil profile residual N after manure application in the 1994 growing season, the two successive additions of clover residues resulted in a build-up of an organic N pool which, through mineralization, maintained higher level of residual NO_3^- -N than for treatments without underseeded clover. Therefore the reduction in profile NO_3^- -N by clover cover crop is not sustainable when clover is grown repeatedly in association with wheat.

Post-Emergence NO_3^- to 20 cm as Wheat N Availability Index

For 1994 and 1995, the NO_3^- -N measured in the 0-20 cm soil layer 30 days after seeding was not related to wheat N uptake (Fig. 2.4). In 1994, NO_3^- -N contents ranged between 8 and 140 kg N ha^{-1} and in 1995, between 6 and 114 kg N ha^{-1} . The reported NO_3^- -N values were for all combinations of treatment factors (manures or NH_4NO_3 combined or not with red clover) and, this explains the wide range of NO_3^- -N soil contents observed. Under the conditions of the study, 30 days after seeding corresponds approximately to the beginning of tillering. It has been found that 80% of the N uptake occurred during tillering and

heading (Delogu *et al* 1998) and that wheat response to N applied during early development was very limited (Sylvester-Bradley *et al.* 1997). Therefore the available N at the beginning of tillering should strongly impact on N uptake. The lack of relationship could be related to the presence of available N in other forms than NO_3^- such as NH_4^+ adsorbed on soil colloids or fixed by phyllosilicates or as labile organic N. Another possible drawback of this index is that it is a punctual estimation that does not integrate over time field conditions and mechanisms of N transport which strongly affect what will be effectively taken up by the plant.

CONCLUSIONS

Clover in combination with manure can increase plant available N in the soil profile so that recovery of the manure N by crops will be strongly reduced. The added N which is not taken up by plants may result in higher level of residual soil profile NO_3^- which is susceptible to denitrification or leaching in fall and in spring. Manures should preferably be applied in spring rather than after harvest for agronomic and environmental purposes. It was found that it is not possible to match precisely N requirements of wheat with the same rate of a N source applied on total N basis each year since the N recovery of the manures and of the mineral N fertilizer varied as a function of the year. Therefore, the finding of a N index would have improved of the status recommendations prevailing in Quebec. Developed at first in Vermont for a large spectrum of soils submitted to climatic conditions close to those prevailing in Eastern Canada, the pre-sidedressed NO_3^- used in corn production appeared as a possible index of N availability. Nevertheless, the post-

emergence measurement of NO_3^- at the 0-20 cm layer presented no relationship with N wheat uptake. Therefore research should turn to alternative indices to estimate the N availability for soil submitted to cool and humid climates and receiving large input of N through organic sources.

ACKNOWLEDGEMENTS

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Table 2. 1. Amounts of total C and N added and relative proportions of N fractions in manure treatments

| Treatments | Dry matter | Total C added | Total N added | Organic N | NH ₄ ⁺ -N | NO ₃ ⁻ -N |
|---------------------------------|------------|---------------------|---------------------|-----------|---------------------------------|---------------------------------|
| | % | kg ha ⁻¹ | kg ha ⁻¹ | % | % | % |
| 1994 | | | | | | |
| SLM ₁ ^{x,y} | 1.06 | 160 | 80 | 17.9 | 79.6 | 2.5 |
| SLM ₂ | 1.11 | 167 | 80 | 53.3 | 46.7 | - |
| SLM ₃ | 1.23 | 191 | 90 | 26.5 | 73.3 | - |
| DSM ₁ | 25.05 | 4240 | 250 | 96.5 | 1.8 | 1.7 |
| DSM ₂ | 26.72 | 3949 | 230 | 96.2 | 2.8 | 1 |
| DSM ₃ | 28.41 | 3323 | 230 | 95.7 | 3.3 | 1 |
| 1995 | | | | | | |
| SLM ₁ | 1.41 | 105 | 130 | 49.4 | 48.8 | 1.8 |
| SLM ₂ | 1.41 | 105 | 130 | 49.4 | 48.8 | 1.8 |
| SLM ₃ | .84 | 208 | 130 | 27.9 | 69.9 | 2.2 |
| DSM ₁ | 31.34 | 2147 | 250 | 68.1 | 26.6 | 5.3 |
| DSM ₂ | 31.34 | 2147 | 250 | 68.1 | 26.6 | 5.3 |
| DSM ₃ | 25.78 | 1348 | 250 | 89.3 | 9 | 1.7 |

^xSLM₁, swine liquid manure; DSM₁, dairy solid manure

^y ₁, applied at pre-seeding; ₂, applied at Zadoks 12 growth stage; ₃, after harvest of the previous year

Table 2. 2. Mean squares of the variance analysis of variance of treatment effects on manure N apparent recovery

| | 1994 | | 1995 | |
|----------------------------------------------------|------|-------------------|------|-------------------|
| | d.f. | MS | d.f. | MS |
| Treatments | 11 | 56 | 11 | 313** |
| Block | 3 | 190* | 3 | 236** |
| Error | 32 | 51 | 33 | 53 |
| Contrasts | | | | |
| SLM-nc vs DSM-nc | 1 | 96 ^{td} | 1 | 973** |
| SLM ₁ -nc vs SLM ₂ -nc | 1 | 135 ^{td} | 1 | 86 ^{td} |
| SLM _{1&2} -nc vs SLM ₃ -nc | 1 | 103 ^{td} | 1 | 68 |
| DSM ₁ -nc vs DSM ₂ -nc | 1 | 2 | 1 | 12 |
| DSM _{1&2} -nc vs DSM ₃ -nc | 1 | 5 | 1 | 69 |
| SLM-nc vs SLM-c | 1 | 18 | 1 | 2626** |
| DSM-nc vs DSM-c | 1 | 5 | 1 | 180 ^{td} |
| SLM ₁ -c vs SLM ₂ -c | 1 | 0 | 1 | 36 |
| SLM _{1&2} -c vs SLM ₃ -c | 1 | 38 | 1 | 29 |
| DSM ₁ -c vs DSM ₂ -c | 1 | 2 | 1 | 28 |
| DSM _{1&2} -c vs DSM ₃ -c | 1 | 76 | 1 | 3 |

^xSLM:, swine liquid manure; DSM, dairy solid manure

^y 1, applied at pre-seeding; 2, applied at Zadoks 12 growth stage; 3, after harvest

^z-nc, no clover; -c, clover

** significant at $\alpha=0.01$ * significant at $\alpha=0.05$ ^{td}trend at $\alpha=0.20$

Table 2. 3. Mean squares of the analysis of variance of treatment effects on amounts of NO_3^- -N measured in the soil profile (0-90 cm) at post-harvest, mid-November and early spring from 1994 to 1995

| | d.f. | Early spring NO_3^- -N | Post-harvest NO_3^- -N | Late Fall NO_3^- -N |
|---------------------------------------------------|------|------------------------------------|------------------------------------|---------------------------------|
| 1994 | | | | |
| Treatments | 11 | 253* | 564 ^{td} | 438** |
| Bloc | 3 | 651** | 1431** | 148* |
| Error | 33 | 105 | 298 | 44 |
| <i>Contrasts</i> | | | | |
| SLM vs DSM ^x | 1 | 93 | 8 | 202* |
| SLM ₁ ^y vs SLM ₂ | 1 | 187 ^{td} | 328 | 17 |
| SLM _{1&2} vs SLM ₃ | 1 | 1037** | 311 | 1873** |
| DSM ₁ vs DSM ₂ | 1 | 22 | 160 | 14 |
| DSM _{1&2} vs DSM ₃ | 1 | 1187** | 70 | 446** |
| SLM-nc ^z vs SLM-c | 1 | 197 ^{td} | 1940* | 122 ^{td} |
| DSM-nc vs DSM-c | 1 | 23 | 1948* | 22 |
| SLM1-c vs SLM2-c | 1 | 16 | 95 | 63 |
| SLM _{1&2} -c vs SLM ₃ -c | 1 | 4 | 123 | 1650** |
| DSM ₁ -c vs DSM ₂ -c | 1 | 48 | 25 | 4 |
| DSM _{1&2} -c vs DSM ₃ -c | 1 | 8 | 1189* | 2 |
| 1995 | | | | |
| Treatments | 11 | 1574** | 1033** | 506 ^{td} |
| Bloc | 3 | 141 | 1673** | 42 |
| Error | 33 | 421 | 361 | 270 |
| <i>Contrasts</i> | | | | |
| SLM vs DSM | 1 | 287 | 665 ^{td} | 172 |
| SLM ₁ vs SLM ₂ | 1 | 168 | 617 ^{td} | 21 |
| SLM _{1&2} vs SLM ₃ | 1 | 6686** | 95 | 383 |
| DSM ₁ vs DSM ₂ | 1 | 38 | 401 | 198 |
| DSM _{1&2} vs DSM ₃ | 1 | 71 | 893 ^{td} | 108 |
| SLM-nc vs SLM-c | 1 | 2099* | 6340** | 1106* |
| DSM-nc vs DSM-c | 1 | 3649** | 519 | 2061** |
| SLM1-c vs SLM2-c | 1 | 1153 ^{td} | 398 | 1 |
| SLM _{1&2} -c vs SLM ₃ -c | 1 | 1332 ^{td} | 183 | 1113* |
| DSM ₁ -c vs DSM ₂ -c | 1 | 1177 ^{td} | 1175 ^{td} | 481 ^{td} |
| DSM _{1&2} -c vs DSM ₃ -c | 1 | 749 ^{td} | 729 ^{td} | 1 |

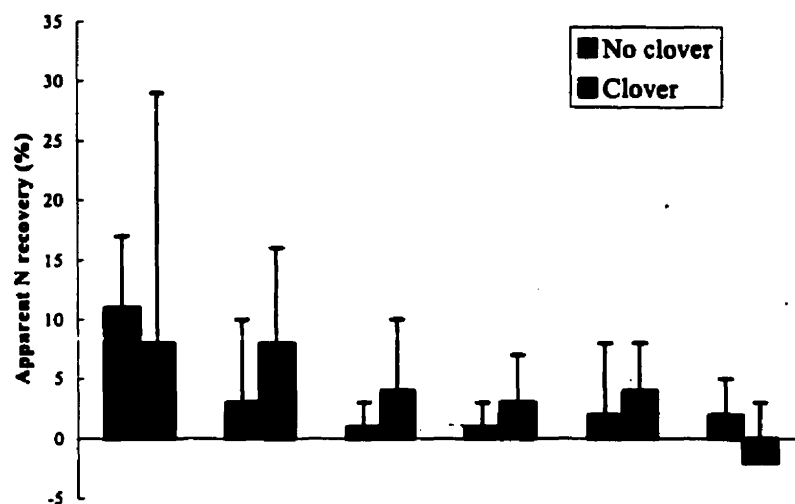
^xSLM: swine liquid manure; DSM, dairy solid manure

^y 1, applied at pre-seeding; 2, applied at Zadoks 12 growth stage; 3, after harvest

^z-nc, no clover; -c, clover

**significant at $\alpha=0.01$ *significant at $\alpha=0.05$ ^{td}trend at $\alpha=0.20$

a)



b)

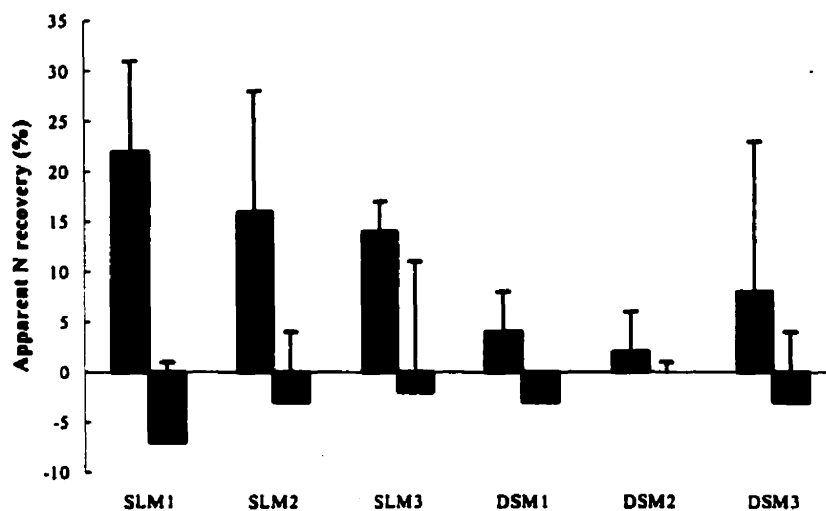


Fig. 2. 1. Apparent N recovery by spring wheat (*Triticum aestivum* L. cv. Algot) of swine liquid manure (SLM) or dairy solid manure (DSM) applied in pre-seeding (1), at Zadoks 12 growth stage (2) or after harvest (3) in a) 1994 and b) 1995. Treatments were applied with or without interseeded red clover (*Trifolium pratense* L. cv. Arlington). Each bar is the mean of four replicates. Error bars are standard errors of the mean.

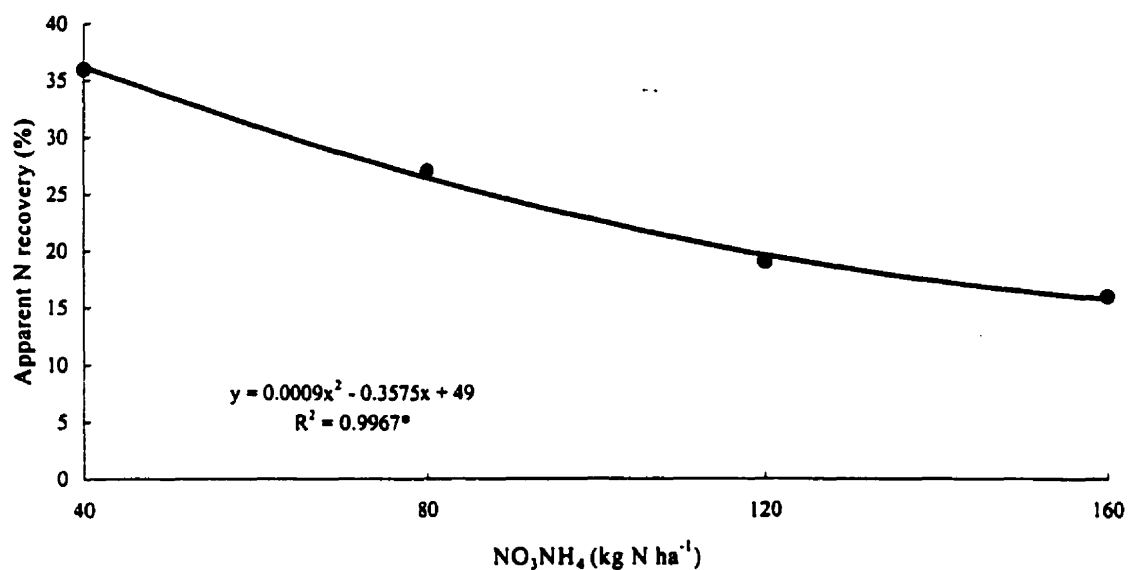


Fig. 2. 2. Apparent N recovery by wheat (*Triticum aestivum* L. cv. Algot) as a function of NH_4NO_3 application rates in 1995. Each datum point is the mean of four replicates.

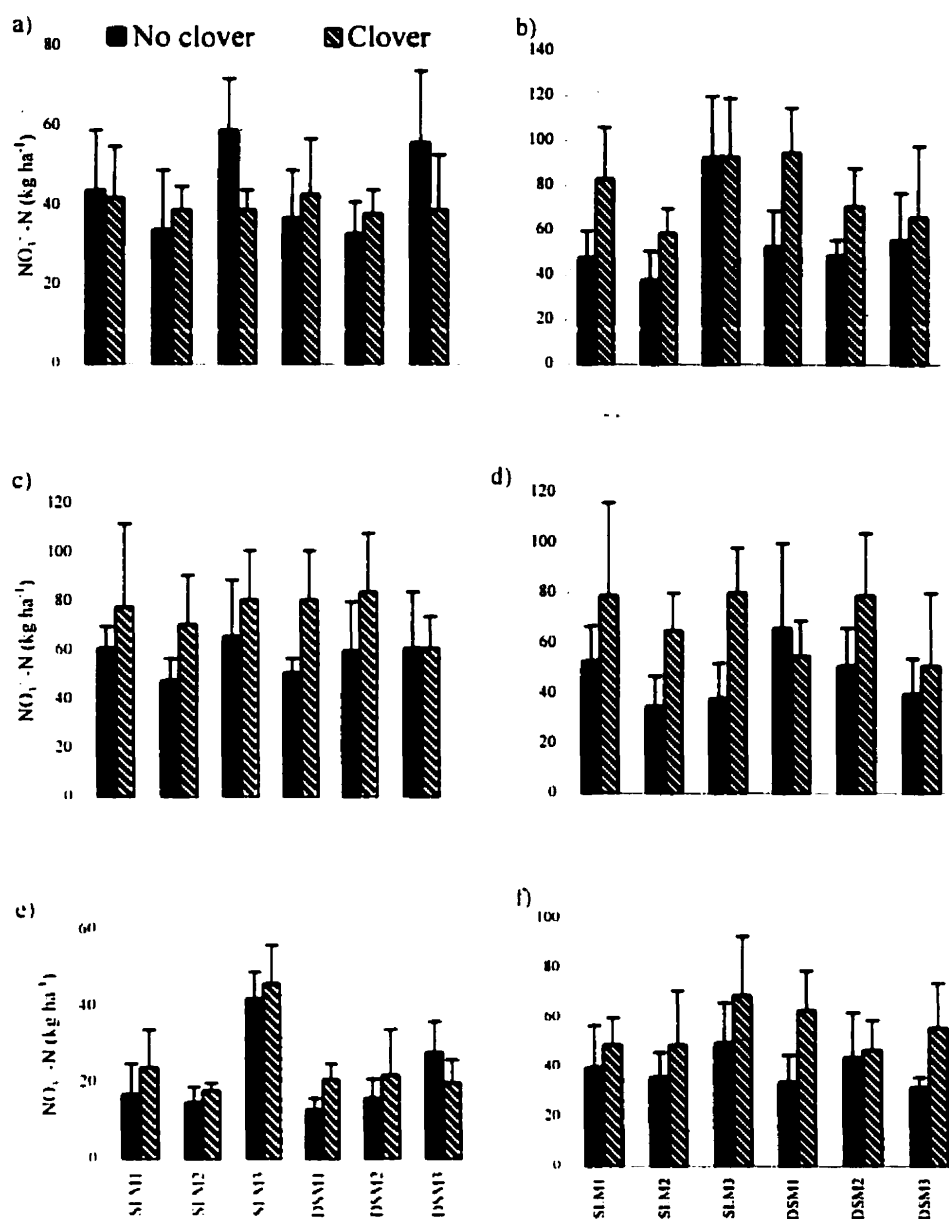


Fig. 2. 3. Nitrate-N in the profile (0-90 cm) of a St. Urbain soil at the end of April of a) 1994 and b) 1995, after harvest of c) 1994 and d) 1995 and at mid-November of e) 1994 and f) 1995. Treatments applied on spring wheat (*Triticum aestivum* L. cv. Algot) combined manures (swine liquid manure (SLM) or dairy solid manure (DSM)) applied at pre-seeding (1), at Zadoks 12 growth stage (2) or after harvest (3)) with or without red clover (*Trifolium pratense* L. cv. Arlington) as companion crop. Each datum bar is the mean of four replicates. Error bars shown are standard errors of the mean.

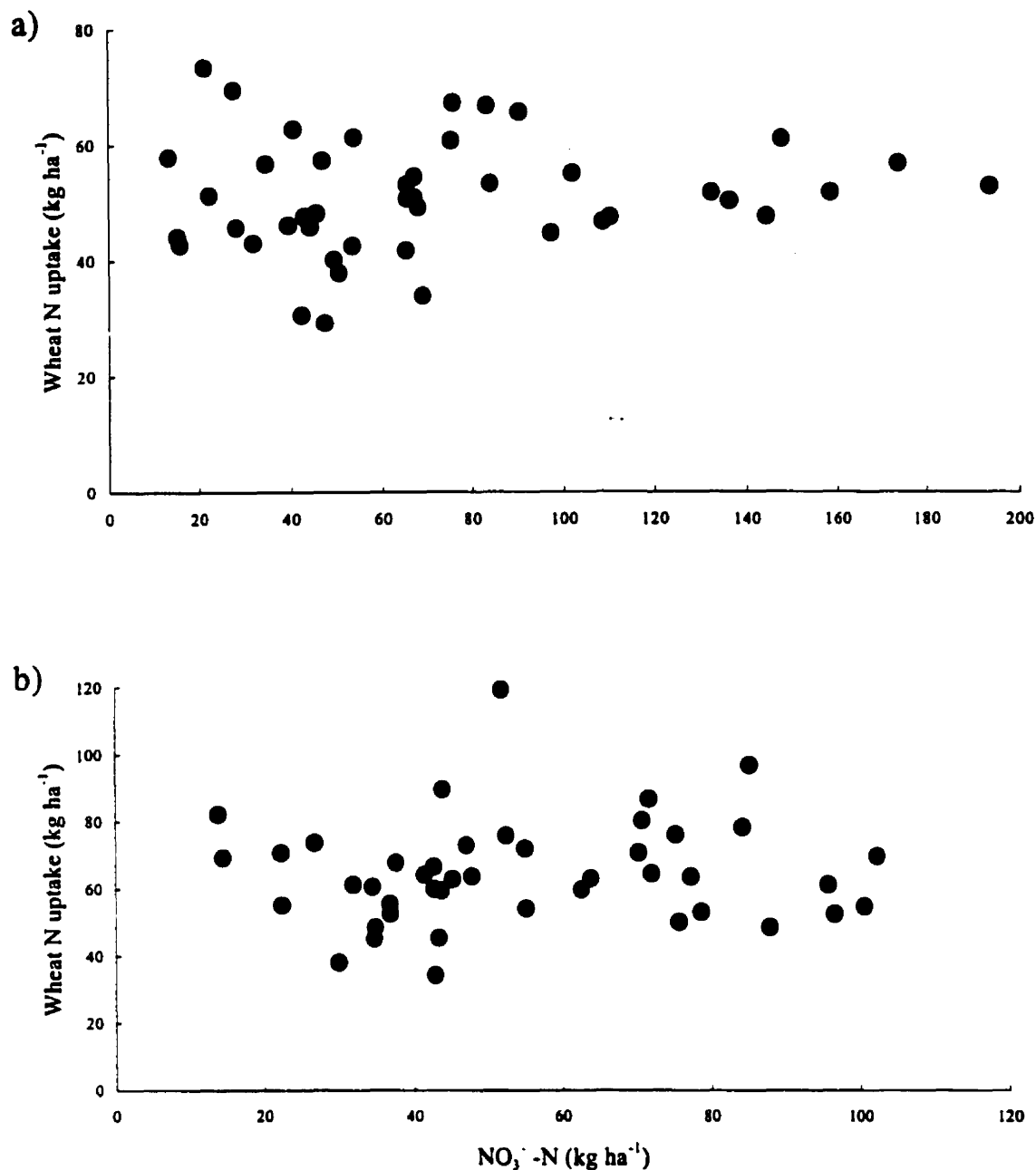


Fig. 2. 4. Nitrogen uptake of spring wheat (*Triticum aestivum* L. cv. Algot) as a function of N-NO_3^- measured in the 0-20 cm layer of a St. Urbain clay 30 days after seeding for organic and mineral N fertilization treatments combined or not with interseeded red clover (*Trifolium pratense* L. cv. Arlington) in a) 1994 and b) 1995. Each datum point represents one measurement.

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FOREWORD THAT PROVIDES A LOGICAL BRIDGE BETWEEN PAPER 2 AND PAPER 3

Nitrogen credits of clover and apparent N recovery of manures estimated in this study are the means of a specific field trial. Averaged over many years, they could provide a gross estimation of the N availability of those sources for poorly drained clay soils. The total amount of N to be applied from a specific source to meet plant N requirements is conditioned by its relative N availability. But recommendations based solely on mean value of N availability of fertilizer, manures or clover cannot be made from one year to another without tremendous imprecision. The amount of fertilizer or manure N required for a crop depends also on the soil N available to the crop during the growing season. The residual N in the soil profile at the beginning of a growing season, the N mineralized during a growing season and the N lost in transfers to water and air are highly dependent of climate. Unpredictable climatic conditions will determine, for each growing season, a different level of soil available N. Those considerations underlined the necessity of a N index that could be use to adjust the rate of N applied as fertilizer to soil available N and, therefore correct N applications on a yearly basis. The absence of a relationship that was observed between water soluble NO_3^- content of the 0-20 cm soil layer measured in post emergence created the need to turn toward other alternatives (Paper 2). The object of the third paper was to test, under field conditions prevailing in this study, a promising alternative to usual chemical extractants: ionic exchanges membranes (IEMs). They can be placed *in situ* for a specific contact period and the ions sorbed are function of diffusion

process which affects nutrient uptake by the plants. Therefore, it was assumed that they could provide a better index of N availability than measurements of mineral N pools as estimated by chemical extractions.

**PAPER 3 : *IN SITU* N FLUXES ESTIMATED BY IONIC-EXCHANGE
MEMBRANES AS NITROGEN BIOAVAILABILITY INDEX IN SPRING
WHEAT**

ABSTRACT

Monitoring the nitrogen (N) released from labile organic and mineral sources in soils subjected to cool spring and highly unpredictable rainfall is a prerequisite to an efficient N management in spring cereal production. An approach based on *in situ* N fluxes as estimated by ionic-exchange membranes (IEMs) is a promising alternative to measurement of mineral N pools by chemical extraction: membranes could provide measurements of N which integrate for a given period of time soil factors that influence N bioavailability such as diffusion and spatial organization. An experiment was set up i) to test the capacity of IEMs compared to 2M KCl soil extractions to discriminate between organic and mineral N sources on their release of mineral N in spring time and ii) to provide reliable indices of N availability to spring wheat (*Triticum aestivum* L. cv. Algot). The study took place on a St. Urbain clay (Orthic Humic Gleysol), at St. Bruno de Montarville, in the province of Quebec. Nitrogen was supplied as 80 kg N ha⁻¹ of NH₄NO₃, underseeded clover residues, swine liquid (SLM) and composted dairy (CDM) manure. The manures were applied either at seeding or the previous fall. Nitrate sorbed on anionic-exchange membranes (N-NO₃⁻_{AEM}) better discriminated between treatments than NO₃⁻ (N-NO₃⁻_{KCl}) and NH₄⁺ extracted with 2M KCl (N-NH₄⁺_{KCl}) or NH₄⁺ sorbed on cationic exchange membranes (N-NH₄⁺_{CEM}) in the end of April, at 20 and at 30 days after seeding. Fall application of CDM and SLM resulted in a lack of synchronization between N release and wheat N uptake. After spring fertilization, the highest N-NO₃⁻_{AEM} values were observed with SLM or NH₄NO₃ applied at 80 kg N ha⁻¹. The best relationships between soil N measurements and wheat yields were obtained for N-NH₄⁺_{CEM} in the end

of April when anaerobic conditions are prevailing and, for N-NO_3^- AEM at 20 and at 30 days after seeding. This study suggests that under wet and cold spring climatic conditions, monitoring of N in the soil should assess fluxes of both NH_4^+ and NO_3^- . Ionic exchange membranes can be useful tools for monitoring these fluxes.

Key words: available N index, cationic-exchange membrane, anionic-exchange membrane, swine liquid manure, composted dairy manure, underseeded clover

INTRODUCTION

Managing added N efficiently by synchronizing its release to plant N uptake and modulating N application with regard to the readily available soil N are priorities if agronomic and environmental goals are to be met in Quebec spring cereal production. Mineral N fertilizer could be replaced by manures and/or legumes to fulfil plant N requirement. The pattern of N released from organic sources is more complex than that from mineral ones since N is found in multitude of chemical compounds that react differently to *in situ* field conditions.

Repeated applications of manure (Klausner *et al.* 1994; Gao and Chang 1996) and agricultural systems in which high amounts of N is returned to soil *via* legume residues (Harris *et al.* 1994; Hossain *et al.* 1996) can lead to the build-up of a large pool of mineralizable organic N. Therefore, the reliance on organic N sources increases the necessity to monitor adequately the fate of N by meaningful measurements representative of biochemical processes involved in soil N cycling and plant N bioavailability.

Many authors have presented nutrient fluxes as measured by *in situ* ionic exchange membranes as an alternative to conventional approaches based on mineral pools of nutrient estimated by chemical extraction. Ions sorbed on IEMs provided relevant observations of nutrient bioavailability and reaction kinetics over a defined period of time (Abrams and Jarrell 1992; Cooperband and Logan 1994; Subler *et al.* 1995). Ionic-exchange membranes were found to have many advantages over chemical extractants. They can be inserted in the soil with minimal soil disturbance (Cooperband and Logan 1994; Qian *et al.* 1996; Huang and Schoenau 1996) and therefore reflect the impact of the soil spatial micro-heterogeneity on N fluxes. The sorption of nutrients on IEMs is largely influenced by diffusion processes (Vaidyanathan and Nye 1966) which often condition their absorption by plants (Barber 1995).

Nitrate is stable only under strongly oxidizing conditions while NH_4^+ is stable under strongly reducing conditions which are related to high soil moisture content (Sposito 1989). Although anaerobic or aerobic conditions determine the speciation of mineral N in the soil solution, until now, only NO_3^- sorbed on AEMs was considered in field experiments. To our knowledge, there is no study reporting the combined use of cationic and anionic exchange membranes to assess N bioavailability and kinetics of release of organic sources in field conditions. Therefore, the objectives of this study were i) to assess the capacity of IEMs to monitor the mineral N released by clover residues and manure into the soil during spring compared to NO_3^- and NH_4^+ extracted with 2M KCl and ii) to assess the potential of N sorbed on IEMs as an index of bioavailable N.

MATERIALS AND METHODS

Site

The experiment took place at St. Bruno de Montarville. Details on the soil classification and physico-chemical characteristics appear in the first and second papers. Although the study was conducted from May 1993 to November 1996, field evaluation of IEMs was carried out only in 1996. Results presented in this paper are therefore limited to one year. Daily total precipitation and average air temperatures data were provided by Environment Canada from the nearby St. Hubert meteorological station (45°31'N; 73°25'W).

Treatments

We selected only seven treatments of the original study (Simard *et al.* 1997) in order to provide a diversity in the type of N sources and the time of application. The treatments were mineral N (NH_4NO_3) applied at 0 and 80 kg N ha⁻¹ before spring wheat (*Triticum aestivum* L. cv Algot) seeding, swine liquid (SLM) and composted dairy manure (CDM) applied either the previous fall or in pre-seeding and, underseeded clover (*Trifolium pratense* L. cv Arlington) ploughed down in late fall as green manure. Spring fertilization and seeding were done the 24th of May. Swine liquid manure was obtained from local farmers and CDM was produced at the farm of the St. Hyacinthe Agricultural Institute. It was a well-decomposed compost containing straw bedding. Manure composition was reported in Table 3.1. The amount of total N of the aerial part of clover incorporated to the soil by chisel ploughing the preceding fall was estimated at 45 kg N ha⁻¹ from yield

data and mineral N content. The experimental design was a randomized complete block with four replicates.

Procedure for In Situ Use of IEMs

The selected IEMs were CR67 (Ionics, Watertown, MA) for cationic exchange membranes (CEMs) and AR204 (Ionics, Watertown, MA) for anionic exchange membranes (AEMs). Membranes are electrolytic three-dimensional matrix composed of homogeneous cross-linked vinyl copolymer reinforcing fabric embedded with quaternary ammonium exchange groups for AEMs and sulfonate groups for CEMs. They behave as strong base (AEMs) or strong acid (CEMs) compounds. Membrane properties are reported in Table 3.2.

The membranes were prepared by cutting a sheet of material into 6.25 X 2.5 cm-strips, which were thoroughly washed with distilled water to remove impurities and lightweight particles, washed with 0.5 M HCl for 5 minutes and then washed with distilled water. The AEM strips were conditioned by shaking for 1 hr in 1M NaCl (Ziadi *et al.* unpublished). The CEM strips were conditioned as with the AEMs but in 1M LiCl. Ion-exchange membranes were saturated with ions (Cl^- or Li^+) reported to have a lesser affinity for the membranes than the target ions and therefore favoring exchanges with target ions in the soil solution (Skogley and Doberman 1996). The strips were allowed to soak one to three days in distilled water until needed.

In the field, membranes were placed in the plough layer approximately between 5 and 15 cm depth by opening a vertical slit with a hand trowel and gently sliding the membrane vertically into the slit (Cooperband and Logan 1994). The vertical position

was preferred in order that ions sorbed on IEMs reflect the depth gradient and would not create a reduced zone by restricting downward water movement. The slit was closed by firmly pressing both sides together to ensure uniform membrane-soil contact. When membranes were later retrieved from the soil, large aggregates that adhered to the membranes were removed by washing with distilled water. The membranes were returned to the laboratory in 50 mL Nalgene tubes containing 25 mL of a 1M NaCl solution for AEMs and 1M HCl for CEMs. Nitrate and ammonium sorbed on membranes were then desorbed in the respective extracting solutions by 1 hr shaking on a reciprocal shaker. Nitrate was measured by high performance liquid chromatography (HPLC) and UV detection at 214 nm. Ammonium was measured colorimetrically according to Nkonge and Ballance (1982). Further details on NH_4^+ and NO_3^- determination were given in previous papers.

Ionic-exchange membrane strips were used for variable lengths of time: in the end of April (April 28th until May 2nd), 10 days after seeding (June 3rd to 13th) and 20 days after seeding (June 13th to 25th). Four AEM and CEM strips were placed in each plot. The soil NH_4^+ and NO_3^- sorbed on IEMs was expressed as fluxes ($\mu\text{g N cm}^{-2} \text{ d}^{-1}$) by dividing the amount of N measured on IEMs ($\mu\text{g N}$) by two times the membranes surface (cm^2) and by the length of IEMs contact period in days (d) with the soil. Nitrogen fluxes reported were averaged for each plot.

Soil and Plant Sampling and Analysis

Composite soil samples (0-15 cm, 3 to 4 cores (7-cm diameter) per plot) were collected each time IEMs were removed from the field. Soil NO_3^- -N and NH_4^+ -N contents were

estimated with 2M KCl extraction performed on fresh soil subsamples. Readers should refer to previous papers for details on soil mineral N analysis and calculations. Wheat N uptake was determined after harvesting of aerial parts at plant maturity. Nitrogen in plant tissues (grain and straw) was determined with wet-digestion using the sulphuric acid-hydrogen peroxide mixture (Richards 1993). Ammonium was determined colorimetrically (Nkonge and Ballance 1982). Further details of the methodology and calculations of wheat N uptake figure in Paper 1.

Statistical Analysis

The procedure GLM (SAS Institute 1990) was used to perform analysis of variance (ANOVA) on mineral N sorbed on IEMs and extracted by 2M KCl for each sampling date and on wheat N uptake data. Pearson coefficients of correlation between soil N variables and wheat N uptake were estimated by the procedure CORR (SAS Institute 1990). Regression analyses were performed to describe the relationship between soil N variables and wheat N uptake. The procedures used were PROC REG or PROC NLIN (SAS Institute 1990).

RESULTS AND DISCUSSION

Weather

Both mean temperature and total precipitation were reported on a daily basis (Figure 3.1). Air temperatures showed erratic fluctuations with steady increases followed by rapid

decreases. Differences of more than 10°C were observed in periods of 2 to 4 days were not uncommon. Nevertheless the general trend of temperature was to increase from mid-April until the end of June. The pattern of total precipitation showed sporadic episodes that were unevenly distributed with an average of 9 mm by events. The largest episode occurred the 29th of May with 42 mm rainfall.

Effects of Treatments on Mineral N Sorbed on IEMs and Extracted with 2M KCl

At all samplings, the analysis of variance demonstrated that $\text{N-NO}_3^-_{\text{AEM}}$ was the soil variable the most apt to statistically discriminate between treatments (Table 3.3). This was true even though NO_3^- fluxes ranged from 0.22 to 0.87 $\mu\text{g N cm}^{-2} \text{ d}^{-1}$ in the end of April, from 3.24 to 12.14 $\mu\text{g N cm}^{-2} \text{ d}^{-1}$ at 20 days after seeding and from 0.23 to 2.49 $\mu\text{g N cm}^{-2} \text{ d}^{-1}$ at 30 days after seeding. This demonstrated the sensitivity of the method to detect treatments effects over a wide range of values of fluxes even if differences were small. Paré *et al.* (1995) reported a higher sensitivity of AEM method to low NO_3^- soil content compared to 2M KCl extraction; our results confirmed their findings. Since, except for $\text{N-NO}_3^-_{\text{KCl}}$ at 30 days after seeding, it was not possible to discriminate between treatments on the basis of $\text{N-NO}_3^-_{\text{KCl}}$, $\text{N-NH}_4^+_{\text{CEM}}$, and $\text{N-NH}_4^+_{\text{KCl}}$ (Table 3.3), $\text{N-NO}_3^-_{\text{AEM}}$ was our best tool to follow release of N by the various N sources. This provides further evidence to the growing literature which reported that ions sorbed on IEMs are good indicators of changes in nutrient bioavailability induces by treatments or site factors (Wander *et al.* 1995; Paré *et al.* 1995; Jowkin et Schoenau 1998; Ziadi *et al.* unpublished data).

At the end of April, the flux of N under CDM applied the previous fall was the highest with a value of $0.87 \mu\text{g N cm}^{-2} \text{ d}^{-1}$ (Fig 3.2a) and was significantly different than those measured under SLM applied in fall and CDM applied at seeding (Table 3.3). The great amount of carbon added with CDM (Table 3.1) at the beginning of September 1995 may have allowed a more intensive microbial activity over an extended period compared to other treatments and therefore, the higher flux of nitrate observed for this treatment would be the result of a higher N mineralization. This hypothesis is supported by Lalande *et al.* (unpublished data) which observed higher CO_2 emission and microbial biomass C in soil receiving composted de-inking paper sludges at 40 Mg ha^{-1} than for unamended controls (in the present study, 30 to 40 Mg ha^{-1} of CDM were applied) for a period of 16 weeks. Hadas and Portnoy (1994) also observed that soil incubated with various composts showed increased CO_2 emission for an extended period of 32 weeks and that it corresponded to a higher release of inorganic N.

At 20 days after seeding, it was not possible to perform an analysis of variance for $\text{N-NO}_3^- \text{KCl}$ (Table 3.3) due to a large number of measurements under the detection limit. The only treatments in which it was possible to measure nitrate was for SLM applied in spring (Fig. 3.2) and 80 kg N ha^{-1} applied as NH_4NO_3 with respective values of 60 and 37 kg N ha^{-1} . The large precipitation that occurred between the 24th of May (when the wheat was seeded and spring fertilization) and the time at which the IEMs were recovered (14th of June) had probably caused the loss of NO_3^- from the 0-20 cm layer *via* leaching and denitrification (Fig. 3.1). Immobilization of N by soil micro-organisms could also have occurred due to spring wheat residue decomposition (Subler *et al.* 1995; Mary *et al.* 1996).

Twenty days after seeding, $\text{N-NO}_3^-_{\text{AEM}}$ measured for SLM applied in spring was significantly higher than for SLM applied in fall and CDM applied in spring. The NO_3^- fluxes were comparable for SLM applied in pre-seeding and NH_4NO_3 at 80 kg N ha^{-1} even though the amount of NO_3^- applied initially was less than 1 kg N ha^{-1} for SLM and 40 kg N ha^{-1} for NH_4NO_3 . This suggests that intense nitrification occurred with SLM treatments. The high NO_3^- fluxes observed in the SLM applied in spring and NH_4NO_3 at 80 kg N ha^{-1} corresponded to the treatments for which it was possible to measure $\text{NO}_3^-_{\text{KCl}}$. Ziadi *et al.* (unpublished data) found in grasslands that $\text{NO}_3^-_{\text{AEM}}$ increased with N fertilizer rates. Paré *et al.* (1995) also observed the highest amount of NO_3^- sorbed on AEMs for treatments receiving N on mineral form (200 kg N ha^{-1} as NH_4NO_3) compared to 560 kg N ha^{-1} applied as stockpiled manure. The fact that we were able to measure fluxes of NO_3^- on AEMs under treatments where we detected nothing with 2M KCl extraction could be attributed to the high capacity of AEMs (2.40 meq g^{-1} dry resin) to retain NO_3^- compared to the soil matrix which let NO_3^- to be leached with infiltrating water or to be denitrified by the anaerobic microflora.

At 30 days after seeding, the significant differences between treatments observed on NO_3^- fluxes were the same as found at 20 days (Table 3.3). The $\text{N-NO}_3^-_{\text{KCl}}$ showed a comparable pattern, but the differences between treatments were less marked (a trend for the difference between SLM and CDM applied in spring and a significant difference at the $\alpha=0.05$ for SLM applied in spring versus SLM applied in fall). The NO_3^- fluxes were on average much lower than at the previous contact period but were larger than in the end of April (Fig. 3.2a, c and e). Paré *et al.* (1995) also reported a decline of $\text{N-NO}_3^-_{\text{AEM}}$ during the growing season. They observed that this decrease was in relation to that of the

$\text{N-NO}_3^-_{\text{KCl}}$. The decline in both NO_3^- extracted by 2M KCl and sorbed on AEM was attributed the plant N uptake that depleted the soil solution in N. In opposition to the finding of Paré *et al.*, the highest $\text{N-NO}_3^-_{\text{KCl}}$ content in the present experiment was observed at 30 days after seeding with a value of 26 kg N ha^{-1} averaged over all treatments. In our case, results suggested that even if mineralization slowed down probably due to the lack of labile C, the NO_3^- was allowed to accumulate since it was not in the period corresponding to the highest N uptake rate by wheat which normally occurs after tillering (Delogu *et al.* 1998).

Mineral N Indices and Wheat N Uptake Relationships

Pearson correlation coefficients clearly indicated that soil N fluxes measured on IEMs were related more closely to wheat N uptake than to soil N mineral pools extracted with 2M KCl (Table 3.4). The highest correlation coefficients between wheat N uptake and soil N measurements were for $\text{NH}_4^+_{\text{CEM}}$ at the end of April ($r=0.45$) when anaerobic conditions were most probably prevailing due to a high gravimetric soil water content (36% w/w) and, $\text{NO}_3^-_{\text{AEM}}$ for contact periods occurring after seeding ($r=0.48$ to 0.49). The gravimetric soil water contents at 20 and 30 days after seeding were of 29 and 24% (w/w). This suggests, that the temporal changes observed in soil water content, may have promoted nitrification at the two last sampling events.

The relationship between $\text{N-NH}_4^+_{\text{CEM}}$ at the end of April and wheat N uptake was linear (Fig. 3.3a). The average NH_4^+ flux was $0.08 \mu\text{g N cm}^{-2} \text{ d}^{-1}$. Although the NH_4^+ flux was lower than that of the NO_3^- , it may be more significant in terms of N that wheat could recover during the growing season. At this period, the pool of exchangeable NH_4^+ , as

estimated by $\text{N-NH}_4^+_{\text{KCl}}$, was the highest with a mean value of 34 kg N ha^{-1} compared to 4 and 8 kg N ha^{-1} measured at 20 and 30 days after seeding, respectively. An increasing body of evidence suggests that release of recently fixed NH_4^+ during a growing season is important in many agricultural soils (Kowalenko and Ross 1980; Drury *et al.* 1991). Other studies have indicated that fixation of added NH_4^+ is much more rapid than its subsequent release due to soil solution depletion in NH_4^+ (Kowalenko 1978; Drury and Beauchamp 1991). Green *et al.* (1994) demonstrated that, in anaerobic conditions, high exchangeable NH_4^+ contents resulted in significant amounts of recently fixed NH_4^+ . They observed the release of exchangeable and fixed NH_4^+ when the shift from anaerobic to aerobic conditions promoted nitrification. This phenomenon may explain why we observed a significant correlation between $\text{N-NH}_4^+_{\text{AEM}}$ measured before seeding and wheat N uptake.

The relationship between $\text{NO}_3^-_{\text{AEM}}$ at 20 days after seeding and wheat N uptake was best described by a quadratic curve (Fig. 3.3d). A significant ($\alpha=0.05$) linear relationship was also found between $\text{NH}_4^+_{\text{CEM}}$ at 20 days and wheat N uptake but the r^2 value was only of 0.11 (Fig. 3.3c). The $\text{NO}_3^-_{\text{AEM}}$ measured at 30 days after seeding was linearly related to wheat N uptake (Fig. 3.3f).

Two weeks after spring fertilization, $\text{N-NO}_3^-_{\text{AEM}}$ correlated significantly ($\alpha=0.001$) with $\text{N-NO}_3^-_{\text{KCl}}$. Pearson correlation coefficients were of 0.72 at 20 days after seeding and 0.66 at 30 days after seeding. Paré *et al.* (1995) and Ziadi *et al.* (unpublished data) reported comparable observations. They concluded that $\text{N-NO}_3^-_{\text{AEM}}$ could be used instead of 2M KCl to measure NO_3^- concentrations in agricultural soils. Under controlled conditions, Subler *et al.* (1995) found that $\text{N-NO}_3^-_{\text{AEM}}$ was highly significantly correlated

with soil NO_3^- concentrations and net nitrification under widely varying conditions of soil N mineralization and immobilization. Our results demonstrate similar conclusions under the conditions prevailing in this study after wheat seeding.

The content of mineral N measured in the plough layer (Magdoff *et al.* 1984; Paul and Beauchamp 1993) 4 to 6 weeks after corn seeding was considered as an effective index of plant N availability that integrates the effects of soil and weather (Magdoff *et al.* 1984). In the second paper of this thesis, we reported the lack of relationship between soil $\text{N-NO}_3^-_{\text{water}}$ measured at 30 days after seeding and wheat N uptake in 1994 and 1995. The Pearson correlation coefficient obtained for soil $\text{N-NO}_3^-_{\text{KCl}}$ and wheat N uptake in 1996 was significant but was smaller than with $\text{N-NO}_3^-_{\text{AEM}}$ (Table 3.4). This suggests that $\text{N-NO}_3^-_{\text{AEM}}$ may be a more reliable index of bioavailable soil N than the other selected measurements under the conditions of 1996 which are specific to our study.

In previous studies, the assumption that IEMs behave as an infinite sink was proven to be wrong for both PO_4^- and NO_3^- as they were found to act rather as dynamic exchanger (Cooperband and Logan 1994; Subler *et al.* 1995). Although this complicated interpretation of nutrient fluxes measured across the sampling interval, many studies found that several ions sorbed on IEMs correlated strongly with plant nutrient uptake (Abrams and Jarrell 1992; Qian *et al.* 1996; Qian and Schoenau 1995). It suggests that ions of a target nutrient sorbed *in situ* on IEMs could represent an average of all its fluxes for the contact period as influenced by factors affecting its bioavailability such as diffusion (Barber 1995) and soil spatial organization (Davidson and Hackler 1994; Strong *et al.* 1997). Our results support that hypothesis.

The wheat N uptake was the highest under SLM applied at seeding with a value of 50 kg N ha^{-1} (Fig. 3.4). Significant differences were found between the SLM treatment and CDM applied at seeding, SLM applied in fall and 80 kg N ha^{-1} applied as NH_4NO_3 (Table 3.5). We attributed this effect to the higher $\text{N-NO}_3^-_{\text{AEM}}$ and $\text{N-NO}_3^-_{\text{KCL}}$ contents observed at 20 and 30 days after seeding compared to other treatments (Fig. 3.2c, d, e and f).

CONCLUSIONS

Inorganic N extracted by neutral salt solutions are the most common measurements used to monitor the release of N during the growing season and to estimate N availability to plants. It is well known that under field conditions, extractable NH_4^+ and NO_3^- soil contents in the plough layer can change rapidly and widely in response to variation in water content, addition of labile organic matter or of organic/inorganic N compounds and plant N uptake. This was observed in the present experiment. Therefore, an intensive sampling strategy over an extended period of time is required to follow adequately the release of manure N. It also appears that, in a climate subjected to cool spring and sporadic episodes of heavy rainfall such as in Quebec and for fine-textured clay soils, an adequate monitoring of the fate of N in the soil should assess fluxes of both NH_4^+ and NO_3^- . Many factors make the IEMs a promising alternative to chemical extraction. The IEMs methodology is simpler and faster than conventional extraction because no soil sampling, preparation and weighing is required. Although the relationship between mineral N sorbed on IEMs and wheat N uptake was not strong, it was better than what we

obtained when we tried to relate wheat N uptake to mineral N estimated with 2M KCl extraction. Furthermore, the greater capacity of IEMs to discriminate between fertilization treatments in regard to their N release could be used to determine when N sources should be applied to synchronize N release to plant N uptake.

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Table 3. 1. Amounts of total C and N added by manure treatments and their relative proportions of N fractions

| Treatments | Dry matter | Total C added | Total N added | Organic N | N-NH ₄ ⁺ | N-NO ₃ ⁻ |
|---------------------------------|------------|---------------------|---------------------|------------|--------------------------------|--------------------------------|
| | % | kg ha ⁻¹ | kg ha ⁻¹ | % | % | % |
| | | | | of total N | | |
| SLM ₁ ^{x,y} | 1.39 | 369 | 133 | 20 | 80 | <1 |
| SLM ₃ | 1.62 | 208 | 133 | 28 | 70 | 2 |
| CDM ₁ | 38.27 | 5866 | 320 | 99 | 1 | 1 |
| CDM ₃ | 37.08 | 5005 | 320 | 89 | 1 | 10 |

^xSLM:., swine liquid manure; CDM, composted dairy manure

^y 1, applied at pre-seeding; 3, after harvest

Table 3. 2. Ionic-exchange membrane characteristics

| Type | Specific weight | Thickness | Water content | Exchange capacity |
|------|---------------------|-----------|---------------|-------------------------------|
| | mg cm ⁻² | mm | % | meq g ⁻¹ dry resin |
| AEM | 13.7 | 0.5 | 46 | 2.40 |
| CEM | 13.7 | 0.56 | 46 | 2.10 |

Table 3. 3. Mean squares of the analysis of variance of treatment effects on mineral N extracted with 2M KCl or sorbed on IEMs

| | df | N-NH ₄ ⁺ CEM | N-NO ₃ ⁻ AEM | N-NH ₄ ⁺ KCL | N-NO ₃ ⁻ KCL |
|---------------------------------------------------|----|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| <i>End of April</i> | | | | | |
| Treatment | 6 | 0.003 | 0.19 | 255 | 8 |
| Block | 3 | 0.013 ^{td} | 0.34 ^{td} | 1213** | 22 ^{td} |
| Error | 18 | 0.005 | 0.13 | 192 | 10 |
| <i>Contrasts</i> | | | | | |
| MIN0 ^x -nc ^z vs MIN0-c | 1 | 0.006 | 0.12 | 133 | 7 |
| SLM ₁ ^y vs CDM ₁ | 1 | 0.001 | 0.02 | 93 | 4 |
| SLM ₃ vs CDM ₃ | 1 | 0.001 | 0.46 ^{td} | 303 | 1 |
| SLM ₁ vs SLM ₃ | 1 | 0.007 | 0.02 | 98 | 0.02 |
| CDM ₁ vs CDM ₃ | 1 | 0.000 | 0.77* | 4 | 11 |
| SLM ₁ vs MIN80 | 1 | 0.007 | 0 | 1 | 9 |
| <i>20 days after seeding</i> | | | | | |
| Treatment | 6 | 0.0007 | 57** | 19 | 16331** |
| Block | 3 | 0.0002 | 0.2 | 56 ^{td} | 1925 |
| Error | 18 | 0.0006 | | 20 | 2011 |
| <i>Contrasts</i> | | | | | |
| MIN0-nc vs MIN0-c | 1 | 0.0003 | 0.002 | 13 | 0 |
| SLM ₁ vs CDM ₁ | 1 | 0.0001 | 60.17** | 0.6 | 57763** |
| SLM ₃ vs CDM ₃ | 1 | 0 | 5 | 34 | 10 |
| SLM ₁ vs SLM ₃ | 1 | 0.0006 | 155** | 14 | 57763** |
| CDM ₁ vs CDM ₃ | 1 | 0.0010 | 6 | 10 | 10 |
| SLM ₁ vs MIN80 | 1 | 0 | 2 | 43 | 57763** |
| <i>30 days after seeding</i> | | | | | |
| Treatment | 6 | 0.002 | 3.71** | 15 | 148 |
| Block | 3 | 0.005 | 0.26 | 70* | 39 |
| Error | 18 | 0.004 | 0.41 | 18 | 121 |
| <i>Contrasts</i> | | | | | |
| MIN0-nc vs MIN0-c | 1 | 0.002 | 0 | 9 | 75 |
| SLM ₁ vs CDM ₁ | 1 | 0.002 | 8.34** | 52 ^{td} | 490 ^{td} |
| SLM ₃ vs CDM ₃ | 1 | 0.003 | 0.05 | 22 | 0 |
| SLM ₁ vs SLM ₃ | 1 | 0.001 | 10.24** | 4 | 611* |
| CDM ₁ vs CDM ₃ | 1 | 0.006 | 0.01 | 0.4 | 1 |
| SLM ₁ vs MIN80 | 1 | 0.001 | 0.41 | 11 | 147 |

^xSLM: swine liquid manure; CDM, composted manure; MIN0, 0 kg applied as NO₃NH₄; MIN80, 80 kg N applied as NO₃NH₄

^y 1, applied at pre-seeding; 3, after harvest

^z-nc, no clover; -c, clover

**significant at $\alpha=0.01$ *significant at $\alpha=0.05$ ^{td}trend at $\alpha=0.20$

Table 3. 4. Pearson correlation coefficients between wheat N uptake and mineral N extracted with 2M KCl or sorbed on IEMs

| | N-NH ₄ ⁺ CEM | N-NO ₃ ⁻ AEM | N-NH ₄ ⁺ KCL | N-NO ₃ ⁻ KCL |
|-----------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| End of April | 0.45** | -0.20 | -0.02 | -0.26 |
| 20 days after seeding | 0.37* | 0.49** | 0.15 | 0.34 ^{td} |
| 30 days after seeding | 0.11 | 0.48** | -0.02 | 0.41* |

**significant at $\alpha=0.01$ *significant at $\alpha=0.05$ ^{td}trend at $\alpha=0.15$

Table 3. 5. Mean squares of the analysis of variance of the treatment effects on wheat N uptake

| | df | ms |
|--------------------------------------|----|-------------------|
| Treatments | 6 | 213** |
| Block | 3 | 96 |
| Error | 18 | 57 |
| <i>Contrasts</i> | | |
| MIN0-nc vs MIN0-c | 1 | 151 ^{td} |
| SLM ₁ vs CDM ₁ | 1 | 238** |
| SLM ₃ vs CDM ₃ | 1 | 74 |
| SLM ₁ vs SLM ₃ | 1 | 873** |
| CDM ₁ vs CDM ₃ | 1 | 31 |
| SLM ₁ vs MIN80 | 1 | 466** |

¹SLM₁: swine liquid manure; CDM₁, composted manure;

MIN0, 0 kg applied as NO₃NH₄; MIN80, 80 kg N applied as NO₃NH₄

²₁, applied at pre-seeding; ₃, after harvest

³-nc, no clover; -c, clover

**significant at $\alpha=0.01$ *significant at $\alpha=0.05$ ^{td}trend at $\alpha=0.20$

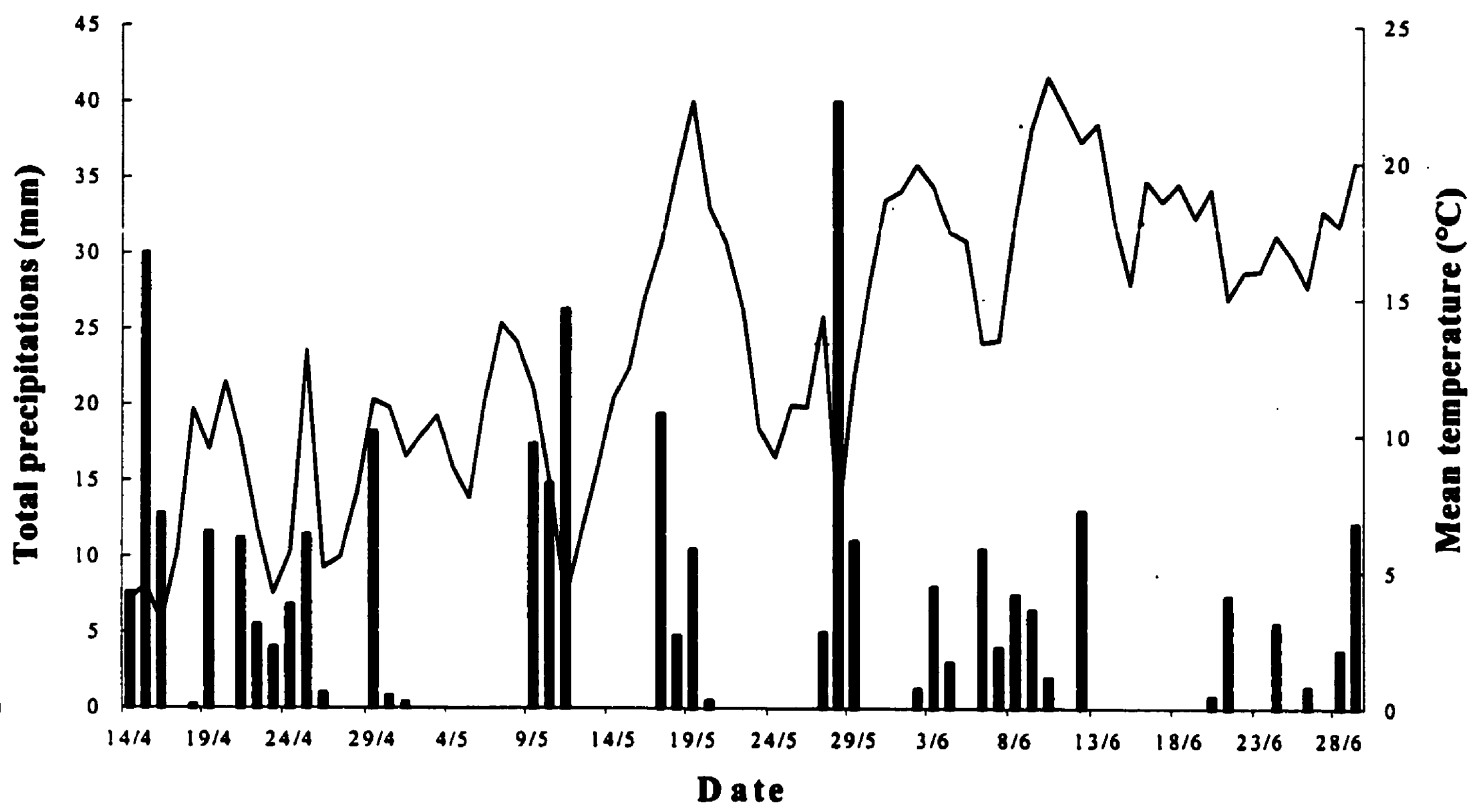


Fig. 3. 1. Total precipitation and average air temperature expressed on a daily basis in spring 1996 at the St. Hubert meteorological station.

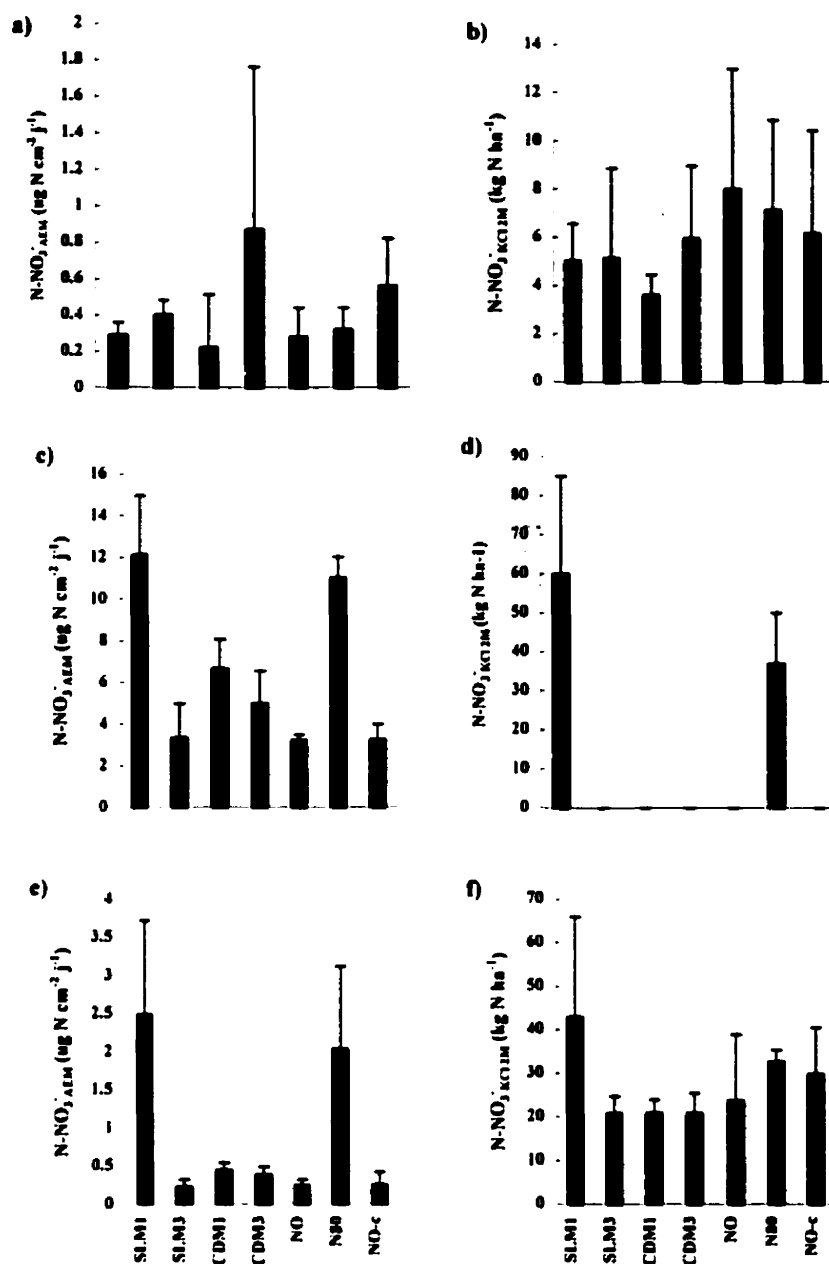


Fig. 3. 2. $\text{N-NO}_3^-_{\text{AEM}}$ measured a) at the end of April, c) at 20 days after seeding and e) at 30 days after seeding and $\text{N-NO}_3^-_{\text{KCl}}$ in the 0–20 cm soil layer b) at the end of April, d) at 20 days after seeding and f) at 30 days after seeding. Treatments applied on spring wheat (*Triticum aestivum* L. cv. Algot) were swine liquid manure (SLM) or composted dairy manure (CDM) applied at pre-seeding (1) or after harvest (3), red clover (*Trifolium pratense* L. cv. Arlington) as companion crop (NO-c), 80 kg N ha⁻¹ applied as NH_4NO_3 in spring (N80) and a control (N0). Each datum bar is the mean of four replicates. Error bars shown are standard errors of the mean.

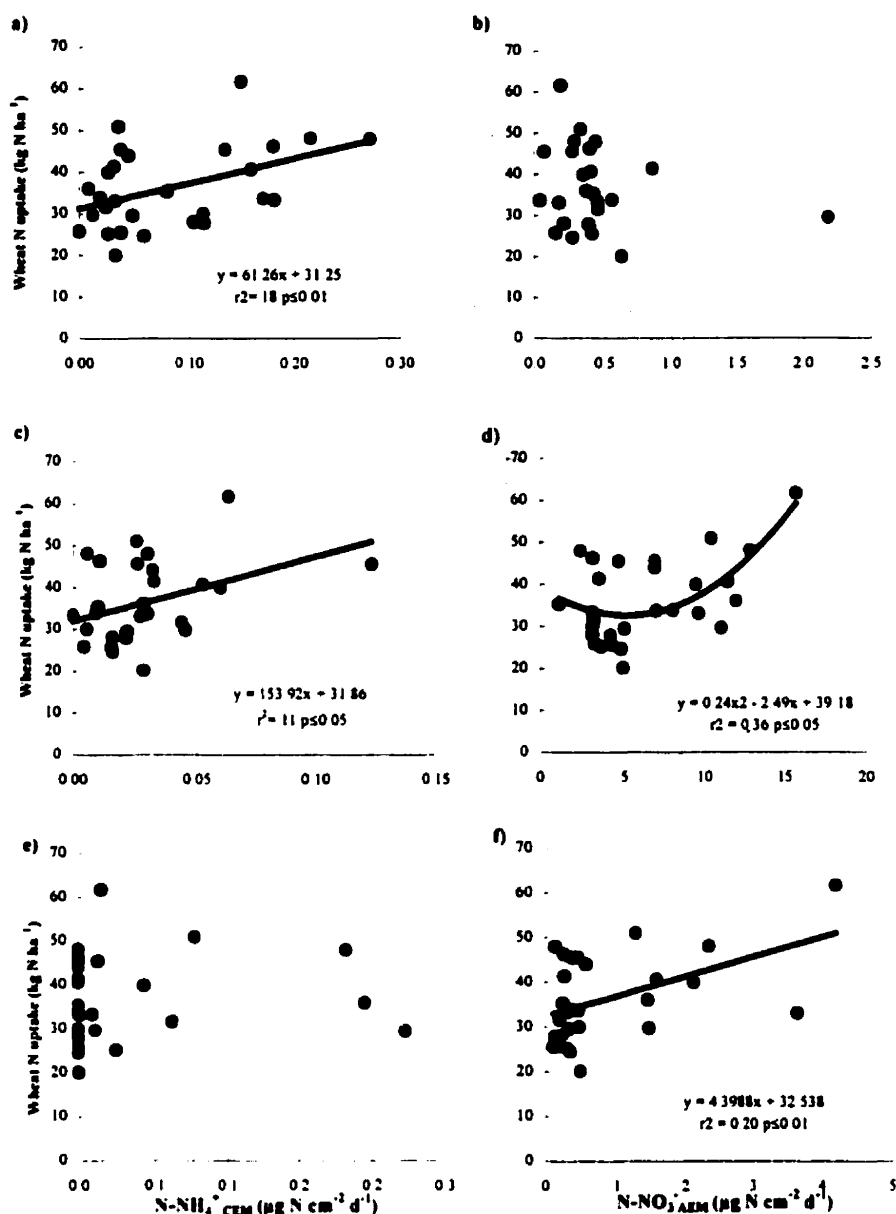


Fig. 3. 3. Nitrogen uptake of spring wheat (*Triticum aestivum* L. cv. Algot) as a function of N-NO₃⁻_{AEM} measured a) at the end of April, c) at 20 days after seeding and e) at 30 days after seeding and N-NO₃⁻_{KCl} in the 0–20 cm soil layer b) at the end of April, d) at 20 days after seeding and f) at 30 days after seeding. Each datum point represents one measurement.

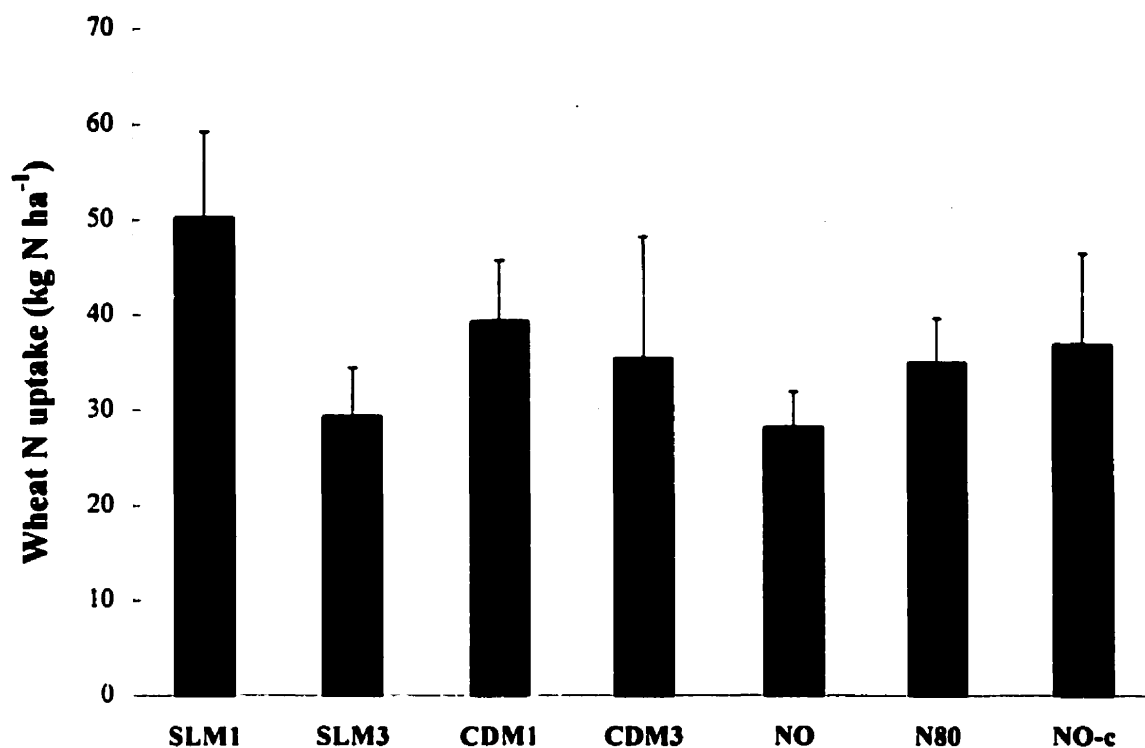


Fig. 3. 4. Nitrogen sources and application time effects on wheat N uptake. Treatments applied on spring wheat (*Triticum aestivum* L. cv. Algot) were swine liquid manure (SLM) or composted dairy manure (CDM) applied at pre-seeding (1) or after harvest (3), red clover (*Trifolium pratense* L. cv. Arlington) as companion crop (NO-c), 80 kg N ha⁻¹ applied as NH₄NO₃ in spring (N80) and a control (N0). Each datum bar is the mean of four replicates. Error bars shown are standard errors of the mean.

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CONCLUSIONS

Summary

In regard to an original contribution to knowledge, the most important conclusions of this study are the following:

1. It was found that in spring wheat (*Triticum aestivum*, L. cv Algot) production, the beneficial effect of an underseeded red clover (*Trifolium pratense* L. cv Algot) ploughed down in late fall as green manure was restricted to N nutritional effects. Nitrogen fertilizer replacement value of clover was 81-85 kg N ha⁻¹. To the author's knowledge, this was the first time that this specific system has been studied in a way that would allow discrimination between non-N and N nutritional effects due to legume incorporation.

2. A red clover cover crop, allowed to grow until late fall after wheat harvest, did not reduce N potential loss risk, as NO₃⁻ measured in the soil profile in fall and in the following spring was equal or higher in the presence of clover than with bare soil. Since the literature was contradictory on the effect of legume cover crop as a scavenger of residual NO₃⁻, this study clarifies this point for the specific system under study.

3. In the presence of red clover, the recovery of N from swine liquid manure and dairy solid manure is diminished by more than 100% because the N supplied by legume and manure is additive. It was known that, in rotational systems, corn yields were less

responsive to manure additions after legume. We confirmed those findings in a cereal-underseeded legume system.

4. While manure application time (pre-seeding, post-emergence or fall) did not significantly affect the apparent N recovery of manures, fall application increased the risk of N transfer to water or atmosphere associated to higher residual NO_3^- soil profile content. The actual status of knowledge on the effects of the application times of manures does not allow generalization to be drawn from available information. In regard to literature reporting very little but conflicting evidence, this study provided information that documents the N recovery of manure N for the specific agroecosystem under study.

5. Fluxes of NO_3^- estimated *in situ* in spring by ionic exchange membranes (IEMs) better discriminated between treatments involving various organic and mineral N sources than N extracted by 2M KCl. Fluxes of NH_4^+ and NO_3^- better related to wheat N uptake than N chemically extracted. To our knowledge, those results are completely new findings. Also, it is the first reported study involving cationic exchange membranes to trap NH_4^+ in field conditions. Our results confirmed that IEMs are a promising alternative to chemical extractants in soil testing.

Conclusions 1 to 4 could be relevant to improve, in a near future, the current practices of N management in spring cereal production in agroecosystems with climatic and pedologic conditions similar to those studied in this project. Although results reported

in conclusion 5 indicated that there is a potential for IEMs to be used in soil testing, but there is much work to be done before it is developed into a useful analytical technique.

Future work

The integration of an estimation of the various pools that replenish the soil solution in N during the growing season and of their dynamics would improve the actual Quebec system of agronomic recommendations. A valid approach should rely on a limited number of *in situ* data. A budget or a model approach too complex and requiring an intensive strategy of data collection cannot be applied currently. Ionic exchange membrane methodology is less labour intensive than chemical extractions and the information provided could possibly integrate many factors influencing nutrient availability such as diffusion and soil spatial organization. Their possible use in a simple model which allows prediction of available N deserves to be investigated.

Among the pools that can be very significant in regard to plant N availability are labile organic N and fixed ammonium. Very little is known about the latter and, traditionally, was considered as being unimportant in terms of plant N nutrition. Soils of the St. Lawrence lowlands, which contain a significant amount of vermiculite, can fix the ammonium fraction in the interlayer space of phyllosilicates and then release it as the soil solution is depleted in NH_4^+ . The importance of this pool should be assessed in terms of N nutrition. This could be particularly significant for the fine-textured gleysolic soils which are reduced for extended periods each spring.

APPENDIX A

Candidates have the option of including, as part of the thesis, the text of one or more papers submitted or to be submitted for publication, or the clearly-duplicated text of one or more published papers.

If this option is chosen, connecting texts that provide logical bridges between the different papers are mandatory. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.

The thesis must still conform to all other requirements of the "Guidelines for Thesis Preparation". The thesis must include the following: a table of contents; an abstract in English and French; an introduction which clearly states the rationale and objectives of the research, a comprehensive review of the literature (in addition to that covered in the introduction of each paper); a final conclusion and summary.

Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. Supervisors must attest the accuracy of such statements at the doctoral

oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all authors of the co-authored papers.