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MANIPULATING NITROGEN RELEASE FROM VEGETABLE CROP RESIDUES BY USE OF ON- AND OFF-FARM ORGANIC WASTES

Thesis submitted in fulfillment of the requirements
For the degree of Doctor (PhD) in Applied Biological Sciences: Land Management and Forestry

Dutch translation of the title:
BEINVLOEDING STIKSTOFVRIJSTELLING UIT OOGSTRESTEN VAN GROENTEN DOOR AANWENDING VAN BEDRIJFS- EN NIET-BEDRIJFSGEBONDEN ORGANISCHE RESTSTOFFEN

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Voorwoord

Op een dinsdagmorgen ergens in oktober 2001 kreeg ik tijdens mijn ontbijt (en met mijn mond vol cornflakes) een telefoontje van Stefaan met de boodschap dat ik op 1 november 2001 mocht ik beginnen werken aan de vakgroep Bodembeheer en -hygiëne. Blijkbaar had ik Stefaan tijdens het sollicitatiegesprek overtuigd met een groot enthousiasme om een doctoraat te schrijven …

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Table of contents

Symbols and abbreviations

BD	Bulk density
C	Carbon
C_{tot}	Total C content
Cell	Cellulose fraction
CD	Coefficient of determination
CRM	Coefficient of residual mass
C:N	Carbon-to-Nitrogen ratio
$C:N_{WS,\ Cell,\ H,\ L}$	C:N ratio of the specific fraction
DM	Dry matter
EF	Modelling efficiency
FC	Field capacity
FM	Fresh matter
H	Hemicellulose fraction
κ	Temperature dependence parameter
k_i	mineralization/immobilization rate constant corrected for the temperature at day *i*
k_{org}	Organic N mineralization rate constant
k_{opt}	N mineralization/immobilization rate constant at optimum temperature
k_{tot}	Total N mineralization rate constant
k (T)	N mineralization/immobilization rate constant as a function of temperature
L	Lignin fraction
L:N	Lignin-to-Nitrogen fraction
N	Nitrogen
NMIT	N mineralization-immobilization turnover
N_{tot}	Total N content
$N_{tot}(t)$	Amount of total N mineralized at time t
$N_{A,tot}$	Amount of potentially mineralizable total N
N_{org}	Organic N content

$N_{org}(t)$	Amount of organic N mineralized at time t
$N_{A,org}$	Amount of potentially mineralizable organic N
N_{min}	Mineral N content
$N_{WS, H, Cell, L}$	N content in the specific fraction
ΔN_i	N mineralization at day *i*
OM	Organic matter
PP	Polyphenol content
R	Correlation coefficient
SAT	Gravimetric moisture content at saturation point
TSOC	Total soluble organic carbon
T	Temperature
T_{opt}	Optimum temperature
Δt	one day.
WFPS	Water-filled pore space
WP	Gravimetric moisture content at wilting point
WS	Water-soluble fraction

Chapter 1

General introduction

1.1 N losses from agriculture

During the last 50 years, European agriculture has shown a trend towards greater intensification and higher productivity accompanied by a significant increase in fertilizer use, especially inorganic fertilizers. Inorganic N use reached a peak of 11 millions tons annually in the mid 1980s before falling to approximately 9-10 millions tons more recently (Anonymous, 2002b). Animal numbers increased during most of this period, contributing to a greater overall N burden through manure. In general, the N pressure on EU agricultural soils from animal husbandry (mainly cows, pigs, poultry and sheep), is approximately 8 millions tons annually spread on agricultural soils, and the total diffuse N pressure (livestock manure, mineral fertilizers, atmospheric deposition, biological fixation) reaches almost 18 millions tons (Anonymous, 2002b).

Due to the trends in this 50 years period, the N losses from agriculture to the environment significantly increased. Agriculture contributes to a large extent to the environmental problems in Europe and is considered as the major cause of the enrichment of soil, water and air with nutrients and soil acidification in Flanders (Table 1.1). Growing public concerns about the environment have forced farmers to produce in a more ecological and sustainable manner. One of the major points of action is the reduction of nutrient losses from agriculture, especially of N. The most important N losses from agriculture are NH_3 volatilization, NO_3^- leaching and denitrification (N_2, N_2O, NO_x) (Fig. 1.1).

Table 1.1 Contribution (%) of different target groups to environmental problems in Flanders (Vandeweerd, 1999; Van Steertegem, 2000)

Environmental impact	Agriculture	Energy supply	Industry	Population	Traffic and transport
Acidification	31	18	27	8	16
Enrichment of soil, water, air with nutrients	66	-	20	10	4
Greenhouse effect	12	27	28	18	15

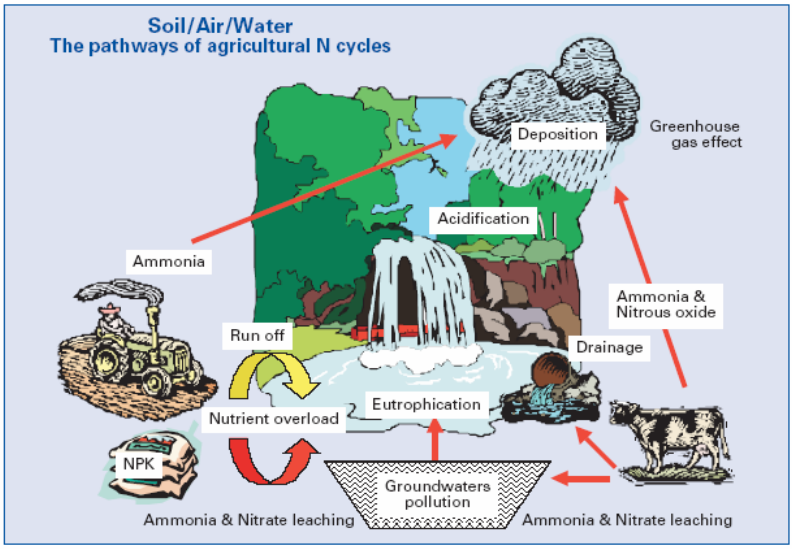

Fig. 1.1 The agricultural N air, soil and water exchanges and possible impacts on the environment (Anonymous, 2002b)

For many EU countries, about 90% of the total emission of NH_3 to the atmosphere comes from agriculture (Pain and Misselbrook, 1997; Berckmans et al., 1998). Intensive livestock production systems are the single largest contributors to N loss through volatilization from agriculture because of the quantities of manure produced (De Clercq et al., 2001), but also mineral N fertilizers can be a source of NH_3 volatilization. The application of liquid animal manure or inorganic fertilizer to soils will increase the potential for volatilization depending on the method of application to land. NH_3 losses from ammonium nitrate fertilizers spread on a calcareous clay soil can be as high as 37% of the added N (applied amount 200 kg N ha^{-1}), while the losses are almost completely reduced when these fertilizers are incorporated at 2 cm depth (losses reduced to 4% of added N) or 4 cm depth (losses reduced to 0.5% of added N) (Demeyer, 1993). NH_3 lost from soil ecosystems to the atmosphere is generally re-deposited onto soil or aquatic ecosystems. The increasing density of livestock with concomitant manure storage and manure

spreading during the last 50 years has resulted in very large NH_3 volatilization, and atmospheric deposition on neighbouring soils and waters with values up to 50-60 kg of nitrogen per hectare per year being recorded in regions with intensive livestock activities (Anonymous, 2002b).

Gaseous N losses, especially N_2O and NO, are also important pollutants since they enhance acidification, contribute to global warming and affect ozone concentrations. N_2 has no direct negative impact on the environment, but the conversion of reactive N into N_2 means an economical loss of N. Agriculture is responsible for almost half of the anthropogenic N_2O emissions (EPA, 2001; IPCC, 2001). In soil two processes - nitrification and denitrification - can lead to the release of N_2O. Denitrification is the microbial reduction of NO_3^- to NO_2^- to NO to N_2O to N_2. Since the bacteria responsible for denitrification are facultative anaerobic, denitrification is more likely to occur in poorly drained, fine-textured soils and in situations with high water tables where anaerobic conditions are more likely to be present. Nitrification is the oxidation of NH_4^+ to NO_3^-, and occurs under aerobic conditions. Agricultural management has a major influence on N_2O emission through for example fertilizer applications, livestock waste handling, residue management or operations affecting structure, aeration and pH of soils. Agricultural N_2O emissions derive from three principal sources: direct emissions from soil nitrogen (e.g. applied fertilizers, N mineralization from soil organic matter and crop residues), emissions from livestock wastes in store and indirect emissions from N lost to the agricultural system (e.g. through leaching, runoff, and atmospheric deposition) (IPCC, 1996). Annual N_2O emissions from arable lands (coarse- and middle-textured) in Belgium range from 0.3 to 1.5 kg N ha^{-1} y^{-1} (0.3 to 1.0% of the fertilizer N applied), while emissions from intensively managed grasslands can range from 14 to 32 kg N ha^{-1} y^{-1} (3 to 11% of fertilizer N applied) (Goossens et al., 2001). Also crop residues are an important source of N_2O, especially easily decomposable N-rich crop residues (Aulakh et al., 1991; Baggs et al., 2000a). Total N_2O emissions from white cabbage, Brussels sprouts, mustard, sugar beet and broccoli residues can range from 0.13 to 14.6% of the added residue-N (Velthof et al., 2002).

Another major diffuse pollutant that is sourced from agriculture is NO_3^-, which, due to its high solubility, is easily leached from the soil to both ground and surface waters and leads to contamination of these waters and eutrophication of surface waters. There is considerable evidence that in the past two decades, the NO_3^- concentration in Western European ground and surface waters has increased as a result of agricultural practices (VMM, 1998; De Clercq et al., 2001; Dunn et al., 2004). The average N concentrations in the measuring points of the Belgian Manure Action Plan (MAP) Measuring Network for surface waters in Flanders during the period June 1999 - November 2000 are shown in Fig. 1.2. During that period, 59% of the measuring points exceeded the EU norm of 11.3 mg NO_3^--N l^{-1} in Flanders. Recently, the number of measuring points exceeding the EU norm dropped, but during July 2003 and May 2004, still 44% of the measuring points exceeded the EU norm of 11.3 mg NO_3^--N l^{-1} in Flanders (VMM, 2004). N losses out of the rooting zone are highly influenced by the soil type/texture and structure, rainfall volumes and patterns, agronomic management and the amount and distribution of residual mineral N in the soil profile (De Clercq et al., 2001). Additions of N from inorganic and organic fertilizers above the requirements for optimum production and/or incorporation of crop residues add to the potential for N leaching losses. Fig. 1.2 shows that intensive horticulture, open air as well as glass-covered vegetable production, has a problem with NO_3^- leaching which is illustrated by high concentrations in Mid West-Flanders during June 1999 and November 2000 (74% of the measuring points exceeded the EU norm of 11.3 mg NO_3^--N l^{-1} in West-Flanders during June 1999 and November 2000; VMM, 2004). More recently, during July 2003 and May 2004, still 72% of the measuring points exceeded the EU norm of 11.3 mg NO_3^--N l^{-1} in West-Flanders (VMM, 2004).

Runoff, or overland flow, is the amount of precipitation in excess of infiltration and evapotranspiration which can transport materials away from fields into surface and water systems. Transport of soil particles (organic as well as inorganic) by wind or water, i.e. erosion, can also remove nutrients associated with soil particles. Compared to N drainage losses, losses through

surface run-off and erosion are rather small and limited to certain regions with topography and soil types sensitive to erosion. De Cooman et al. (1994) made an estimation of these losses for all hydrographical basins in Flanders and came up with a maximum annual loss of 10 kg N ha^{-1}.

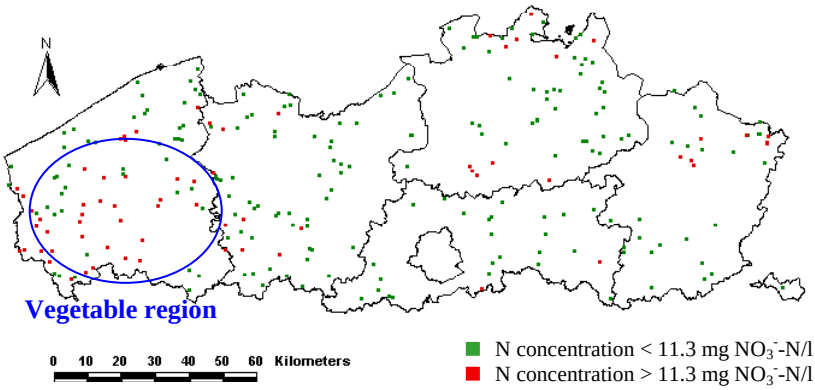

Fig. 1.2 Average NO_3^- concentrations in surface water for the measuring points of the Belgian Manure Action Plan (MAP) (June 1999 - November 2000) (VMM, 1998)

1.2 Intensive vegetable production and N losses

Intensive field vegetable production in Flanders (Belgium) is located mostly on soils of high chemical fertility, what in combination with an excessive use of organic N fertilization or slurry, results in high soil N mineralization rates and high NO_3^- concentrations in soil after harvest (autumn). N mineralization rates from 0.9 up to 1.6 kg N ha^{-1} day^{-1} have been found for soils used for vegetable production in Flanders (Demyttenaere, 1991). Furthermore, vegetables leave large amounts of crop residues on the field after harvest (Table 1.2). The amount of crop residues varies according to the type of crop, but for some important crops, like cabbages, it can go up to several dozens of t ha^{-1} (Rahn et al., 1992). The average N content of these crop residues is high and varies in general between 2 and 4% on a dry matter basis. Upon

mineralization of these crop residues, mineral N amounts up to 150 kg N ha^{-1} can be released into the soil (De Neve and Hofman, 1998), resulting in large NO_3^- concentrations, and potential problems with respect to N losses, in particular NO_3^- leaching and gaseous N losses.

Table 1.2 Fresh matter left on the field as crop residues and the N content in crop residues for a number of field grown vegetables (Scharpf, 1991; De Neve en Hofman, 1993)

Crop residues	Fresh matter (ton ha^{-1})	N content (kg N ha^{-1})
Brussels sprouts	50-60	140-200
White cabbage (processing)	40-50	170
Broccoli		180
Chinese cabbage		100
White cabbage (fresh)	30-40	170
Cauliflower		130
Fennel		90
Peas		85
Beans		90
Carrots	20-30	90
Celery		110
Iceberg lettuce		85
Leek	10-20	60
Spinach		30
Lettuce	< 10	35

The present Belgian Manure Action plan (MAP) states that the NO_3^- content in soil (from 0 to 90 cm), between the 1st of October and the 15th of November, must be below 90 kg N ha^{-1}. This threshold is often exceeded after the growth of vegetables first of all because vegetables have a high latent mineral N residue (Table 1.3). The latent mineral N residue is the amount of mineral N remaining in the rooting zone at the moment of maximum N uptake by the crop and this is considered as a mineral N buffer necessary to secure an optimum growth (Schlaghecken, 1986). The latent mineral N residue after vegetables is mostly high because of several reasons: the rooting depth of most

vegetables is limited to 60 cm or shallower and the rooting density is less dense than for many arable crops, and therefore, the mineral N available in deeper soil layers can not be taken up and the profile becomes less depleted. Furthermore, a number of vegetables is harvested before maturation, on one hand, and can leave a large amount of crop residues on the field after harvest, on the other hand. In the policy-supported N-(eco)² project (Anonymous, 2002a) research was done on the relation between the NO_3^- content in soil at harvest (between the 1st of October and 15th of November) and the amount of NO_3^- leached during winter, in order to evaluate the present NO_3^- standards (both European Nitrate Directive and Belgian MAP) and to adapt and differentiate them for different crops and soil types. The (eco)²-project showed that in future the NO_3^- problem will become even more acute for vegetables, for example, the permitted NO_3^- norm in soil on the 1st of October for cauliflowers grown on a coarse-textured soil without the removal of crop residues will be maximum 40 kg N ha^{-1} (Anonymous, 2002a). Even with a very precise and well-timed fertilization, it will be almost impossible to comply with these standards. This would threaten the future production of some economically very important vegetables such as cauliflower and leek.

Table 1.3 Latent mineral N residue in soil after harvest of some vegetables (Salomez et al., 1995)

Vegetable	Sampling/rooting depth (cm)	Latent mineral N residue (kg N ha^{-1})
Spinach	90	75
Celery	60	100
Peas	30	50
Cauliflower	60	75
Carrots	60	50
Leek	60	60
Beans	30	60
Celeriac	60	50
Cabbage	90	50
Brussels sprouts	90	25

Despite the fact that the intensive vegetable production in Flanders occupies only a limited part of the total area of agricultural land, economically it is a very important sector (Table 1.4). The production value per hectare is much larger for vegetable growing than for arable farming. Approximately 15% of the total production value of agriculture in Flanders originates from vegetable production, corresponding to ca. 700 millions of euro, exclusive the value of the vegetable processing industry (VOLT, 2003). To secure the future of vegetable growing and its processing industry in Flanders, there is an urgent need for methods to reduce the soil mineral N concentrations at harvest.

Table 1.4 Production value of the different agfricultural sectors in Flanders in 2002 (VOLT, 2003)

Agricultural sector	Production value (million euro)
Arable farming	425
Horticulture	1359
Vegetables	675
Fruit farming	297
Livestock	2720
Total	4504

Several strategies could be considered for reducing N losses after the harvest of vegetable crops. Catch crops can reduce mineral N contents in soil after harvest. However, the dry matter yield and N uptake of catch crops depends on the length of the growth period and the prevailing temperatures, and their N uptake efficiency is low when they are sown later then the end of August (Sörensen, 1992; Ninane et al., 1994; Geypens and Honnay, 1995; Fig. 1.3). Therefore, catch crops often give poor results when sown after vegetables harvested from September on.

Removal of crop residues from the field is another option to avoid N losses from crop residues. However, removal of crop residues has several drawbacks such as extra labour and machinery costs for the farmer and an increased risk

of soil compaction if residues would be removed in wet conditions (autumn). Furthermore, in many agro-ecosystems in Flanders, a loss of organic matter is observed with detrimental effects on soil physical and biological properties. A regular supply of crop residues may help to maintain soil fertility and contribute to the preservation of a stable soil structure and to the reduction of soil erosion (Blevins and Frye, 1993). Finally, if the N in crop residues could be preserved until the next spring, this N could be taken into account in the N balance which would allow a reduction of the fertilizer N rates and improve economic returns (Rahn et al., 1992).

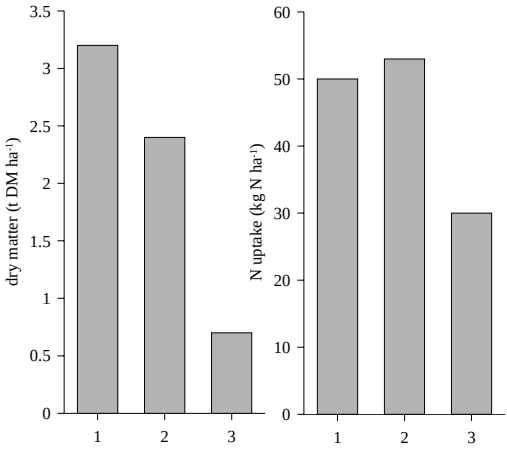

Fig. 1.3 Dry matter and N uptake of white mustard sown in the middle of August (1), at the end of August (2) and in the middle of September (3) (Ninane et al., 1994; Geypens and Honnay, 1995)

A possible alternative method to reduce mineral N concentrations in soil is manipulating the N release from N-rich crop residues by the addition, in the field, of other organic materials, and the main objective of this work was to examine this method as a possible management option (see 1.4).

1.3 N transformations during decomposition of crop residues in soil

In order to manipulate the N release from crop residues, a good understanding of the decomposition and N release from crop residues is needed. When crop residues are incorporated in soil, they will be colonized by micro-organisms, which will decompose the residues and use the crop residue-C and -N for energy and biomass production. During decomposition C and N cycles are strongly linked. The C assimilation rate depends on the rate of decomposition of plant materials and the assimilation yield of the decomposed C by the microflora (Mary et al., 1996). The N assimilation requirements are then determined by this C-flow and the C:N ratio of the decomposers (Mary et al., 1996; Fig. 1.4).

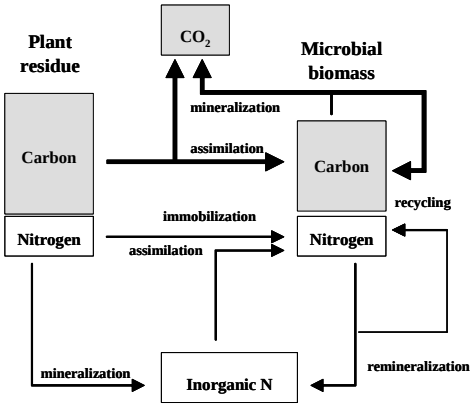

Fig. 1.4 Flow chart of C and N transformations during initial decomposition of crop residues in soil; thick lines: C fluxes; thin lines: N fluxes (Mary et al., 1996)

During the decomposition of crop residues, a continuous N mineralization-immobilization turnover occurs in soil, i.e. the continuous transfer of mineralized N into organic components and of immobilized N into inorganic components, as a result of cycling through the microbial biomass. Net N mineralization is the balance between gross N immobilization and gross N

mineralization. When the incorporated crop residues contain a large amount of N, the N that is not needed for biosynthesis will be released as mineral N into soil (i.e. *net N mineralization*). However, if the crop residues contain a small amount of N, below the threshold of the microbial requirement for biosynthesis, the micro-organisms will use mineral N present in soil (i.e. *net N immobilization*). The critical C:N ratio, i.e. the break point between net N mineralization and net N immobilization, depends on the length of the incubation period (larger when time considered is longer), but in general the incorporation of organic materials with a C:N ratio larger than 20-40 promotes net N immobilization (Iritani and Arnold, 1960; Fox et al., 1990; Vigil and Kissel, 1991).

The first step in the N mineralization process of crop residues is ammonification or the formation of NH_4^+ from organic N forms, and this involves a large diversity of micro-organisms and occurs under a wide range of temperatures, pH and moisture contents (Hansen et al., 1995). The second step is nitrification or the oxidation of NH_4^+ to NO_2^- followed by the oxidation of NO_2^- to NO_3^-. The first reaction is carried out by *Nitrosomonas sp*. and the second reaction by *Nitrobacter sp*., and these micro-organisms are active in a much more narrow range of temperature, pH and moisture content.

N mineralization of crop residues is influenced by soil biological and chemical properties, temperature, moisture and the nature and chemical composition of the crop residues.

Important (bio)chemical characteristics in relation to the N release of crop residues are the N content and C:N ratio (Fox et al., 1990; Vigil and Kissel, 1991). Other important characteristics are the content of water-soluble compounds, hemicellulose, cellulose, lignin which determine the degradability of the crop residues, and hence, the N mineralization/immobilization rate (De Neve and Hofman, 1996; Bending et al., 1998). Polyphenolic compounds in some crop residues can reduce the N mineralization rate (Palm and Sanchez, 1991; Oglesby and Fownes, 1992; Constantinides and Fownes, 1994) since

they are toxic for several micro-organisms, including bacteria, fungi and microfauna involved in N mineralization, and since they have a strong protein binding capacity through their strong affinity for amide groups (Scalbert, 1991; Capasso, et al. 1995; Hewlett et al., 1997). Other residue properties, like residue particle size, can also determine the rate of decomposition. Chopped or ground residues are more accessible to micro-organisms than intact plant parts due to the increased surface area of the residues exposed to decomposition (Angers and Recous, 1997) and the lack of intact lignified barrier tissue (Summerell and Burgess, 1989). Therefore, this speeds up the initial colonization rate of the residues and increases the N release.

Several environmental factors also influence the N release from crop residues, but soil moisture content and temperature are the two most important ones. Under the climatological conditions of the study area, and considering the time of incorporation of crop residues of vegetables (September-December), the influence of the soil moisture content on the N mineralization is more or less negligible, since N mineralization has a wide plateau of optimal moisture contents (Cassman and Munns, 1980; Goncalves and Carlyle, 1994) and the moisture contents at that time usually fall within the plateau. On the other hand, at the time of incorporation, the occurring temperatures are rather low and may have a crucial influence on the N mineralization. However, several studies showed that the decomposition and N mineralization of easily decomposable N-rich crop residues can still be substantial at low temperatures (<5°C) (Breland, 1994; Van Scholl et al., 1997). The N mineralization rate of recalcitrant crop residues is more limited by low temperatures than of easily decomposable crop residues (Fig. 1.5; De Neve et al., 1996; Andersen and Jensen, 2001).

Finally, also soil-related factors, including soil texture, pH and salinity, have an effect on N mineralization from crop residues. Crop residues incorporated in coarse-textured soils generally release more N than in fine-textured soils (Hassink, 1993; Thomsen et al., 2001). However, the soil-related factors are

less important than the biochemical composition of crop residues and environmental factors.

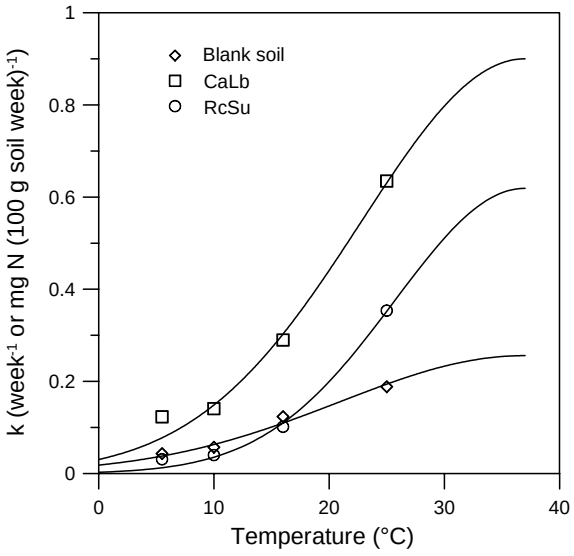

Fig. 1.5 Gaussian temperature model fitted to the N-mineralization rate constants of an unamended loamy sand and two types of crop residues: easily decomposable cauliflower leave blades (CaLb) and the more recalcitrant upper part of red cabbage stems (RcSu) (De Neve et al., 1996)

1.4 Manipulating the N release from N-rich crop residues

The main objective of this thesis was to examine whether manipulating the N release from crop residues by mixing them with organic wastes is a good alternative management option to reduce NO_3^- concentrations in soil after the harvest of vegetables. Manipulating the N release from N-rich crop residues by using organic wastes includes two phases: a N immobilization phase and a remineralization phase, which are initiated by the addition of a suitable organic material to the soil. The organic materials can include on-farm wastes, such as straw, and off-farm wastes from agricultural industries, mostly food industries, which can be used as amendments in agriculture on the condition that they contain no or small tolerable amounts of toxic compounds.

When the crop residues are incorporated in autumn, the N immobilization phase is initiated by the addition of an organic waste that should either immobilize the N released from the crop residues or delay the N mineralization, and hence, protect the crop residue-N from leaching. Organic wastes with a potential to immobilize N from crop residues will hereafter be referred to as *immobilizer wastes.* Their immobilization potential will mainly depend on their (bio)chemical composition like a high C:N ratio, a high lignin and/or a high polyphenol content, since these parameters have proved to be indicators for N immobilization or decreased N mineralization (Fox et al., 1990; Palm and Sanchez, 1991; Vigil and Kissel, 1991, Oglesby and Fownes, 1992; Constantinides and Fownes, 1994; Bending et al., 1998).

The remineralization phase should start in spring, some time before a new crop is sown or planted, by the incorporation in soil of another type of organic waste with the intention to stimulate remineralization of immobilized N (i.e. priming effect). This type of organic wastes will hereafter be referred to as *remineralizer wastes.* Their remineralization potential could be linked to a high content of easily decomposable C, since it has been shown that addition of C in the form of sugars can lead to a marked increase in soil microbial activity (Falih and Wainwright, 1996).

If we are able to find effective combinations of immobilizer and remineralizer wastes, we might further enhance the synchronization between the N release from crop residues and the crop N demand, and hence, increase the N use efficiency.

1.5 Objectives

In order to be able to manipulate the N release of crop residues, a quantitative knowledge of the N mineralization of crop residues is needed. For most vegetables, a good knowledge of the amounts of above ground crop residues left on the field and their N mineralization is available (Iritani and Arnold, 1960; De Neve et al., 1994; De Neve and Hofman, 1996). However, much less

information is available on the N mineralization of roots (Frankenberger and Abdelmagid, 1985; Wivstad, 1999; Malpassi et al., 2000), especially of vegetable roots. Therefore, the aim of the first study was to develop a predictive dynamic relationship for the N mineralization of vegetable root residues and green manures (*Chapter 2*).

An experiment was set up to screen a large number of organic wastes for their potential to immobilize crop residue-N (straw, two green waste composts, saw dust, paper sludge and tannic acid) or to remineralize immobilized residue-N (molasses, vinasses, malting sludge, dairy sludge) (C*hapter 3*).

An increase in N_2O emission may occur when immobilizer wastes are incorporated together with N-rich crop residues as compared to crop residues alone due to the large availability of NO_3^--N released from the crop residues in combination with a large amount of easily decomposable C from the immobilizer wastes. In this case, the increase in N_2O emissions would partially offset the beneficial effect of reduced NO_3^- leaching. Knowledge concerning the N_2O emissions is therefore crucial before the use of organic wastes can be further stimulated. Therefore, the effect of mixing crop residues with immobilizer wastes on the N_2O emission from soil was tested under controlled conditions (*Chapter 4*).

Incorporating ^{15}N labelled material in soil allows to track the N released from the celery residues into the different N fractions (Müller and Sundman, 1988; Jensen et al., 1997). To increase the understanding of the mode of action of the immobilizer or remineralizer wastes, a laboratory experiment where ^{15}N labelled celery residues were incorporated together with organic wastes, was set up (*Chapter 5*). This information may help to explain why certain organic wastes have got an effect on N mineralization-immobilization turnover (NMIT) of crop residues and others have not.

For the incubation experiments, the calculations for the amounts of applied organic material were based on the area i.e. from 1 ha to the area of the PVC tube (= 16.8 cm^2) or to the area of the plastic containers (= 1320 cm^2).

In the following studies, different organic wastes were tested for their potential to manipulate the N release of N-rich crop residues under field conditions. The first field experiment was set up on a silt loam, lasted one year, and the main purpose was to screen the suitability of several organic wastes (straw, saw dust, green waste compost and paper sludge as immobilizer wastes and dairy sludge and vinasses as remineralizer wastes) under field conditions (*Chapter 6*).

The above experiments do not give answers on questions such as what is the effect of texture on the N immobilization and remineralization potential of organic wastes? Can continuous incorporation of immobilizer wastes lead to more NO_3^- leaching on the long-term due to a build-up of relatively labile organic N? What is the effect of manipulating the N release of crop residues on the following crop? To answer those questions, additional field experiments were set up in different soil textures (silt loam, sandy loam, loamy sand) and lasting two years (*Chapter 7*).

Incorporating ^{15}N labelled crop residues together with organic wastes under field conditions may reveal some of the mechanisms that take place in soil during the decomposition and may help to explain some unexpected phenomena observed in the previous experiments. In a final field experiment, ^{15}N labelled celery residues were incorporated in soil together with straw and vinasses, and the distribution of celery-^{15}N over different N fractions in soil was followed (*Chapter 8*).

Chapter 9 gives the general discussion and *Chapter 10* the summary and conclusions.

Chapter 2

N mineralization of vegetable root residues and green manures as related to their (bio)chemical composition

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Chaves B, De Neve S, Hofman G, Boeckx P, Van Cleemput O (2004) Modelling the N mineralization of vegetable root residues and green manures using their (bio)chemical composition. Eur J Agron 21: 161-170.

Abstract Little is known concerning the N mineralization or N immobilization of (vegetable) root residues in soil. The (bio)chemical composition of a range of vegetable root residues (large and fine roots of red cabbage, white cabbage, Brussels sprouts, savoy cabbage, leek), and of plant parts of two green manures (ryegrass, white mustard) was determined by standard chemical analysis (total C, total N, C:N ratio) and by a modified Stevenson fractionation method (water-soluble, hemicellulose, cellulose, lignin fraction). Fresh chopped crop residues, homogenously mixed with a sandy loam soil, were incubated for 119 days at constant temperature (21°C) and constant moisture content (14% w/w). The net N mineralization of each residue was determined by destructive sampling. All fine roots, except these of Brussels sprouts, showed a net N release throughout the incubation. All large roots showed a slight N immobilization at the start of the incubation, but at the end of the incubation a small net N release was observed, except for Brussels sprouts. The roots of Brussels sprouts immobilized N throughout the entire incubation. The leaves of the green manures released more N than both stems and roots. It was possible to fit a first-order kinetics model to eight of the fourteen N mineralization patterns: $N(t) = N_A(1 - \exp(-kt))$, with N_A the maximum amount of mineralized N and k the mineralization rate constant. For the six other residues where the first-order model could not be fitted, the amount of N mineralized at the end of the incubation was taken as an estimate of the N_A parameter. Both the mineralization parameters N_A and k were correlated to a large number of (bio)chemical parameters. The amount of mineralized N, N_A, was best correlated with the C:N ratio ($R = -0.86$), and the rate constant k was best correlated with the lignin:N ratio ($R = -0.94$). The predictive relationship between the N mineralization of vegetable root residues and green manures and their (bio)chemical composition has the advantage that is independent of the length of the incubation time. The critical C:N ratio, i.e. the break point between net N mineralization and net N immobilization ($N_A = 0$) was found to be 36.6.

2.1 Introduction

Nitrogen present in roots has often been ignored in the N economy of cropping systems, because they are thought to contain only small amounts of N (10 to 15% of the total plant-N as determined in physical excavation studies) (Kumar and Goh, 2000). However, recent ^{15}N studies involving crop and pasture legumes, suggested that only a small proportion of roots may be physically recovered (Kumar and Goh, 2000), so that the proportion of plant-N contained in roots is in fact larger, up to 40% of total plant-N (Rochester et al., 1998). Furthermore, in some studies involving the N contribution of cover crops to a following crop (Harris and Hesterman, 1990; Thomsen, 1993), the specific N contribution of roots seemed of some importance. The recovery of N released from rye roots (as catch crop) in the subsequent crop (barley) was more than one third of the recovery of N released from rye leaves in barley (respectively 13% and 35%) (Thomsen, 1993). The recovery of N released from alfalfa roots (as catch crop) in the subsequent crop (corn) was almost the same as the recovery of N released from alfalfa leaves in corn (respectively 14% and 19%) (Harris and Hesterman, 1990). This indicates that the N mineralization of roots may be of importance and should be studied in more detail.

An important factor governing the N mineralization of crop residues is their (bio)chemical composition (e.g. N content, C:N ratio, water-soluble, hemicellulose, cellulose, lignin and polyphenol content). Many studies have tried to relate the N mineralization of crop residues to their (bio)chemical composition, but most of this research dealt with the major agricultural crops, legumes and green manures (Fox et al., 1990; Palm and Sanchez, 1991; Vigil and Kissel, 1991; Constantinides and Fownes, 1994; Quemada and Cabrera, 1995; Trinsoutrot et al., 2000). Some research has been done on the N mineralization of above ground vegetable crop residues (Iritani and Arnold, 1960; De Neve et al., 1994; De Neve and Hofman, 1996). However, much less information is available on the N mineralization of roots (Frankenberger and Abdelmagid, 1985; Wivstad, 1999; Malpassi et al., 2000), especially of vegetable roots.

Most researchers established a static relationship between the (bio)chemical composition and the amount of N mineralized at the end of the incubation period. However, differences in incubation time lead to differences in amounts of net N mineralized, so that the length of the incubation also affects the relation with the (bio)chemical composition. Therefore a dynamic relationship (considering incubation time) between the (bio)chemical composition and the N mineralization of crop residues is more valuable. The N mineralization of crop residues in function of time can be often described by a first-order kinetics model: $N(t) = N_A(1 - \exp(-kt))$.

The aim of this study was to develop a predictive dynamic relationship for the N mineralization of vegetable root residues and green manures under fixed environmental conditions by fitting a first-order kinetics model to the N mineralization patterns of the crop residues first and then relating their (bio)chemical composition to the model parameters.

2.2 Materials and methods

Crop residues

The crop residues chosen for this study were fresh vegetable root residues from red cabbage (*Brassica oleracera* convar. *capitata* var. *rubra*), white cabbage (*Brassica oleracera* convar. *capitata* var. *alba*), Brussels sprouts (*Brassica oleracera* var. *gemmifera*), savoy cabbage (*Brassica oleracera* convar. *capitata* var. *sabauda*) and leek (*Allium porrum* L.), and two green manures, ryegrass (*Lolium perenne* L.) and white mustard (*Sinapis alba* L.). The cabbage roots were separated in fine (diameter < ca. 1 cm) and large roots (diameter > ca. 1 cm), and the green manures into leaves, stems and roots. The reason for this division was the expected difference in the (bio)chemical composition and the net N mineralization of the different roots and plant parts.

Samples of the plant material were dried at 55 °C until constant weight for the determination of the dry matter content, and were then ground for further chemical analysis. The total C and N contents were determined using a CNS

elemental analyser (Variomax CNS, Elementar, Germany). The water-soluble, hemicellulose (+ structural protein), cellulose (+ structural protein) and lignin (+ structural protein) fractions were determined by Stevenson fractionation as modified by De Neve and Hofman (1996). For each fraction obtained by the Stevenson fractionation, the C and N content (CNS elemental analyser) and the ash content (combustion for 4h at 450°C) were determined. For the extraction of inorganic N in the plant material, 60 ml KCl (*1N*) was added to 5 g of fresh plant material (ratio KCl:plant material 1:12), this solution was shaken for one hour, filtered, and analysed for NO_3^--N and NH_4^+-N with a continuous flow auto-analyser (Chemlab System 4, Skalar, The Netherlands).

Soil

The soil used in the incubations was the top layer (0-20 cm) of a sandy loam soil collected at Beitem (West-Flanders, Belgium) after the harvest of Brussels sprouts. The soil contained 9.1% clay, 36.1% silt and 54.9% sand, had a pH_{KCl} of 5.7, a total N content of 0.066%, a C content of 0.74% a NO_3^- content of 2.7 mg N kg^{-1} and a NH_4^+ content of 1.7 mg N kg^{-1}. The soil was sampled at a moisture content of 19% (w/w) (79% of field capacity (FC)), which was too high for the incubations. Therefore the soil was allowed to dry to a moisture content of 10% (w/w) (42% of FC). During the drying period, visible impurities (e.g. stones, roots) were removed from the soil. The soil was not sieved and not air-dried in order to minimize the disturbance of the microbial activity.

Incubation experiment

For the incubation, fresh soil (equivalent to 283 g dry soil) was mixed thoroughly with 6 g of fresh chopped crop residues (equivalent to 36 t fresh matter (FM) ha^{-1}) and incubated in PVC tubes (diameter = 4.63 cm; filling height = 12 cm; bulk density = 1.4 g cm^{-3}). After filling the tubes, distilled water was added to obtain a moisture content of 14% (w/w) (58% of FC) and the tubes were covered with a single layer of gas permeable parafilm to minimize water loss. The tubes were incubated for 119 days at constant

temperature (21°C). The reason for this rather high temperature, compared to soil temperatures at the time of incorporation of crop residues, was to ensure that mineralization and immobilization of the vegetable root residues would be more pronounced and more accurately measurable.

In total, there were fourteen incubation treatments with crop residues and one control treatment (containing only soil). The sampling was destructive, by removing tubes in triplicate for each treatment at each sampling date. Samples were taken 10, 21, 36, 48, 69, 92 and 119 days after the start of the incubation. The soil was removed from the tubes and mineral N (NO_3^--N + NH_4^+-N) was extracted by adding 60 ml of (*1N*) KCl to 30 g of fresh soil (ratio KCl:soil 1:2) and this extract was analysed with a continuous flow auto-analyser (Chemlab System 4, Skalar, The Netherlands).

Data analysis

The net N mineralization of the crop residues (as % of total N) was calculated as the difference between the amounts of inorganic N released in the amended soil and those released in the unamended soil, divided by the total amount of crop residue-N added.

The (bio)chemical composition of the large and fine vegetable roots were compared with each other using a variance analysis (One-Way ANOVA, SPSS). Fitting of a single first-order kinetics model to the net N mineralization was performed using non-linear regression (Levenberg-Marquardt algorithm) in SPSS. A correlation analysis of the (bio)chemical parameters with the first-order model parameters was done using a Pearson's correlation matrix and a stepwise multiple linear regression.

2.3 Results

(Bio)chemical composition

The fourteen crop residues had a large variation in (bio)chemical composition (Table 2.1 and 2.2).

Table 2.1 Chemical characteristics of the vegetable root residues and green manures

Crop residue	DM	Ash	C_{tot}	N_{tot}	N_{min}	C:N ratio
	%	% DM		g kg^{-1} DM		
Large vegetable root residues						
RC	23.3	12.0	442.0	14.3	0.026	30.9
WC	26.5	10.1	458.1	13.3	0.016	34.5
BS	32.3	15.7	433.1	9.3	0.007	46.5
SC	29.8	7.6	448.0	14.8	0.020	30.3
Fine vegetable root residues						
RC	22.1	26.4	370.6	17.2	0.046	21.6
WC	27.0	32.3	320.2	15.4	0.024	20.7
BS	31.5	39.8	289.4	8.5	0.024	33.8
SC	25.2	20.6	415.0	20.3	0.008	20.5
L	11.2	19.3	398.4	35.7	0.012	11.2
Green manure leaves						
RG	21.2	28.4	395.7	29.7	0.98	12.1
WM	16.7	36.0	354.9	37.7	1.46	9.4
Green manure stems						
WM	17.0	14.4	398.4	20.8	1.99	19.2
Green manure roots						
RG	15.9	29.0	384.1	16.9	0.97	22.7
WM	20.1	13.2	450.1	10.6	1.20	42.5

RC: red cabbage; WC: white cabbage; BS: Brussels sprouts; SC: savoy cabbage; L: leek; RG: ryegrass; WM: white mustard; DM: dry matter; Ash: ash content; C_{tot}: total C content; N_{tot}: total N content; N_{min}: mineral N content

Table 2.2 Results of the biochemical fractionation (in %OM), N content (% of total N) and C:N ratio of the different fractions

Crop residue	WS	N_{WS}	$(C:N)_{WS}$	H	N_H	$(C:N)_H$	Cell	N_{Cell}	$(C:N)_{Cell}$	L	N_L	$(C:N)_L$	L:N
Large vegetable root residues													
RC	15.6	48.6	3.8	35.2	32.7	37.2	7.5	9.2	28.4	41.7	9.5	150.1	29.2
WC	16.0	50.8	6.2	39.1	32.2	42.9	26.1	2.7	80.5	18.8	4.3	170.5	14.2
BS	21.4	38.6	12.2	32.3	37.8	42.1	30.5	18.6	92.5	15.8	5.0	172.6	17.0
SC	16.3	35.4	12.0	37.6	45.7	23.3	7.9	8.7	26.6	38.2	10.2	128.1	25.8
Fine vegetable root residues													
RC	20.8	47.3	6.6	33.9	38.3	18.9	9.4	6.0	46.2	35.9	8.4	99.5	20.9
WC	21.5	42.9	2.3	33.3	35.7	27.2	8.0	4.6	25.3	37.2	8.0	109.2	24.2
BS	20.2	11.4	58.2	40.0	66.1	16.0	6.7	2.0	12.4	33.1	10.6	142.5	38.9
SC	30.3	53.2	12.3	35.5	32.6	18.9	24.3	11.6	48.8	10.0	2.7	79.0	4.9
L	28.4	65.1	4.0	36.6	27.1	14.4	15.9	2.8	79.2	19.1	5.1	48.8	5.4
Green manure leaves													
RG	19.2	18.9	9.1	36.3	59.4	6.5	25.0	15.4	24.7	19.6	6.4	43.0	6.6
WM	24.0	29.0	8.6	44.2	52.9	6.5	6.6	8.0	7.8	25.2	10.1	28.1	6.7
Green manure stems													
WM	10.0	36.6	2.9	32.2	38.5	15.6	29.3	17.4	35.4	28.5	7.6	78.6	13.7
Green manure roots													
RG	17.6	27.5	22.2	32.4	50.3	11.8	27.4	14.1	37.1	22.6	8.1	67.7	13.4
WM	5.7	23.0	3.0	37.2	41.5	40.8	4.6	18.7	26.2	52.6	16.8	119.3	49.6

-WS = water-soluble; -H = hemicellulose; -Cell = cellulose; -L = lignin; L:N = lignin:N ratio; See Table 2.1 for further abbreviations

The total N content varied from 8.5 g kg^{-1} dry plant material to 37.7 g kg^{-1} and the C:N ratio varied from 9.4 to 46.5. Mineral N contents in the vegetable roots were very small (always less than 0.3% of total N) whereas the mineral N contents were larger in the green manure plant parts (between 3 and 12% of total N). The (bio)chemical fractions resulting from the Stevenson fractionation, also varied largely among the residues, except the hemicellulose fraction, which was relatively constant (varying between 30 and 45% of OM (organic matter)). The water-soluble and hemicellulose fraction contained the major part of N in all residues. The C:N ratio was, in most residues, the lowest in the water-soluble fraction and the highest in the lignin fraction (Table 2.2).

The (bio)chemical composition of the fine roots differed from the composition of the large roots. The fine roots had significantly lower C:N ratios of the hemicellulose fraction ($P< 0.01$), lower C contents ($P< 0.05$), lower C:N ratios ($P< 0.05$), higher water-soluble contents ($P< 0.05$) and lower C:N ratios of the lignin fraction ($P< 0.05$) than the large root residues. The different (bio)chemical composition of the large roots of the cabbages is due to the fact that they have a more woody structure than the roots of most other annual crops. The roots of most annual plants have a N content varying between 2% and 3% on dry matter, a C:N ratio of ca. 13 and lignin contents varying between 8 and 30% (Frankenberger and Abdelmagid, 1985; Bending et al., 1998; Wivstad, 1999). Compared to these roots, the large cabbage roots in this study had a lower N content (< 1.5% DM), a higher C:N ratio (30 – 47) and a higher lignin content (16 to 42% OM). Also the plant parts of the green manures differed in their (bio)chemical composition: the green manure leaves had higher N contents and lower C:N ratios than both stems and roots.

N mineralization

In general, nitrification was very rapid and only trace amounts of NH_4^+ were found during the incubation. In the following, the net N mineralization is presented in terms of the sum of NH_4^+-N and NO_3^--N.

The net N mineralization or N immobilization curves of the vegetable root residues as a function of time are presented in Fig. 2.1 (a,b). The large differences in mineralization pattern between the large and fine roots indicated that it was useful to study them separately, which was also suggested by the results of the (bio)chemical analysis. The large cabbage root residues showed a small immobilization period during the first 48 to 92 days, and only at the end of the incubation net N mineralization occurred, except for Brussels sprouts, where N immobilization continued during the entire incubation period. The fine cabbage root residues released N during the whole incubation period, except for Brussels sprouts. The N release from the leek roots started very rapidly: after 21 days already 41.6% of the total N was released (by day 119: 50% of total N).

The N mineralization of the green manures is given in Fig. 2.1 (c,d). From day 21 till day 48, the ryegrass leaves released significantly more N than the roots. This was due to an initial lag phase in the N release of the ryegrass roots. The N release from the white mustard leaves was significantly larger than from its stems at all sampling dates (except at day 92), and also significantly larger than from its roots (except after 21 days). In general, the N release from the roots was small during the whole incubation period.

The net N mineralization of the fourteen crop residues at the end of the incubation (in % of total N) is summarized in Table 2.3. All roots showed a net mineral N release, except the roots of Brussels sprouts. There was a clear difference between the large roots, where less than 15% of total N was mineralized after 119 days, and the fine roots which released between 20 and 25% of total N. Leek roots released most N (50% of total N). However, in absolute terms under field conditions, the total amount of N released by the leek roots will not differ much from the cabbage roots, because for leek the amount of root residues left on the field after harvest is far less than for cabbages.

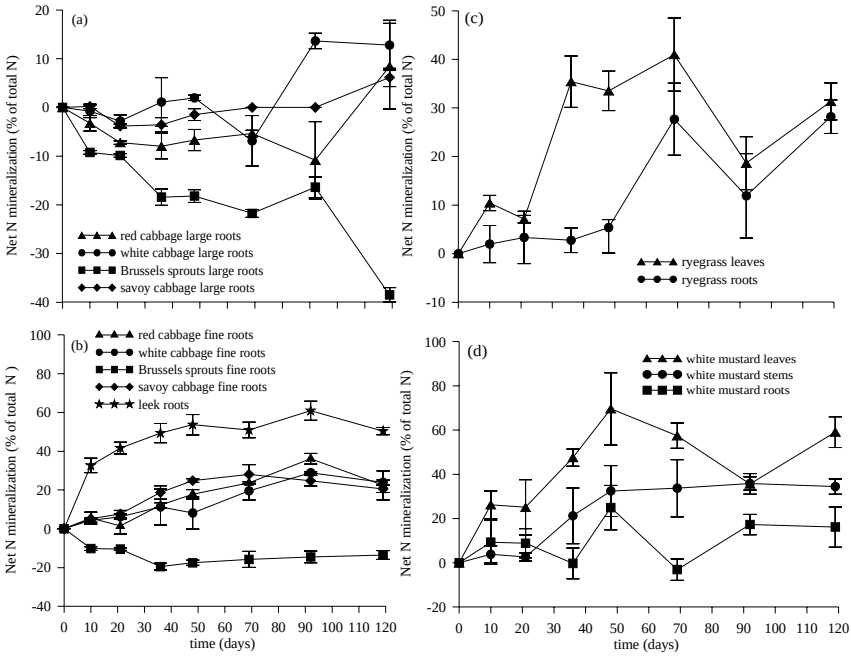

Fig. 2.1 Net N mineralization of the vegetable root residues and green manures: (a) large vegetable root residues; (b) fine vegetable root residues; (c) ryegrass leaves and roots and (d) white mustard leaves, stems and roots; error bars are standard deviations

We tried to fit a single first-order kinetics model to the net N mineralization patterns of the crop residues:

$$N_{tot}(t) = N_{A,tot}(1-\exp(-k_{tot}t)) \qquad (2.1)$$

in which $N_{tot}(t)$ is the percentage of total N mineralized at time t; $N_{A,tot}$ is the amount of potentially mineralizable total N (% of total N) and k_{tot} is the first-order N mineralization rate constant (day^{-1}). The results of the curve fitting are given in Table 2.3. A negative value of $N_{A,tot}$ means that net N immobilization occurred. The first-order model could not be fitted to six of the fourteen crop residues, namely large roots of red, white and savoy cabbage, fine roots of Brussels sprouts and roots of ryegrass and white mustard. The reason was

either a period of immobilization preceding the net N mineralization, or a very small net N mineralization (initial lag phase) during the first 48 days. For these six residues, the N mineralization at the end of the incubation (119 days) was used as an alternative estimate for the parameter $N_{A,tot}$ and no value of the k_{tot} parameter could be estimated.

Table 2.3 Observed net N mineralization (%N_{min}) of the crop residues 119 days after the incorporation (in % of total N) and the parameters of the first-order model fitted to the N mineralization data; standard error of parameter estimates between brackets

Crop residue	%N_{min}	k_{tot} day^{-1}	$N_{A,tot}$ % of N_{tot}	R^2
Large vegetable root residues				
RC[b]	8	-	8.0 (9.0)	-
WC[b]	13	-	13.0 (5.0)	-
BS	-38[a]	0.013 (0.006)	-39.7 (10.4)[a]	0.751
SC[b]	6	-	6.0 (2.0)	-
Fine vegetable root residues				
RC	22	0.012 (0.007)	38.4 (11.9)	0.759
WC	24	0.007 (0.006)	50.1 (31.6)	0.763
BS[b]	-14[a]	-	-13.5 (2.3)[a]	-
SC	21	0.033 (0.008)	26.0 (2.1)	0.835
L	50	0.082 (0.010)	53.8 (1.4)	0.937
Green manure leaves				
RG	31	0.044 (0.017)	32.4 (3.7)	0.620
WM	59	0.054 (0.017)	54.9 (4.6)	0.679
Green manure stems				
WM	34	0.017 (0.008)	43.6 (9.3)	0.742
Green manure roots				
RG[b]	28	-	28.2 (3.4)	-
WM[b]	16	-	16.2 (9.2)	-

[a] negative data indicates N immobilization; [b] first-order kinetics model was not a good fit: $N_{A,tot}$ is the percentage N mineralized at day 119 (between brackets: standard deviation); as a result k_{tot} could not be estimated; k_{tot} = rate constant for mineralization of total N; $N_{A,tot}$ = potentially mineralizable N (in % of total N); see Table 2.1 for abbreviations

Relationship between N mineralization and (bio)chemical composition

To find correlations between the net N mineralization of crop residues and their (bio)chemical composition, the model parameters $N_{A,tot}$ and k_{tot} were first plotted against either single (bio)chemical parameters or combinations of these parameters (data not shown). These plots revealed linear ($Y = AX + B$) and curvilinear ($Y = AX^B$) relations. Because it is possible to transform curvilinear equations into linear equations ($\ln Y = \ln A + B \ln X$), the correlations were further examined through a Pearson correlation matrix and linear regression (Table 2.4). The amount of mineralizable nitrogen $N_{A,tot}$ and the rate constant k_{tot} were correlated to a large number of (bio)chemical parameters, but only those parameters significant at $P< 0.01$ are given in Table 2.4. The parameter $N_{A,tot}$ was best correlated with the C:N ratio (correlation coefficient $R= -0.86$). Stepwise multiple linear regression was used to test whether additional parameters could improve the regression, but no significant improvement in the prediction of $N_{A,tot}$ could be obtained. The net N mineralization rate constant k_{tot} had both linear and curvilinear relationships with the (bio)chemical parameters. The best correlation was a curvilinear relationship with the lignin:N ratio ($R= -0.94$). Also here stepwise multiple linear regression could not add any other parameter to improve the prediction of k_{tot} significantly.

Substitution of the regression equations of the parameters $N_{A,tot}$ and k_{tot} in the first-order model (Eq. 2.1) yielded following relationship for predicting the N mineralization of vegetable roots and green manures:

$$N_{tot}(t) = [-2.03\ C{:}N + 74.2][1 - \exp(-(0.44\ (L{:}N)^{-1.23})t)] \qquad (2.2)$$

where $N_{tot}(t)$ is the percentage of N mineralized at time t, C:N is the overall C:N ratio of the crop residue, and L:N is the ratio of lignin (in % of organic matter) over total N content (in % of dry matter).

Table 2.4 Pearson correlation coefficients and the linear or curvilinear regression equations between net N mineralization parameters and the (bio)chemical composition; only correlations significant at *P*< 0.01 are shown

Parameter	Biochemical component	Correlation coefficient (R)		Regression equations
$N_{A,tot}$	C:N ratio	-0.86	linear	-2.03 C:N + 74.2
(% of N_{tot})	$(C:N)_L$	-0.79	linear	-0.45 $(C:N)_L$ + 69.1
	N_{tot}	0.73	linear	21.2 N_{tot} – 17.4
	N_{org}	0.73	linear	22.2 N_{org} – 18.2
k_{tot}	L:N	-0.94	curvilinear	0.44 $(L:N)^{-1.23}$
(day^{-1})	N_{org}	0.92	linear	0.025 N_{org} –0.023
	N_{tot}	0.89	linear	0.023 N_{tot} – 0.021
	WS x N_{WS}	0.84	linear	0.001 (WS x N_{WS}) – 0.006

N_{tot} = total N content (% DM); N_{org} = organic N content (% DM); L = lignin; WS = water-soluble fraction (% of OM); N_{ws} = N content of the water-soluble fraction (% of OM).

2.4 Discussion

Most research on roots has been limited to roots of cover crops, since those are considered as being a potential source of N for the following crop (Frankenberger and Abdelmagid, 1985; Bending et al., 1998; Wivstad, 1999; Malpassi et al., 2000). These researchers found N releases ranging from 16% of total N for alfalfa roots (Frankenberger and Abdelmagid, 1985) to 25-30% of total N for clover roots and rye roots (Frankenberger and Abdelmagid, 1985; Bending et al., 1998; Wivstad, 1999) for a temperature range from 15°C to 25°C and for a period from 4 to 8.5 months. All these studies reported a net N release from roots at the end of the incubation period, but some roots had an initial immobilization period, which lasted from 6 days (Bending et al., 1998) up to 6 weeks (Frankenberger and Abdelmagid, 1985). The results obtained in this study for the fine roots of cabbages, the green manure roots and the leek roots were in the same range as the results reported by these previous studies (N releases between 20 and 50% of total N). However, the N release from the large cabbage roots was somewhat less (< 15% of total N). These large roots showed also a much longer initial period of N immobilization (7 to 13 weeks)

than found in previous studies. The patterns of N immobilization of the Brussels sprout roots found here (both large and fine roots), are in contradiction with the results from Bending et al. (1998), who reported an immobilization period of 6 days followed by a net N mineralization of 50% of total N. However, the Brussels sprout roots used by Bending et al. (1998) were roots from a much younger crop (growth period of only four months compared to seven months in this study) and they had a higher N content (2.8% DM), a lower C:N ratio (12.5) and a lower lignin:N ratio (10) compared to the Brussels sprout roots used in this study (N content < 1% DM; C:N = 34-47; lignin:N = 17-39). However, entering the data of Bending et al. (1998) into our relationship gave a correct prediction of the amount of N mineralized from the roots after a period of 6 months, namely ca. 50% of total N.

So far, the large and fine roots of the cabbages were considered separately. However, under field conditions, the N mineralization of all the roots together is of importance. The division of the roots in large and fine roots has been determined, and was on average 63% large roots and 37% fine roots for red cabbage, white cabbage and savoy cabbage, and 75% large roots and 25% fine roots for Brussels sprouts (fresh weight basis). Since the large and fine roots had a different N content, the N content must also be considered when calculating the aggregate N mineralization. Therefore, the N mineralization of the fine and large roots was weighed by the fraction of total N they contained. The overall N mineralization of the complete roots is shown in Table 2.5.

For the roots of red, white and savoy cabbage a net N release was observed during the whole incubation period (except after 21 days for red cabbage), and after 119, days 13% to 17% of total N was released. The complete roots of Brussels sprouts immobilized N during the whole incubation period and after 119 days the total N immobilization was equivalent to 33% of total N added. It should be noted that a temperature of 21°C, as was used here, is higher than the real temperature at the time of incorporation of crop residues (autumn). This means that the actual rate of N release (or N immobilization) from the root residues under field conditions will be lower than found here.

Furthermore, although the complete aboveground part of the plant is removed, the roots can still further develop in soil when no soil cultivation has taken place yet. This means that they are not decomposed yet and that they will not release significant amounts of N.

Table 2.5 Cumulative net N mineralization of the total cabbage roots (large + fine roots) (in % of total N) at each sampling date

Time days	Red cabbage	White cabbage	Brussels sprouts	Savoy cabbage
0	0.0	0.0	0.0	0.0
10	0.4	1.3	-9.5	2.4
21	-3.6	0.9	-10.0	1.3
36	0.4	5.2	-18.7	6.4
48	3.5	4.5	-18.1	10.3
69	6.7	3.8	-20.4	14.9
92	8.6	19.8	-15.9	14.5
119	14.2	17.3	-32.7	12.6

A relationship between the (bio)chemical composition and the net N mineralization of crop residues is limited when it is established after a fixed time, since differences in incubation time affect the amount of N mineralized and thus the relationships with the (bio)chemical composition. Describing the N mineralization of crop residues by a simple mathematical model in function of time first (e.g. by a single first-order model), and then relating the model parameters with the (bio)chemical composition overcomes this problem. Mineralization patterns of crop residues with a low C:N ratio often follow the course of a single first-order model (Eq. 2.1) (Stanford et al., 1973; Frankenberger and Abdelmagid, 1985; Kirchmann and Bergqvist, 1989; De Neve and Hofman, 1996). However, the N mineralization patterns from materials with relatively wide C:N ratios can show an initial immobilization period or a lag phase followed by net N mineralization, and then the first-order model does not give a good fit, as was observed here. More sophisticated mathematical functions may be able to describe the N mineralization patterns

with a lag phase, for example the Gompertz model (Ellert and Bettany, 1988), however, more parameters need to be estimated then which may be difficult when the mineralization data is highly variable. Also the N mineralization patterns with an initial immobilization period followed by a net N release cannot be satisfactory described by any simple function. Since no simple mathematical function was available to describe all the N mineralization patterns found in this study, we used the first-order kinetics model, although it is not optimal, because of its simplicity and the easy parameter fitting.

The N mineralization process is not only influenced by time, but also by environmental factors such as temperature, soil moisture content and soil bulk density. It has to be noted that the predictive relationship found here, can only be used under the same experimental conditions as in this study.

According to this study, the C:N ratio was the best predicting parameter for the potential amount of N that can mineralise from a crop residue ($N_{A,tot}$). The C:N ratio at the break point between net N mineralization and net N immobilization, the so called critical C:N ratio, was obtained by equating $N_{A,tot}$ = – 2.03 C:N + 74.2 to zero, and yielded 36.6. This is a relatively high value, corresponding to critical C:N ratios found by Janzen and Kucey (1988) and Vigil and Kissel (1991) for long-term incubations.

2.5 Conclusions

In general, root residues showed a net N release after a period of four months, except the roots of Brussels sprouts (N immobilization). The large roots of cabbages released maximally 13% of their N and the N released from fine roots was between 20 and 25% of total N. The overall N mineralization (large + fine roots) was on average 15% of total N for the cabbage roots and between 16% and 28% of total N for the green manures roots. Assuming that the amount of roots left on the field is 36 t fresh matter ha^{-1}, the amount of N released will be between 12.4 and 27.3 kg N ha^{-1} for cabbage and green manure roots. Leek roots released 50% of total N, but the actual amount of N

released from leek roots will be comparable to cabbages and green manures since the actual amount of roots left on the field will be far less. Under actual field conditions the N release from roots will be even lower due to lower temperature, etc., and compared to above ground residues, the N release from vegetable root residues will be negligible. Hence, the contribution of vegetable root residues to NO_3^- leaching after the harvest of vegetables is probably limited.

Equation 2.2 is a predictive relationship between the N mineralization of vegetable root residues and green manures under fixed environmental conditions and their (bio)chemical composition, namely their C:N ratio and lignin:N ratio. The relation has two strengths. First, the relationship is independent of the length of the incubation because a first-order kinetics model was fit to the N mineralization data first, and then the (bio)chemical composition was linked to the model parameters. Secondly, the vegetable root residues and green manures differed largely in their (bio)chemical composition and their N mineralization patterns. Hence, we expect that the relationship may have some predictive value for other crop residues not included in this study.

Chapter 3

Screening organic wastes for their potential to manipulate the N release from N-rich vegetable crop residues in soil under laboratory conditions

Parts of this chapter were published in:

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Abstract In a laboratory study, organic wastes were screened for their potential to manipulate the N release from vegetable crop residues in two phases: an immobilization and remineralization phase. During the immobilization phase, celery leaves (*Apium graveolens* L.) were mixed with an *immobilizer waste* (straw, two green waste composts (compost 1 and compost 2), saw dust, paper sludge, tannic acid) in order to immobilize N released from crop residues. During the remineralization phase, the treatments received a *remineralizer waste* (malting sludge, vinasses, molasses, dairy sludge) in order to stimulate remineralization of immobilized N. Straw showed the most pronounced N immobilization (on average 30.2 mg N kg^{-1}). N immobilization with tannic acid, saw dust and compost 2 was slower and less pronounced (on average 16.4, 15.9 and 8.0 mg N kg^{-1}, respectively). Compost 1 and paper sludge immobilized almost no N. Only when compost 1 was mixed with vinasses, remineralization was observed (up to 55.4 mg N kg^{-1}) during a 30 day period. For all other remineralizer wastes, positive priming effects were scarce and short-lived. Manipulating the N release of N-rich crop residues may be a suitable method to reduce the NO_3^- concentration in soil after incorporation of crop residues. Especially easily decomposable wastes (i.e. low lignin content) with a large C:N ratio seem to have a potential to immobilize N. However, stimulating remineralization of immobilized N seems not easy to accomplish.

3.1 Introduction

A possible method to reduce mineral N concentrations in soil is manipulating N mineralization of N-rich crop residues, i.e. simultaneously mixing of crop residues with on- and off-farm organic wastes which will either immobilize N released from crop residues or delay N mineralization (= N immobilization potential). Organic wastes include e.g. urban waste composts, composts from on-farm organic residues or green waste (hedge trimmings, grass clippings, …) or wastes from agricultural industries, mostly food industries, which can be used as amendments in agriculture on the condition that they contain no or small tolerable amounts toxic compounds. There is evidence that certain organic wastes have a N immobilization potential (*immobilizer wastes*). Rahn et al. (2003) found that the mineral N content was lower in a soil where sugar beet leaves or Brussels sprouts leaves were mixed with straw or compactor paper waste compost compared to soil with only sugar beet or Brussels sprouts leaves. De Neve et al. (2004) showed that next to materials with a high C:N ratio such as straw and green waste compost, also materials with a high polyphenol content, like tannic acid, can immobilize N from crop residues, and that the period of N immobilization depends on the type of added material.

An additional advantage would be achieved if remineralization of immobilized and preserved N could be stimulated by the time a new crop is sown or planted in the following spring by incorporating a second organic waste (*remineralizer waste*). This would further enhance synchronization between N release and crop N demand. Several authors have observed an extra N release (i.e. priming effect) from soil organic matter after the incorporation of fresh organic matter, like glucose (Asmar et al., 1994; Wheatly et al., 2001), shoots of *Lolium perenne* (Kuzyakov et al., 1999) and sugar beet disks (Falih and Wainwright, 1996). Rahn et al. (2003) found that N mineralization of sugar beet leaves and Brussels sprouts leaves mixed with molasses doubled after 56 and 112 days, respectively. The addition of molasses caused a remineralization of 73% of the

N initially immobilized in combination with a green waste compost of a high C:N ratio (De Neve et al., 2004).

The aim of this study was a systematic screening of different organic wastes under laboratory conditions for their potential to either immobilize N released from N-rich crop residues (*immobilizer wastes*) or to stimulate remineralization of immobilized N at a time when crop N demand starts again (*remineralizer wastes*).

3.2 Materials and methods

Crop residues and organic wastes

The N-rich crop residues and the organic wastes used in the incubations are presented in Table 3.1. Celery residues (*Apium graveolens* L.) were chosen as crop residues because of their high N mineralization potential (Scharpf, 1991; De Neve and Hofman, 1996). Only fresh leaf blades were added in order to have a more homogenous N mineralization.

Widely available organic wastes, of which we presumed that they would have a high C:N ratio or lignin content, were chosen as *immobilizer wastes* since these characteristics have proved to be indicators for a decreased N mineralization or N immobilization (Fox et al., 1990; Vigil and Kissel, 1991). The following organic wastes were tested: cereal straw, two green waste composts (compost 1 and compost 2), saw dust and paper sludge. Both green waste composts were collected early in the composting process (i.e. before maturing) because we presumed that immature compost would have a higher N immobilization capacity. Compost 1 was one month older than compost 2. Paper sludge was waste of a paper factory producing magazine and newspaper paper. In addition to these organic wastes, tannic acid was used as a model compound for organic wastes containing important amounts of water-soluble polyphenols, as has been done in previous studies (Bending and Read, 1996; Rahn et al., 2003; De Neve et al., 2004).

Table 3.1 (Bio)chemical composition of the N-rich crop residues and organic wastes

	DM	N_{tot}	N_{min}	C:N	Fractions (%OM)				PP
	%	g kg^{-1} DM			WS	H	Cell	L	%OM
Crop residues									
Celery leaves	13.1	46.8	3.04	7.7	29.2	42.7	7.4	20.7	1.25
Immobilizer wastes									
Straw	91.4	4.7	0.04	99.4	4.7	38.8	21.7	34.8	0.50
Compost 1 [a]	52.5	14.4	0.40	19.3	4.3	25.7	4.8	65.2	0.18
Compost 2 [a]	55.5	13.0	0.54	18.7	4.2	22.2	22.2	51.4	0.25
Saw dust	95.0	0.6	0.08	863.6	2.7	27.4	26.9	43.0	0.62
Paper sludge	29.0	6.1	0.19	50.6	3.5	26.6	20.8	49.1	0.11
Tannic acid	94.0	0.9	0.13	573.3	100.0	0.0	0.0	0.0	100.0
Remineralizer wastes									
Malting sludge	9.1	29.9	0.41	8.4	14.5	66.8	6.7	12.0	0.11
Vinasses	65.0[c]	58.9	5.22	7.0	100.0	0.0	0.0	0.0	n.d.[b]
Molasses	72.3[b]	22.6	0.86	19.6	100.0	0.0	0.0	0.0	n.d.[b]
Dairy sludge	8.8	51.4	4.77	8.3	18.9	47.9	4.8	28.4	0.17[f]

[a] compost 1 was one month older than compost 2; [b] not determined; DM: dry matter; N_{tot}: total N content; N_{min}: mineral N content; WS: water-soluble fraction; H: hemicellulose fraction; Cell: cellulose fraction; L: lignin fraction; PP: polyphenol content; OM: organic matter

As *remineralizer wastes*, widely available organic wastes of which we presumed that they would cause a priming effect, because of their high content of easily decomposable C, were chosen. It has been shown that addition of C in the form of sugars can lead to a marked increase in soil microbial activity (Falih and Wainwright, 1996). The following organic wastes were chosen: molasses, vinasses, malting sludge and dairy sludge. Molasses and vinasses were both wastes from the sugar industry. Malting sludge and dairy sludge were wastes from the malting and dairy industry, respectively.

Subsamples of the crop residues and the organic wastes were dried at 55°C until constant weight for determination of the dry matter content, and ground for further chemical analysis. Total C and N content of the materials was determined by dry combustion using a CNS elemental analyser (Variomax CNS, Elementar, Germany). Mineral N in 5 g of fresh crop residues or organic

wastes was extracted with (*1N*) KCl (ratio 1:12), and the extract was analysed for NO_3^--N and NH_4^+-N colorimetrically with a continuous flow auto-analyser (Chemlab System 4, Skalar, The Netherlands). Water-soluble, hemicellulose (+ structural protein), cellulose (+ structural protein) and lignin (+ structural protein) fractions were determined by a Stevenson fractionation as modified by De Neve and Hofman (1996). Polyphenol content was determined by the Folin-Denis method adapted by King and Heath (1967).

Incubation experiment

The soil used in the incubation experiment was the top layer (0-20 cm) of a sandy loam soil collected at Beitem (West-Flanders, Belgium) after harvest of spinach. The soil contained 8.6% clay, 30.9% silt and 60.6% sand, had a pH_{KCl} of 5.45, a total N content of 0.11%, a C content of 1.52%, a NO_3^- content of 1.4 mg N kg^{-1} and a NH_4^+ content of 2.0 mg N kg^{-1}. Visible impurities (e.g. stones, roots) were removed from the soil, but the soil was not sieved and not air-dried in order to minimize the disturbance of the microbial activity.

The incubation experiment consisted of two phases: an immobilization phase and a remineralization phase. At the start of the immobilization phase, fresh soil (equivalent to 283 g dry soil) was thoroughly mixed with fresh chopped (0.5-1 cm) celery leave blades (equivalent to 36 t FM ha^{-1}) and a fresh, chopped immobilizer waste (equivalent to ca. 2.5 t C ha^{-1}) and incubated in a PVC tube (diameter = 4.63 cm; filling height = 12 cm; bulk density = 1.4 g cm^{-3}). After filling the tubes, distilled water was added to obtain a moisture content of 50% WFPS (water-filled pore space) and the tubes were covered with a single layer of gas permeable parafilm to minimize water loss. The actual moisture content in de tubes was 49% WFPS. The tubes were incubated during 99 days at constant temperature (15°C). The treatments were (1) unamended soil, (2) celery only (=soil + celery residues), (3) straw (=celery only + straw), (4) compost 1 (=celery only + compost 1), (5) compost 2 (=celery only + compost 2), (6) saw dust (=celery only + saw dust), (7) paper sludge (=celery only + paper sludge) and tannic acid (=celery only + tannic acid). The sampling was destructive by removing tubes in triplicate for each

treatment at each sampling date. Samples were taken 7, 14, 29, 38, 52, 72 and 99 days after the start of the incubation. The mineral N in 30 g fresh soil was extracted with (*1N*) KCl (ratio 1:2) and the NO_3^--N and NH_4^+-N content was determined colorimetrically as for the organic wastes.

Ninety nine days after the start of the experiment, half of the tubes were amended with a remineralizer waste in quantities equivalent to 3 t C ha^{-1} for molasses and 1.5 t C ha^{-1} for vinasses, malting sludge and dairy sludge. The other half received only distilled water (= control immobilizer waste treatments). Therefore, the PVC tubes were emptied, the soil mixture was thoroughly mixed with the remineralizer waste or water, and the tubes were refilled and incubated as in the immobilization phase. Not all possible combinations were followed in order to maintain a surveyable experiment. It resulted in seven treatments from the immobilization phase together with sixteen new treatments: (1) straw + malting sludge (=celery only + straw + malting sludge), (2) compost 1 + malting sludge (= celery only + compost 1 + malting sludge), (3) compost 2 + malting sludge (= celery only + compost 2 + malting sludge), (4) saw dust + malting sludge (= celery only + saw dust + malting sludge), (5) paper sludge + malting sludge (= celery only + paper sludge + malting sludge), (6) tannic acid + malting sludge (= celery only + tannic acid + malting sludge), (7) compost 1 + molasses (= celery only + compost 1 + molasses), (8) paper sludge + molasses (= celery only + paper sludge + molasses), (9) compost 1 + vinasses (= celery only + compost 1 + vinasses), (10) paper sludge + vinasses (= celery only + paper sludge + vinasses), (11) compost 1 + dairy sludge (= celery only + compost 1 + dairy sludge), (12) paper sludge + dairy sludge (= celery only + paper sludge + dairy sludge), (13) malting sludge (= blank soil + malting sludge), (14) molasses (= blank soil + molasses), (15) vinasses (= blank soil + vinasses) and (16) dairy sludge (= blank soil + dairy sludge). Sampling was done in the same way as in the immobilization phase and took place 107, 113, 127, 141, 155, 169 and 190 days after the start of the incubation. The samples were analysed for mineral N as in the immobilization phase.

Statistics

Analysis of variance and a Duncan's post hoc test (One-way ANOVA in SPSS) was used to determine variances attributable to treatments. For non-linear regression the Levenberg-Marquardt algorithm (SPSS) was used.

3.3 Results

Biochemical composition

The immobilizer wastes were chosen for their high C:N ratio, high lignin content or high polyphenol content. Straw, saw dust and paper sludge had a high C:N ratio of > 50 (Table 3.1). The two green waste composts had a high lignin ratio (> 50% on organic matter). Tannic acid consisted of 100% polyphenol content on organic matter basis.

The remineralizer wastes were chosen for their high content of easily decomposable C. Molasses and vinasses consisted of 100% of water soluble C (Table 3.1). The dairy and malting sludge had a high hemicellulose content, 67% and 48% on organic matter, respectively. The N content of the remineralizer wastes was also quite high, especially for vinasses and dairy sludge where it was above 5% on dry matter.

Blank soil and celery only

The N mineralization rate of the blank soil was 0.08 mg N kg^{-1} soil day^{-1} (~ 0.3 kg N ha^{-1} day^{-1} for a soil depth of 30 cm) which is rather low for a soil used for vegetable production. N mineralization rates between 0.9 and 1.6 kg N ha^{-1} day^{-1} have been found for soils used for intensive vegetable production in Flanders (Demyttenaere, 1991).

The net N mineralization of the celery residues was calculated as the difference between the amounts of mineral N released in the soil amended with celery residues and the amount of mineral N released in the unamended soil (Fig. 3.1). The variability between replicates at a given sampling time was

large, which is typical when fresh organic matter is incubated in soil (De Neve and Hofman, 1996). A first-order kinetics model (Eq. 2.1) could be fit to the net N mineralization data of the celery residues, where $N_{tot}(t)$ was the amount of mineral N released at time t (mg N kg^{-1} soil), $N_{A,tot}$ was the amount of potentially mineralizable N (mg N kg^{-1} soil) and k_{tot} was the N mineralization rate constant (day^{-1}). Non-linear regression yielded the equation (3.1) (R^2= 0.865), and the amount of potentially mineralizable N was 52.9 mg N kg^{-1} soil which was 39% of the amount of added N.

$$N_{tot}(t) = 52.9\ (1-\exp(-0.05t)) \qquad (3.1)$$

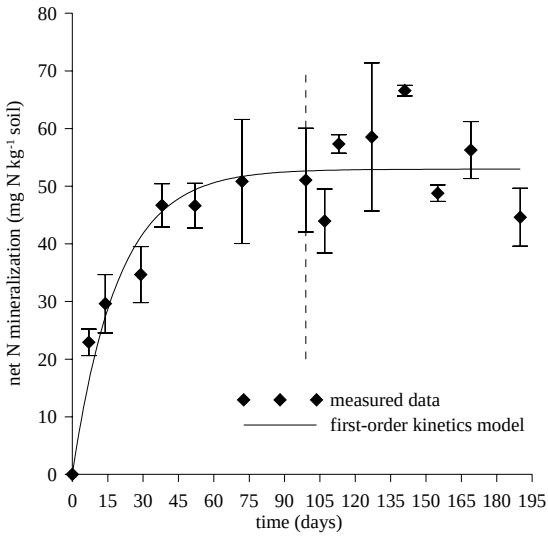

Fig. 3.1 Net N mineralization from the celery only treatment; error bars are standard deviations

Immobilizer wastes

In order to evaluate the N immobilization potential of the immobilizer wastes, the amount of N released by the celery only treatment was subtracted from the amount of N released from the treatments amended with a particular immobilizer (on each sampling date) (Fig. 3.2 and 3.3). The significant

differences between the treatments amended with a particular immobilizer and the celery only treatment are presented in Table 3.2.

Straw showed the fastest and most pronounced N immobilization, which was significant ($P < 0.01$) at all sampling dates. After two weeks, already 30.4 mg N kg^{-1} soil was immobilized. During the rest of the incubation period, the N immobilization varied between 24.7 and 44.8 mg N kg^{-1} soil. In the other treatments, the N immobilization started somewhat slower and was less significant ($P < 0.05$), nevertheless, these immobilizer wastes also showed a tendency towards N immobilization. Tannic acid caused N immobilization varying between 6.5 and 35.4 mg N kg^{-1} soil. Compost 2 showed two immobilization periods: one at the start of the incubation and one after 127 days, and these varied between N immobilization of 23.9 mg N kg^{-1} soil and net N mineralization of 14.1 mg N kg^{-1} soil. Saw dust immobilized N especially after 107 days from the start of the experiment, and the maximum N immobilization was 34.6 mg N kg^{-1} soil. The N immobilization in compost 1 and paper sludge was negligible.

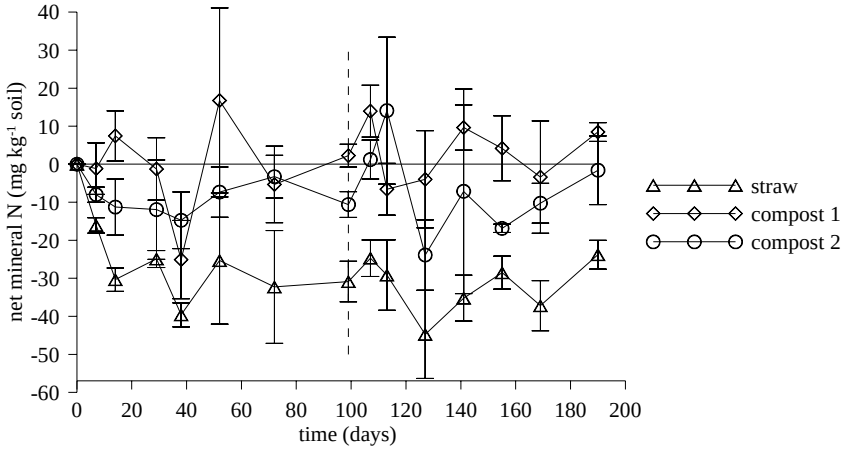

Fig. 3.2 Net N immobilization potential of straw and two green waste composts; the line y = 0 corresponds to the celery only treatment; error bars are standard deviations

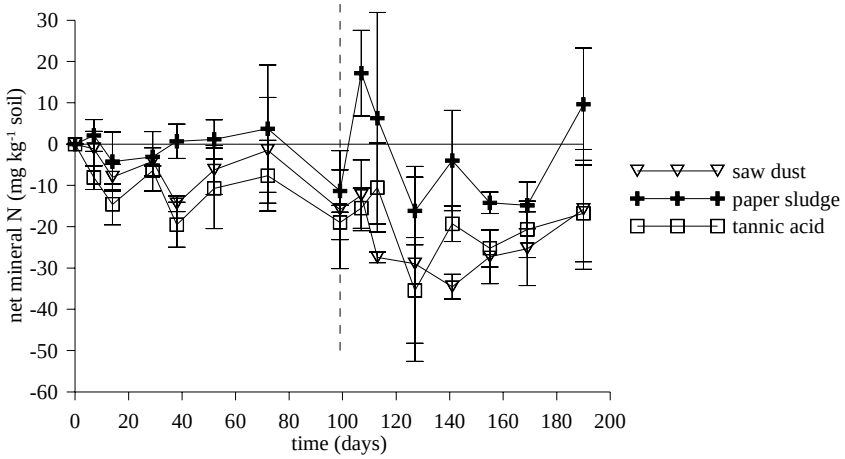

Fig. 3.3 Net N immobilization of saw dust, paper sludge and tannic acid; the line y = 0 corresponds to the celery only treatment; error bars are standard deviations

Table 3.2 The net N immobilization capacity of the immobilizer wastes (mg N kg^{-1} soil)

Time days	Straw	Compost 1	Compost 2	Saw dust	Paper sludge	Tannic acid
7	-16.1 (2.0)**	-1.2 (6.8)	-8.0 (2.0)*	-1.1 (4.2)	2.1 (3.9)	-8.1 (2.9)**
14	-30.4 (3.1)**	7.5 (6.6)	-11.3 (7.4)*	-8.0 (3.1)	-4.2 (7.2)	-14.6 (5.0)**
29	-24.9 (2.2)**	-1.2 (8.2)	-11.9 (13.1)	-4.1 (7.2)	-3.1 (2.2)	-6.5 (1.3)
38	-39.6 (3.2)**	-25.1 (10.3)*	-14.7 (7.4)*	-14.5 (1.9)**	0.7 (4.1)	-19.5 (5.5)**
52	-25.3 (16.4)*	16.8 (24.3)	-7.3 (6.6)	-6.2 (5.9)	1.1 (4.8)	-10.7 (9.8)
72	-32.3 (14.9)**	-5.3 (10.1)	-3.3 (5.6)	-1.5 (12.8)	3.7 (15.8)	-7.7 (8.6)
99	-30.9 (5.3)**	2.2 (3.0)	-10.6 (3.4)	-15.9 (14.3)	-11.4 (5.1)	-19.0 (4.1)
107	-24.7 (4.8)**	14.0 (6.8)*	1.2 (5.1)	-12.4 (8.6)*	17.2 (10.4)*	-15.5 (4.9)*
113	-29.1 (9.3)**	-6.6 (6.8)	14.1 (19.3)	-27.4 (1.3)**	6.3 (25.6)	-10.5 (10.8)
127	-44.8 (11.6)**	-4.0 (12.8)	-23.9 (9.3)*	-29.0 (23.6)*	-16.2 (8.2)	-35.4 (12.8)**
141	-35.2 (6.0)**	9.7 (6.0)	-7.1 (26.9)	-34.6 (3.0)**	-4.0 (12.2)	-19.3 (4.3)**
155	-28.5 (4.3)**	4.2 (8.5)	-16.9 (1.1)*	-27.3 (6.5)**	-14.2 (2.6)**	-25.2 (4.5)**
169	-37.2 (6.6)**	-3.4 (14.8)	-10.3 (5.2)*	-25.3 (8.9)**	-14.8 (5.6)*	-20.7 (6.8)**
190	-23.8 (3.8)**	8.5 (2.5)	-1.6 (9.0)	-15.8 (14.5)	9.6 (13.6)	-16.8 (11.7)*

* and ** indicate that value is significantly different from zero: *P< 0.05; **P< 0.01; between brackets standard deviations

Remineralizer wastes

The priming effect (or remineralization) caused by the remineralizer wastes was calculated as the mineral N content in the 'immobilizer + remineralizer' treatment minus the mineral N content in the immobilizer treatment, minus the net N release of remineralizer waste (Fig. 3.4, Table 3.3). Only compost 1 + vinasses gave significant remineralization amounts up to 55.4 mg N kg^{-1} soil from day 28 till day 70. Significant positive priming effects in the other remineralizer waste treatments lasted only one sampling date, like in compost 2 + malting sludge, saw dust + malting sludge, paper sludge + malting sludge and paper sludge + dairy sludge. In the other treatments no significant positive priming effects occurred.

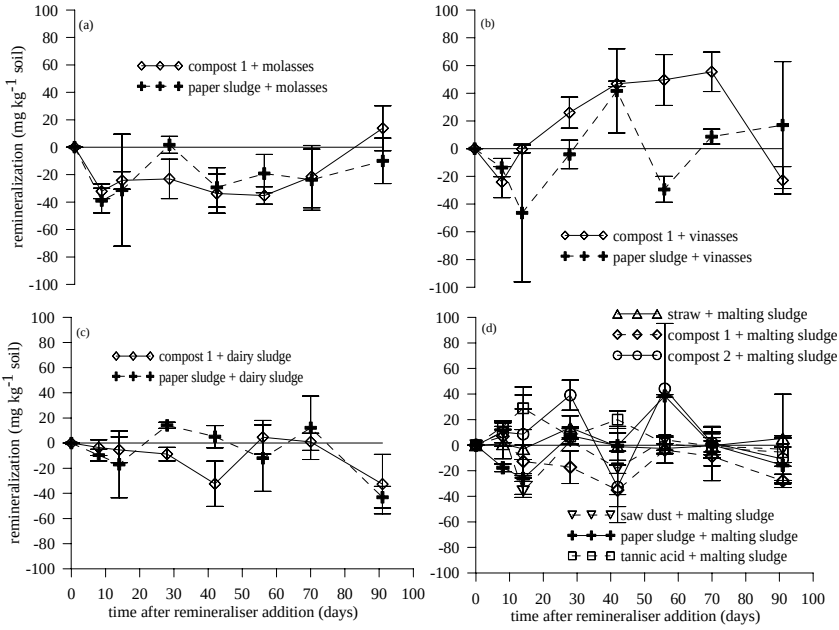

Fig. 3.4 N remineralization patterns of the remineralizer wastes: molasses (a), vinasses (b), dairy sludge (c) and malting sludge (d); errors bars are standard deviations

Table 3.3 The priming effect of the remineralizer wastes (mg N kg^{-1} soil)

		days after addition of remineralizer waste						
		8	14	28	42	56	70	91
Malting sludge +	Straw	0.9 (11.6)	-2.9 (16.6)	13.7 (9.3)	-1.1 (10.7)	-2.5 (3.8)	-0.4 (9.4)	5.3 (34.6)
	Compost 1	7.0 (7.8)	-12.7 (12.9)	-17.2 (12.8)	-35.1 (12.9)*	-3.7 (10.3)	-8.7 (19.0)	-27.9 (5.2)*
	Compost 2	10.2 (8.1)	8.6 (30.7)	39.2 (11.8)*	-32.6 (27.9)	44.2 (51.0)	1.9 (12.1)	-10.8 (16.8)
	Saw dust	14.7 (2.7)*	28.9 (16.7)	6.9 (6.1)	20.1 (6.9)*	1.2 (5.0)	-2.0 (5.3)	-5.5 (11.0)
	Paper sludge	-17.6 (3.0)*	-26.0 (14.9)	7.5 (6.7)	-0.4 (15.1)	38.5 (1.0)**	0.4 (5.7)	-15.8 (14.4)
	Tannic acid	10.6 (8.7)	-35.9 (9.0)**	3.2 (2.5)	-18.1 (20.6)	4.2 (3.1)	-0.7 (15.5)	-3.1 (10.4)
Vinasses +	Compost 1	-24.1 (11.2)	-0.4 (2.7)	25.9 (11.2)	46.8 (2.0)**	49.5 (18.4)*	55.4 (14.3)*	-22.9 (9.9)
	Paper sludge	-13.6 (6.6)	-46.4 (49.7)	-4.1 (10.3)	41.7 (30.4)	-29.4 (9.4)*	8.7 (5.4)	17.0 (45.8)
Molasses +	Compost 1	-32.1 (5.3)**	-24.2 (6.4)*	-23.1 (14.3)	-33.8 (14.3)	-35.2 (6.3)*	-21.4 (22.7)	13.8 (16.4)
	Paper sludge	-38.9 (9.0)*	-31.3 (40.8)	1.8 (6.2)	-29.3 (14.4)	-19.2 (14.0)	-23.7 (22.2)	-10.0 (16.5)
Dairy sludge +	Compost 1	-3.6 (6.3)	-5.4 (10.3)	-8.8 (5.4)	-32.4 (18.0)	4.7 (13.3)	1.0 (6.7)	-32.6 (23.6)
	Paper sludge	-9.5 (4.7)	-16.9 (26.6)	14.1 (2.5)*	5.1 (8.9)	-12.0 (26.4)	12.2 (25.4)	-43.1 (8.6)*

* and ** indicate values significantly different from zero: *P< 0.05; **P< 0.01; positive values indicate positive priming effect (remineralization); negative values indicate negative priming effect (immobilization); between brackets standard deviations

3.4 Discussion

Immobilizer wastes

The critical C:N ratio for N mineralization/immobilization depends on the length of the incubation period considered (higher when time considered is larger) and in general, the application of organic materials with a C:N ratio higher than 20-40 promotes net N immobilization (Iritani and Arnold, 1960; Fox et al., 1990; Vigil and Kissel, 1991). Lignin is a complex molecule with ring-type structures, mostly found in older and woody plant tissues, and is quite difficult to decompose by micro-organisms. Several authors have found a negative correlation between N mineralization and lignin content of organic materials, and N mineralization seems already reduced when the lignin content is above 10% on dry matter for legumes (Oglesby and Fownes, 1992; Constantinides and Fownes, 1994) and above 14% on dry matter for non-legumes (Constantinides and Fownes, 1994).

In this study, three organic wastes had a high C:N ratio: straw (C:N 99.4), paper sludge (C:N 50.6) and saw dust (C:N 863.6), but only straw caused significant N immobilization at all sampling dates. Straw immobilized on average 30 ± 7 mg N kg^{-1} soil during a period of 190 days, which would correspond with 127 kg N ha^{-1} under field conditions. Saw dust only showed a tendency towards N immobilization (on average 15.9 ± 11 mg N kg^{-1} or 67 kg N ha^{-1}) notwithstanding that it had a much higher C:N ratio than straw. The immobilization potential of paper sludge was almost non-existing (on average 1.9 ± 10 mg N kg^{-1} or 8 kg N ha^{-1}). Compost 1 and compost 2 were selected for their high lignin content (65.2% and 51.4% on OM, respectively), but also their N immobilization potential was limited. Compost 1 released N in the soil (1.1 ± 11 mg kg^{-1} ~ 5 kg N ha^{-1}), although it contained the highest lignin content. Compost 2 immobilized only 8.0 ± 9 mg kg^{-1} (~ 33 kg N ha^{-1}). Also the lignin content of saw dust and paper sludge was quite high: 43.0% and 49.1% on OM, respectively. Therefore, a possible explanation for the low N immobilization potential of saw dust, paper sludge, compost 1 and compost 2

could be the influence of the lignin content on the decomposition of organic material. Several studies have found a negative relationship between the decomposition rate and the lignin content (Tian et al., 1992; Vanlauwe et al., 1996; Mueller et al., 1998; Rahn et al., 2002). The high lignin content of saw dust, paper sludge, compost 1 and compost 2 may have slowed down decomposition, decreasing the N demand of the micro-organisms, and hence, decreasing N immobilization. De Neve et al. (2003) and Prat Roibas (2001) indeed showed that the amount of mineralizable C of green waste compost, saw dust and paper sludge (of similar biochemical composition than the wastes in our study) was only 7.7%, 22% and 30.1% of added C_{org}, compared to 45% of added C_{org} for straw. Evidence is also emerging from recent studies (Iglesias-Jiménez, 2001; Nendel et al., 2004) that micro-organisms may first degrade easily decomposable material in soil (i.e. crop residues), and thereafter recalcitrant material (i.e. immobilizer wastes). This would mean that the N release from the crop residues and N immobilization by organic wastes are separated in time, decreasing the probability for immobilizers to immobilize N released from crop residues. So, when organic materials with a high lignin content are incorporated they may have a delayed N mineralization or cause immobilization of soil mineral N (Fox et al., 1990), but these materials seem to have only a low potential to delay N mineralization or immobilize N from N-rich crop residues because their decomposition is too slow or not synchronized with that of the crop residues. This would mean that a good immobilizer must have a high C:N ratio, and must be easily decomposable (i.e. low lignin content).

Another possible explanation for the low N immobilization potential of organic wastes could be the rather low application rate of 2.5 t C ha^{-1} used in this study, especially for the paper sludge. Rahn et al. (2003) used an application rate of 10 t C ha^{-1}, and found reductions in mineral N in a sandy loam soil relative to the crop residue control of 50 to 90% for straw and compactor paper waste, and 10 to 60% for tannic acid. In this study, the reductions relative to the celery treatment were similar to those of Rahn et al. (2003) for straw (55 to 103%) and tannic acid (14 to 60%), but lower for paper

sludge (-42 to 29%). Motavalli and Discekici (2000) added paper waste at a rate of 2.9 t C ha^{-1} and also found limited effects on the N mineralization.

It has been shown that organic materials with a high polyphenol content (> 2-4% on dry matter) have a delayed N mineralization or cause N immobilization (Palm and Sanchez, 1991; Constantinides and Fownes, 1994; Bending et al., 1998). Organic materials with a high polyphenol content have a double effect on N mineralization. First, polyphenolic compounds are toxic for several micro-organisms, including bacteria, fungi and micro fauna involved in the process of N mineralization (Scalbert, 1991, Capasso et al., 1995, Hewlett et al., 1997). Secondly, polyphenols have a strong protein binding capacity through their strong affinity for amide groups. The polyphenol effect on N availability therefore is not an immobilization of mineralized N, but an inhibition of N mineralization through the above mentioned effects. In this study, tannic acid was selected as a model compound for wastes with a high polyphenol content. The N mineralization inhibition potential of tannic acid was not as high as the N immobilization potential for straw, but was still quite good (on average 16 ± 8 mg N kg^{-1} (69 kg N ha^{-1})) and lasted during the complete incubation experiment (190 days). This is in contradiction with previous studies which have shown that polyphenols seem to affect the N mineralization especially in early stages of decomposition (Constantinides and Fownes, 1994; Bending et al., 1998). However, De Neve et al. (2004) also found N immobilization during the complete incubation period after mixing leek residues with tannic acid. The degree of N immobilization caused by tannic acid was comparable with results found by Rahn et al. (2003) (reduction of mineral N of 10-60% compared to crop residue control) and De Neve et al. (2004) (N immobilization of 10-20 mg N kg^{-1} soil), although the application rate in our study was much lower (2.5 t C ha^{-1}) than in these latter studies (10 t C ha^{-1} and 21 t C ha^{-1}, respectively).

Remineralizer wastes

Despite the fact that compost 1 had caused no N immobilization, compost 1 + vinasses caused significant remineralization (positive priming effect) (up to

55.4 mg N kg^{-1}) of soil organic N during several days (from day 42 till day 70), what would correspond with 232 kg N ha^{-1} in field conditions. Although this remineralization was temporary, a new crop could profit from this extra N release if it were sown soon after the addition of vinasses. All other remineralizer wastes caused no or very short-lived positive priming effects, and a following crop would not be able to profit from it. However, in this study, the limited effects must have been real priming effects and not pool substitution effects, since total net N mineralization in treatments with an immobilizer and a remineralizer waste was larger than the sum of net N mineralization in the treatments where the immobilizer and remineralizer wastes were added separately.

The reports of real positive priming effects in case of N are scarce up till now, and only few materials have been tested: fertilizers (Jenkinson et al., 1985; Powlson and Barraclough, 1993) glucose (Asmar et al., 1994; Falih and Wainwright, 1996; Wheatly et al., 2001), molasses (De Neve et al., 2004) and ryegrass shoots (Kuzyakov et al., 1999). Although it is widely accepted that micro-organisms play a crucial role in the process, the mechanisms leading to real positive priming effects remain poorly understood. The most common explanation for priming effects is an increased soil organic matter decomposition as a result of a higher microbial population and activity due to the higher availability of energy and nutrients from added organic materials (Sörensen, 1974; Kuzykov et al., 2000; Fontaine et al., 2003). However, recently, interactions between soil micro-organisms, soil fauna and plants are also regarded as important keys for understanding priming effects (Kuzyakov et al., 2000).

Due to a limited knowledge concerning priming effects, it is difficult to explain why the organic wastes in this study caused only limited priming effects. A possible reason may be the low N immobilization of the immobilizer wastes (especially compost 1 and paper sludge, which were combined with all remineralizer wastes), leading to less immobilized N available for remineralization. In case of molasses, the high C:N ratio was probably also a

determining factor, which led to further N immobilization in stead of remineralization. However, De Neve et al. (2004) tested molasses with an even higher C:N ratio (C:N 24) and did found a positive priming effect. In case of malting sludge and dairy sludge, the biochemical composition was possibly not suitable to induce N priming effects, since most researchers found N priming effects after incorporation of low molecular compounds like glucose (Asmar et al., 1994; Falih and Wainwright, 1996; Wheatly et al., 2001).

If we are not able to stimulate remineralization before a new crop is sown or planted, the immobilized N may become gradually available as a result of N mineralization from microbial biomass during the subsequent period. If this N release occurs in spring or summer a crop will still be able to benefit from it, but if the mineralization occurs in autumn or winter, the risk of NO_3^- leaching may be increased. It has been found that straw incorporation reduces NO_3^- leaching during the first winter, but that it may lead to higher mineral N contents and higher NO_3^- leaching on the long term (Nicholson et al., 1997; Catt et al., 1998; Silgram and Chambers, 2002). Therefore, N remineralization may be a crucial factor within the method of manipulating the N release from crop residues.

3.5 Conclusions

Manipulating the N release of N-rich crop residues by using organic wastes may be a suitable method to reduce mineral N residues after harvest of vegetables. Especially organic wastes with a high C:N ratio and a low lignin content (i.e. easily decomposable material), seem to have a potential to immobilize N released from crop residues. Also organic wastes with a high polyphenol content seem to have a N immobilization potential. However, stimulating remineralization of immobilized N by the start of the following spring might not be easy to achieve. Most positive priming effects in this study were scarce and short-lived.

Chapter 4

The effect of mixing organic wastes and N-rich crop residues on the short time N_2O emission from horticultural soil in laboratory experiments

Parts of this chapter were published in:

Chaves B, De Neve S, Lillo Cabrera MC, Boeckx P, Van Cleemput O, Hofman G (2005) The effect of mixing organic biological waste materials and high N crop residues on the N_2O emission from soil. Biol Fert Soils 41: 411-418.

Abstract Manipulating the N release from N-rich crop residues by simultaneous mixing of these residues with immobilizer wastes, seems to be a possible method to reduce NO_3^- leaching. The aim of this study was to examine whether the incorporation of immobilizer wastes together with a N-rich crop residue (celery) had also an effect on the short term N_2O emission from horticultural soils under laboratory conditions. A sandy loam soil and celery residues were mixed with different immobilizer wastes and incubated in PVC tubes at 80% WFPS and 15°C. Every two hours a gas sample was taken and analysed by gas chromatography for its N_2O concentration. The soil amended with only celery residues had a cumulative N_2O emission of 9.6 mg N kg^{-1} soil in 50 hours. When the celery residues were mixed with an immobilizer waste the N_2O emission was each time lower than the emission in the celery only treatment (between 3.8 and 5.9 mg N kg^{-1} soil during maximum 77 hours), except with paper sludge (17.2 mg N kg^{-1} soil in 100 hours). The higher N_2O emission from the paper sludge treatment was probably due to its unusually low C:N ratio. Straw, two green waste composts (compost 1 and compost 2), saw dust and tannic acid reduced the N_2O emission of the celery treatment by 40 to 60%. Although the N_2O reduction potential can be expected to be lower and with differing dynamics under field conditions, this study indicates that, apart from reducing NO_3^- leaching, organic waste application may at the same time reduce N_2O emissions after incorporation of high N crop residues.

4.1 Introduction

Incorporating N-rich crop residues may increase the risk of N_2O emissions from wet soils, i.e. water-filled pore space (WFPS) is > 60% (autumn and winter), depending on the quantity and quality of the incorporated material (Aulakh et al., 1991; Baggs et al., 2000a). Nitrous oxide emission is not desired since it is a powerful greenhouse gas (Mosier and Schimel, 1991) and it affects the stratospheric ozone layer, resulting in a higher UV-B intensity reaching the earth surface (Cicerone, 1987). Since organic C and NO_3^- are substrates for denitrifier micro-organisms (Aulakh and Rennie, 1987; Weier et al., 1993) adding easily decomposable C (e.g. from immobilizer wastes) to a large NO_3^--N producing reservoir (from N mineralization of crop residues or NO_3^--N still available in the soil after harvest) might increase denitrification in wet soils (autumn and winter). On the other hand, one may expect immobilizer wastes to reduce N_2O emission when incorporated together with crop residues, since they may reduce the mineral N content of soil due to N immobilization. Several researchers indeed found a reduced N_2O emission after incorporation of organic materials with a high C:N ratio due to N immobilization (Baggs et al., 2000b; Hao et al., 2001; Velthof et al., 2002). However, the combined effect of the addition of N-rich crop residues and organic wastes on N_2O emission has received little attention. Rahn et al. (2003) found a tendency for higher N_2O emission in a laboratory study (60% WFPS, 15°C) where sandy loam soil (0.8% organic C) and silt loam soil (1.6% organic C) were amended with fresh sugar beet leaves and organic wastes such as tannin, wheat straw and green waste compost compared to soils with only crop residues, but generally all the N_2O emissions were very low in that study (< 150 µg N_2O-N kg^{-1} day^{-1}) due to a moisture content of only 60% WFPS. Baggs et al. (2002) found high N_2O emissions under field conditions after incorporation of paper mill sludge in the presence of lettuce residues (up to 6.8 kg N_2O-N ha^{-1} over 67 days) probably due to the high organic C input.

The aim of this study was to examine the effect of incorporating immobilizer wastes together with a N-rich crop residue (celery) on the N_2O emission. Our research hypothesis was that an increase in N_2O emission would occur when immobilizer wastes were incorporated together with N-rich crop residues as compared to crop residues alone due to the large availability of NO_3^--N released from the crop residues in combination with a large amount of easily decomposable C from the immobilizer wastes. If this is proven to be the case, the increase in N_2O emissions would partially offset the beneficial effect of the reduced NO_3^- leaching. Knowledge concerning the N_2O emissions is therefore crucial before the use of organic wastes can be further stimulated.

4.2 Materials and methods

Crop residue, immobilizer wastes and soil type

Celery residues were chosen as N-rich crop residues because of the high N mineralization potential. The celery residues were divided into leaves and stalks and added to the soil in a know ratio in order to have a homogenous N mineralization. Widely available immobilizer wastes with high C:N ratio or high lignin content such as cereal straw, two green waste composts (compost 1 and compost 2), saw dust and paper sludge, were chosen since these characteristics have proved to decrease N mineralization and increase N immobilization (Fox et al., 1990; Vigil and Kissel, 1991). Both green waste composts were collected early in the composting process (i.e. before maturing) because we suspected immature compost would have a higher N immobilization capacity; compost 1 was one month older than compost 2. The paper sludge was waste of a paper factory producing magazine and newspaper paper. In addition to these immobilizer wastes, tannic acid was used as a model compound for organic wastes containing important amounts of water-soluble polyphenols, as has been done in previous studies (Bending and Read, 1996; Rahn et al., 2003; De Neve et al., 2004).

Subsamples of the crop residues and the immobilizer wastes were dried at 55°C until constant weight and then the dry matter content was determined; dry residues were ground for further chemical analysis. The total C and N content of the materials was determined by dry combustion using a CNS elemental analyser (Variomax CNS, Elementar, Germany). The mineral N in 5 g of dried crop residues or organic wastes was extracted with (*1N*) KCl (ratio 1:12), and the extract was analysed for NO_3^--N and NH_4^+-N colorimetrically with a continuous flow auto-analyser (Chemlab System 4, Skalar, The Netherlands). The water-soluble, hemicellulose (+ structural protein), cellulose (+ structural protein) and lignin (+ structural protein) fractions were determined by a Stevenson fractionation as modified by De Neve and Hofman (1996). The polyphenol content was determined by the Folin-Denis method adapted by King and Heath (1967). The biochemical characteristics of the celery residues and the organic wastes are shown in Table 4.1.

Table 4.1 (Bio)chemical composition of the celery residues and immobilizer wastes

	DM	N_{tot}	NO_3^--N	NH_4^+-N	C:N	WS	H	Cell	L	PP
	%	g kg^{-1} DM				%OM				
Celery leaves	11.9	42.3	0.59	1.01	9.2	33.3	43.0	7.3	16.2	1.73
Celery stems	5.8	12.5	0.33	0.46	27.4	47.2	28.4	12.5	11.2	0.34
Leaves + stems [a]	6.6	19.6	0.38	0.57	19.1	44.4	31.3	11.5	12.2	0.62
Cereal straw	91.4	4.7	0.01	0.03	99.4	4.7	38.8	21.7	34.8	0.50
Compost 1 [b]	52.5	14.5	0.01	0.02	19.0	4.3	25.7	4.8	65.2	0.18
Compost 2 [b]	55.5	13.0	0.00	0.02	18.7	4.2	22.2	22.2	51.4	0.25
Saw dust	95.0	0.6	0.08	0.05	863.3	2.7	27.4	26.9	43.0	0.62
Paper sludge	29.6	15.4	0.00	1.50	18.4	5.1	36.1	21.2	37.6	0.05
Tannic acid	94.0	0.6	0.00	0.08	876.6	100.0	0.0	0.0	0.0	100.0

[a] ratio leaves:stems = 1:4; [b] compost 1 was one month older than compost 2; DM: dry matter, N_{tot}: total N content, WS: water-soluble fraction, H: hemicellulose fraction, Cell: cellulose fraction, L: lignin fraction, PP: polyphenol content, OM: organic matter

The soil used for the experiments was the top horizon (0-20 cm) of a sandy loam soil collected from a vegetable farm in Poeke (East-Flanders, Belgium). The soil contained 8.8% clay, 47.9% silt and 43.3% sand, had a pH_{KCl} of 6.15,

a total N content of 0.17% and a C content of 1.62%. The initial mineral N content of the soil was 39.4 mg N kg^{-1} soil (28.6 mg NO_3^--N kg^{-1}; 10.8 mg NH_4^+-N kg^{-1}). Visible impurities (e.g. stones, roots) were removed, but the soil was not sieved and not air-dried in order to minimize the disturbance of the microbial activity.

N_2O emission

Fresh soil (equivalent to 238 g oven dry soil) was thoroughly mixed with dried and ground (< 0.3 mm) celery leaves and stems (1.3 g dry leaves kg^{-1} soil; 5.0 g dry stems kg^{-1} soil; ratio leaves:stems = 1:4 on dry matter basis) and a dried and ground (< 0.3 mm) immobilizer waste (equivalent to ca. 5 t C ha^{-1}; 1.7 g dry straw kg^{-1} soil; 2.7 g dry compost 1 kg^{-1} soil; 3.0 g dry compost 2 kg^{-1} soil; 1.5 g dry saw dust kg^{-1} soil; 1.6 g dry paper sludge kg^{-1} soil; 2.4 g dry tannic acid kg^{-1} soil) and incubated in PVC tubes (diameter = 6.70 cm; filling height = 5 cm; bulk density = 1.35 g cm^{-3}). After filling the PVC tubes, distilled water was added to obtain a moisture content of 80% WFPS (water-filled pore space). This moisture content was chosen because it is high enough to have significant denitrification (Doran et al., 1990), and it is a realistic value under field conditions in this soil. The PVC tubes were filled in triplicate and incubated at 15°C. The treatments were (1) unamended soil, (2) celery only (=soil + celery residues), (3) straw (=celery only + straw), (4) compost 1 (=celery only + compost 1), (5) compost 2 (=celery only + compost 2), (6) saw dust (=celery only + saw dust), (7) paper sludge (=celery only + paper sludge) and tannic acid (=celery only + tannic acid).

For the determination of the N_2O emission, the PVC tubes were placed in glass jars (1 l), which were then closed airtight. After two hours a gas sample (1 ml) was taken from the headspace with a syringe through a septum in the lid, and its N_2O concentration was determined immediately with a Shimadzu GC-14B gaschromatoghraph with an electron capture detector (ECD) and two packed columns (1 m and 2 m, respectively; Porapack Q, 80/100 mesh). The operating conditions were as follows: carrier gas N_2 (55 ml min^{-1}), injector temperature 105°C, column and oven temperature 35°C and detector temperature 250°C.

The chromatograph was calibrated using N_2O standard gas (25 ± 1.5 µl l^{-1}in He). Following the GC measurement, the glass jars were opened to allow aeration, closed again and after two hours the N_2O concentration was measured again. The N_2O flux measurements were repeated four times a day: at 10h, 12h30, 15h and 17h30. The measurements continued until no excess N_2O was emitted from soil (N_2O concentration in gas sample = N_2O concentration in air). Table 4.2 gives the duration of the N_2O emission (= incubation time) in the different treatments.

During the first measurements, it became clear that we were not able to record the maximum N_2O flux, because it occurred overnight. Therefore, the measurements were repeated with two series of tubes for each treatment: one series filled at 8 p.m. (day series) and one series at 6 a.m. the evening before (night series). It was hypothesized that, except from the time difference, no other differences occurred between the two series. During the first measurements, the standard deviations between the replicates within treatments were very small, what justified this assumption.

At the start and the end of the N_2O measurements, the mineral N content and the total soluble organic C content (TSOC) in the soil of each treatment were determined. At the start of the measurements, three extra PVC tubes were filled for each treatment and were immediately sampled (destructively). At the end of the measurements, the PVC tubes were removed from the glass jars and also sampled destructively. The mineral N content was determined by extracting 30 g of soil with (*1N*) KCl (ratio 1:2) and the NH_4^+-N and NO_3^--N content was determined colorimetrically as for the organic wastes. Total soluble organic C was determined by extracting 30 g of soil with distilled water (ratio 1:2) and analysing the extract with a Total Organic Carbon Analyser (TOC-V_{CPN}, Shimadzu, Japan).

Statistical analysis

Analysis of variance and Duncan's post hoc test (One-way ANOVA in SPSS) were used to determine significant differences attributable to treatments. A correlation analysis was done using a Pearson's correlation matrix in SPSS.

4.3 Results

N_2O emission

The fluxes of N_2O as a function of time are shown in Fig. 4.1. The soil without amendments showed a negligible N_2O flux (0.0012 mg N_2O-N kg^{-1} soil h^{-1}). When crop residues or immobilizer wastes were added to the soil the N_2O flux increased significantly compared to the unamended soil. When the soil was mixed with celery residues only, the maximum N_2O flux was 0.58 mg N_2O-N kg^{-1} soil h^{-1} and the elevated N_2O emissions lasted 33.5 hours. When the celery crop residues were mixed with an immobilizer waste, the maximum N_2O flux was always lower than the celery only treatment, except for paper sludge. The maximum N_2O flux after addition of paper sludge was 0.76 mg N_2O-N kg^{-1} soil h^{-1} and the elevated N_2O emissions lasted 57.5 hours. The other immobilizer wastes showed maximum N_2O fluxes between 0.16 and 0.29 mg N_2O-N kg^{-1} soil h^{-1} and the elevated N_2O emission lasted 33.5 hours, except for tannic acid (53.0 hours).

The total amount of N_2O emitted during the measuring periods (= cumulative N_2O emission) was calculated for each treatment (Fig. 4.2, Table 4.2). The highest cumulative N_2O emission occurred when celery residues were incorporated together with paper sludge (17.2 mg N_2O-N kg^{-1} soil), followed by the celery treatment (9.6 mg N_2O-N kg^{-1} soil). The other treatments showed a significantly lower cumulative N_2O emission of 6 mg N_2O-N kg^{-1} soil or lower.

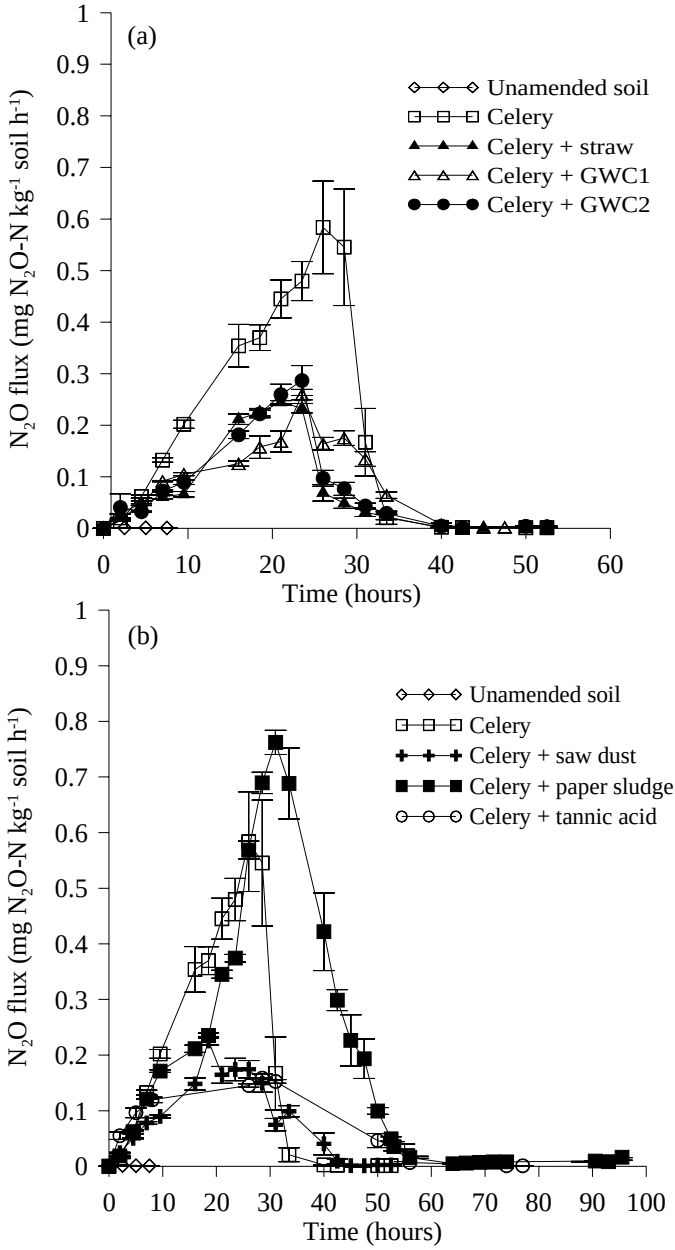

Fig. 4.1 The N_2O fluxes as a function of time for the unamended soil, celery residues and celery plus different immobilizer wastes: straw, compost 1 and compost 2 (a) and saw dust, paper sludge and tannic acid (b); errors bars are half the standard deviations

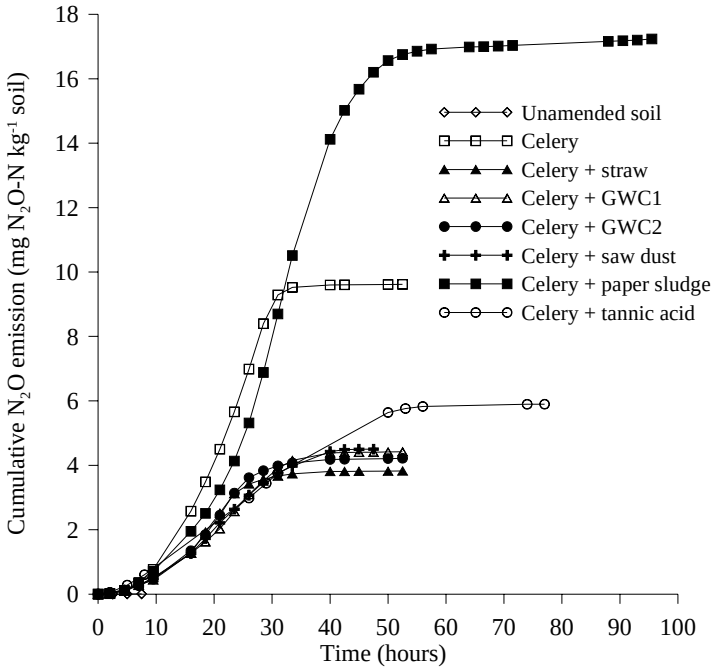

Fig. 4.2 The cumulative N_2O emission for the different treatments

By subtracting the cumulative N_2O emission of the celery treatment from the cumulative N_2O emission of the celery plus immobilizer wastes and dividing this by the N_2O emission in the celery treatment, the reduction in N_2O emission relative to the celery emission was calculated (Table 4.2). Incorporation of straw, compost 1, compost 2 and saw dust decreased the N_2O emission of celery with more than 50% (reduction between 53 and 60%). Tannic acid lowered the N_2O emission of celery residues with almost 40%. On the other hand, incorporating paper sludge together with the celery residues increased the N_2O emission with 80% compared to the celery treatment.

Table 4.2 Duration of N_2O emission, cumulative N_2O emission, reduction in N_2O emission upon addition of immobilizer wastes; between brackets standard deviations; different letters indicate significant differences in a row (P< 0.01)

	Soil	Celery only	Straw	Compost 1	Compost 2	Saw dust	Paper sludge	Tannic acid
Duration of N_2O emission (h) *	7.5	52.5	52.5	52.5	52.5	52.5	95.5	77.0
Cumulative N_2O emission	0.0075^{a}	9.61^{b} (0.98)	3.82^{c} (0.33)	4.42^{c} (0.45)	4.31^{c} (0.38)	4.51^{c}	17.24^{d}	5.90^{c}
(mg N_2O-N kg^{-1} soil)	(0.0019)					(0.75)	(1.25)	(0.09)
Reduction in N_2O emission	-**	-**	-60^{a}	-54^{ab}	-55^{ab}	-53^{ab}	80^{c}	-38^{b}
(% of celery emission)			(8)	(4)	(2)	(10)	(6)	(6)

* duration of N_2O emission is the same as the incubation time; **not applicable

Mineral N

The mineral N content at the start and the end of the N_2O measurement period is shown in Table 4.3. At the start of the N_2O emission, the NO_3^--N content in the soil was comparable across amended treatments and on average 32.7 mg NO_3^--N kg^{-1} soil. Also the NH_4^+-N content was comparable, except when paper sludge was added. The NH_4^+-N content in the paper sludge treatment was significantly higher ($P< 0.01$) because of the large NH_4^+-N content of the paper sludge itself (Table 4.1).

Also at the end of the N_2O emission, no significant differences in NO_3^--N content could be determined between the amended treatments. The NO_3^--N content at the end of the N_2O emission was on average 4% of the NO_3^--N content at the start (min. 0% and max. 9%) and on average 1.3 mg NO_3^--N kg^{-1} soil for all treatments. At the end of the N_2O emission, the NH_4^+-N content was still significantly higher in the paper sludge treatment (36.4% of the NH_4^+ content at the start). For the other amended treatments the NH_4^+-N content at the end of the N_2O emission was on average 13% of the content at the start (min. 5% and max. 18%).

Table 4.3 The mineral N content (mg N kg^{-1} soil) and total soluble organic C (TSOC) (mg C kg^{-1} soil) in the soil at the start and the end of the N_2O measurement period; between brackets standard deviations; different letters indicate significant differences in a column ($P< 0.05$)

	Start			End		
	NO_3^--N	NH_4^+-N	TSOC	NO_3^--N	NH_4^+-N	TSOC
Celery only	31.1^a (4.4)	10.1^a (1.1)	116.8 ^{a} (2.3)	1.3^a (1.0)	1.8^a (0.9)	31.6 ^{a} (12.5)
Straw	33.7^a (0.9)	13.1^a (0.3)	124.2 ^{a} (5.2)	1.1^a (1.5)	0.5^a (0.6)	14.9 ^{b} (2.3)
Compost 1	-*	-*	-*	0.4^a (0.7)	2.3^a (0.7)	18.7 ^{ab} (0.8)
Compost 2	34.5^a (2.6)	10.1^a (2.2)	97.2 ^{a} (12.3)	2.1^a (1.6)	1.8^a (0.6)	18.1 ^{ab} (0.5)
Saw dust	33.3^a (3.6)	12.7^a (1.1)	106.7 ^{a} (14.9)	0.0^a (0.0)	1.3^a (0.6)	12.7 ^{b} (1.2)
Paper sludge	31.0^a (3.1)	28.7^b (2.8)	121.1 ^{a} (9.1)	2.9^a (1.3)	10.0^b (1.9)	21.1 ^{ab} (2.0)
Tannic acid	-*	-*	-*	-*	-*	-*

* not determined

Total soluble organic C

The TSOC content in the soil for the different amended treatments is presented in Table 4.3. At the start of the N_2O emission, the TSOC contents were similar in all amended treatments (on average 113.2 mg C kg^{-1}). During N_2O emission, the TSOC content decreased. The highest amount of TSOC, at the end of the N_2O emission, was found in the celery only treatment (31.6 mg C kg^{-1}), whereas the smallest amount was found in the straw and saw dust treatments (14.9 and 12.7 mg C kg^{-1}, respectively).

4.4 Discussion

Both nitrification and denitrification are sources of N_2O. Nitrification is the dominant process under aerobic conditions (60% WFPS and lower), while denitrification is the dominating process under anaerobic conditions (> 80% WFPS), but both processes can occur simultaneously (Stevens et al., 1997). Under aerobic conditions, N_2O can also be produced by denitrification in anaerobic micro sites within soil aggregates (Renault and Stengel, 1994). At 80% WFPS (as in this study), the intensity of nitrification and denitrification activities is similar (Linn and Doran, 1984), but decomposition of incorporated plant residues in soil increases denitrification through increased microbial activity and uptake of oxygen at a rate exceeding its replacement via diffusion (Aulakh and Rennie, 1987). So, due to the input of organic material, one could expect that N_2O production by denitrification would be more important in this study.

The denitrification potential (N_2 + N_2O) of sandy loam soils from different agro-pedological regions in Flanders varied between 0.72 and 3.72 mg N kg^{-1} soil day^{-1} under saturated conditions and when 50 mg N kg^{-1} soil (as KNO_3) was added as N source, and without addition of a C source (D'Haene et al., 2003). The fact that the conditions for denitrification were not optimal (80% WFPS, no extra N added), and that only N_2O emission was measured, may at least partly explain the difference with this previous study. Nevertheless, the

average N_2O flux of the unamended soil in this study was quite low (0.029 mg N kg^{-1} soil day^{-1}) although the soil had a large total N (0.17%) and mineral content (39.4 mg N kg^{-1} soil).

Denitrifying organisms use organic C compounds as electron donors for energy and for synthesis of cellular constituents. Therefore, denitrification is strongly dependent on the availability of organic compounds such as native soil organic matter, crop residues, etc. Since much of the total soil organic C is highly resistant to decomposition, it has been reported that denitrification correlates better with glucose equivalent C (Stanford et al., 1975), water soluble C (Bijay-Singh et al., 1988; Schloemer, 1991; Hill and Cardaci, 2004) and mineralizable C (Bijay-Singh et al., 1988; Hill and Cardaci, 2004), which are easily decomposable. In this study, the TSOC content (water-soluble) was used as a measure for the amount of readily available C. At the start of the incubations, the TSOC content was similar amongst treatments, probably due to the large input of water-soluble C from the celery leaves (887.5 mg C kg^{-1} soil). During the incubations the TSOC content decreased. Possible explanations for this decrease are adsorption to soil particles, C immobilization into soil microflora and respiration. No correlations were found between TSOC and cumulative N_2O emission, what may indicate that easily available C was not a limiting factor in this study.

The rather high and comparable mineral N content in the soils of the different treatments at the start of the N_2O measurements (on average 47 mg N kg^{-1} soil) was due to the large mineral N content of the soil (39.4 mg N kg^{-1} soil) at the time that it was sampled. The celery residues only added 6 mg of mineral N kg^{-1} (14.6% of the mineral N content at the start). The mineral N input of the immobilizer wastes was negligible, except for paper sludge which supplied 10.0 mg NH_4^+-N kg^{-1} soil (Table 4.1). During the N_2O measurements the mineral N content decreased from start till end. Possible explanations for this decrease in mineral N in this incubation experiment are gaseous N losses (N_2O, NO, N_2, NH_3) and N immobilization. Since the N_2O emission only ranged between 8.2 and 28.9 % of initial mineral N content in the soil, and

NH_3 volatilisation was most probably negligible since the slightly acidic soil, the decrease was mainly due to either N_2 emission or N immobilization. Whether N_2 emission or N immobilization was the dominant process depended on which process was the fastest in using the available mineral N, and on the degree of aeration in the specific treatment. The fraction of N_2 in denitrification increases with increasing organic C, WFPS, pH and temperature, and the effect of organic C depends on the degree of anaerobiosis generated by the microbial activity (Stevens and Laughlin, 1998). Also in the celery only treatment, with a relatively low C:N ratio, N immobilization could have occurred. Jensen (1994b) reported of a short net N immobilization phase (10 to 30 days) followed by net N mineralization after incorporation of pea residues with a C:N ratio of 19.4 and a small particle size (1 to 10 mm). Due to dried and ground celery residues with an overall C:N ratio of 19.1 and incubations lasting maximum 4 days, significant N immobilization may have occurred, even in the celery only treatment.

When the celery residues were mixed with an immobilizer waste, the cumulative N_2O emission was each time lower than in the celery only treatment, except in combination with paper sludge. First, paper sludge delivered a large amount of NH_4^+-N to the soil. Baggs et al. (2002) also found an increased NH_4^+-N content in the soil and a higher N_2O emission after incorporating paper mill sludge together with calabrese residues. Secondly, the paper sludge had a low C:N ratio (C:N 18.4) compared to paper sludge used in some other studies (C:N 86, Aitken et al., 1998; C:N 24.6, Baggs et al., 2002; C:N 520, Rahn et al., 2003), and even lower than the C:N ratio of the celery residues. Therefore, it is reasonable to assume that the paper sludge used in our study had a limited N immobilization potential, and that a larger amount of mineral N in the soil was available compared to the celery only treatment, leading to more denitrification and N_2O emission. This is confirmed by the significantly higher amount of mineral N at the end of the incubations in the paper sludge treatment compared to the other treatments (Table 4.3).

The other immobilizer wastes significantly decreased the N_2O emission, either due to their N immobilization potential or to more reduction of N_2O to N_2. The N immobilization potential of all immobilizer wastes was probably fast and large since the materials were added dried and milled, leading to a fast depletion of the mineral N pool, and hence a reduced N_2O emission. Drying and grounding of residues has certainly a profound effect on residue N mineralization kinetics (Moorhead et al., 1988). By drying and grounding, the specific surface of the residues increases substantially, exposing nearly all of the added material to direct microbial attack. Cell constituents are released immediately in the soil. So, any effect will be more pronounced when materials are added ground and milled, and it must be stressed that in field conditions, the N immobilization can be expected to be much slower and lower, leading to a higher mineral N content in the soil, and possibly to a lower reduction in N_2O emission by the wastes. However, on the other side, adding ground and dried immobilizer wastes could have caused more reduction of N_2O to N_2, and so, a reduced N_2O emission. The dried and ground immobilizer wastes were probably decomposed quite rapidly, leading to fast oxygen depletion and more anaerobic conditions, favourable for reduction to N_2. Although N_2 emission means an economical loss of N, it has no direct negative impact on the environment. Therefore, even if N_2 emission was the major process, incorporating immobilizer wastes together with N-rich crop residues would still be beneficial for the environment, since losses of reactive N to the environment (N_2O emission and NO_3^- leaching) would be reduced.

The N_2O flux pattern of tannic acid (Fig. 4.1 and 4.2) was quite different from the pattern of the other immobilizer wastes with a N_2O reduction potential. The N_2O emission started faster (at 4.5 h the N_2O fluxes were 0.1 and <0.05 mg N_2O kg^{-1}soil h^{-1} for tannic acid and the other immobilizer wastes, respectively) and lasted longer (at 52.5 h the N_2O-N fluxes were 0.036 and 0.002 mg N_2O-N kg^{-1}soil h^{-1} for tannic acid and other immobilizer wastes, respectively), but the maximum N_2O flux was lower (0.16 and >0.23 mg N_2O-N kg^{-1} soil h^{-1} for tannic acid an the other immobilizer wastes, respectively). The reason for the different N_2O emission pattern was probably the different mechanism of action

of the tannic acid compared to the other immobilizer wastes. Polyphenolic compounds are toxic for several micro-organisms, including bacteria, fungi and micro fauna involved in N mineralization, on one side (Scalbert, 1991; Capasso et al., 1995; Hewlett et al., 1997), and have a strong protein binding capacity through their strong affinity for amide groups, on the other side. The polyphenol effect on N availability therefore is not immobilization of mineralized N as for the other immobilizer wastes, but inhibition of N mineralization through the above mentioned effects.

Finally, it should be stressed that the experiment in this study was a short time laboratory incubation, and that long term field experiments are necessary for verification. First, the immobilizer wastes in this study were added dried and ground, and hence, the effect of incorporation of fresh materials under field conditions should be further studied to pass from 'reduction potential' to the actual emission reduction to be expected. Secondly, it is mostly found that freshly added N is first immobilized into microbial biomass, then transformed by microbial activity in other forms, and finally, released into the soil as mineral N. Hence, it is possible that on the long term N_2O emission will restart.

4.5 Conclusions

In the past, immobilizer wastes have been shown to have a potential to reduce NO_3^- leaching after incorporation of high N crop residues. However, the effect of immobilizer wastes on N_2O emission has received only limited attention. This study has shown that immobilizer waste application, in general, reduced N_2O emission when applied simultaneously with a high N crop residue like celery (N_2O reduction between 40 and 60% compared to the treatment with only celery residues under optimised laboratory conditions). The paper sludge, used here, increased the N_2O emission, but this was due to an unusually low C:N ratio. Long term field experiments are necessary to pass from 'reduction potential' to the actual emission reduction to be expected.

Chapter 5

Manipulating the N release from ^{15}N labelled celery residues by using straw and vinasses under laboratory conditions

Chaves B, De Neve S, Boeckx P, Berko C, Van Cleemput O, Hofman G (2006) Manipulating the N release from ^{15}N labelled celery residues by using straw and vinasses (submitted).

Abstract The aim of this laboratory study was to investigate the effect of straw and vinasses on the N mineralization-immobilization turnover of celery residues during two simulated periods under laboratory conditions. During the first simulated period, ^{15}N labelled celery residues were incubated together with straw, in order to immobilize the N released from celery residues, followed by an incorporation of vinasses after 84 days, aiming to remineralize the immobilized celery-N. During the second period, the set-up of the experiment was repeated, except that non-labelled celery residues were used. Total N, mineral N and their ^{15}N enrichments as well as microbial biomass N were determined at regular time intervals. During both periods, mixing celery residues with straw significantly increased microbial biomass N (90.5 and 40.5 mg N kg^{-1} extra compared to celery only treatment) and decreased the amount of total mineral N (reduction of 56.1 and 45.9 mg N kg^{-1} soil compared to celery only treatment) and celery derived mineral ^{15}N (-1.2% and -0.3% of celery derived ^{15}N in mineral N pool in straw treatment compared to 36% of celery derived ^{15}N in celery only treatment). After *ca.* 100 days, remineralization occurred in the straw treatment (32.2 and 11.1 mg N kg^{-1} soil), but the mineral N content remained significantly lower than in the celery only treatment during the complete experiment. The amount of remineralized celery-^{15}N was very low (5.6% of celery derived ^{15}N after 380 days). Vinasses caused no real priming effect, although it did slightly increase the amount of remineralized celery-^{15}N (+6.6% of celery derived ^{15}N after 380 days compared to the straw treatment) probably due to a pool substitution effect.

5.1 Introduction

Immobilizer wastes like straw, paper waste, green waste compost and saw dust have already been shown to possess an N immobilization potential under controlled conditions (Vityakon et al., 2000; Rahn et al., 2003; De Neve et al., 2004; Chaves et al., 2005a). The intensity of immobilization seems to be manageable by the right choice of organic wastes (De Neve et al., 2004). Especially easily decomposable wastes (i.e. low lignin content) with a large C:N ratio seem to have a potential to immobilize N (Chaves et al., 2005a). Some laboratory studies also suggested that it is possible to remineralize immobilized N by incorporating other organic wastes, like molasses (Rahn et al., 2003; De Neve et al., 2004) and vinasses (Chaves et al., 2005a).

Incorporating ^{15}N labelled crop residues in soil allows to follow the released residue-N into different soil N fractions (Müller and Sundman, 1988; Thomsen, 1993; Jensen et al., 1997; Wivstad, 1999). Comparing the distribution of released residue-^{15}N across the different N fractions when these crop residues are incorporated with or without an immobilizer or remineralizer waste, may indicate in which N fraction the crop residue-N is immobilized, how remineralization of immobilized N occurs and how a remineralizer waste influences this remineralization.

The aim of this study was to examine the effect of straw (as *immobilizer waste*) and vinasses (as *remineralizer waste*) on the N mineralization-immobilization turnover (NMIT) of celery residues. The incubation experiment imitated two periods, each with an immobilization and a remineralization phase. During the first period, ^{15}N labelled celery residues were incubated together with straw, in order to immobilize N released from celery residues, followed by an incorporation of vinasses after 84 days, aiming to remineralize the immobilized N. During the second period, the set-up of the experiment was repeated, except that non-labelled celery residues were used,

in order to be able to further follow the distribution of celery-^{15}N added in the first period.

5.2 Materials and methods

Crop residues and organic wastes

Celery residues (*Apium graveolens* L.) were chosen as crop residues because of their high N mineralization potential (Scharpf, 1991; De Neve and Hofman, 1996). The residues were separated into leaves and stems, dried at 55°C, ground and added in a known ratio (leaves:stems= 1:1.2 on dry matter) in order to obtain a more homogeneous N mineralization. Cereal straw was chosen as *immobilizer waste* since it has a high C:N ratio and has already proven to have a N immobilization potential (Rahn et al., 2003; De Neve et al., 2004; Chaves et al., 2005a). As *remineralizer waste* vinasses, from the sugar industry, was chosen, because of its high content of easily decomposable C, since it has been shown that addition of C in the form of sugars (= readily available C) can lead to a marked increase in soil microbial activity (Falih and Wainwright, 1996).

Subsamples of celery residues, straw and vinasses were dried at 55°C until constant weight for determination of the dry matter content, and ground for further chemical analysis. Total C and N content of the materials was determined by dry combustion using a CNS elemental analyser (Variomax CNS, Elementar, Germany). ^{15}N atom% of total N was determined using a elemental analyser (ANCA-SL, PDZ Europa, UK) connected to an Isotope Ratio Mass Spectrometer (IRMS) (Model 20-20, Serlon, UK) (Table 5.1).

Incubation experiment

The soil used in the incubation experiment was the top layer (0-20 cm) of a silt loam soil collected from the vegetable growing region in East-Flanders (Belgium). The soil contained 13.6% clay, 51.8% silt and 34.6% sand, had a pH_{KCl} of 6.4, a total N content of 0.17%, a C content of 1.62% a NO_3^- content

of 36.0 mg N kg^{-1} and a NH_4^+ content of 1.2 mg N kg^{-1}. Visible impurities (e.g. stones, roots) were removed from the soil, but the soil was not sieved and not air-dried in order to minimize the disturbance of microbial activity before the start of the experiment.

Table 5.1 Chemical composition of celery residues and organic wastes

	First period						*Second period*				
	DM_{ap} g kg^{-1}	N mg kg^{-1}	DM %	N_{tot} g kg^{-1} DM	^{15}N atom%	C:N	DM_{ap} g kg^{-1}	N mg kg^{-1}	DM %	N_{tot} g kg^{-1} DM	C:N
Celery residues											
Leaves	0.50	29.8	12.1	59.4	8.57	6.45	0.50	24.5	10.8	49.4	7.52
Stems	0.58	25.0	5.67	41.7	10.8	7.44	0.58	17.6	7.15	30.2	11.6
Leaves+stems[a]	1.08	54.8	8.61	49.8	9.75	6.99	1.08	42.1	8.82	39.0	9.70
Immobilizer waste											
Straw	8.10	38.3	94.0	4.7	n.a.[b]	98.7	7.90	52.2	91.4	6.6	65.3
Remineralizer waste											
Vinasses	1.88	110.5	65.0	58.9	n.a.[b]	7.00	1.88	110.5	65.0	58.9	7.00

[a] sum/weighed average; [b] n.a. not applicable; DM_{ap}: applied dry matter to soil; N: applied total N to soil; DM: dry matter; N_{tot}: total N content)

At the start of the first simulated period, fresh soil (equivalent to 17 kg dry soil) was thoroughly mixed with dried ^{15}N labelled celery leaves and stems (equivalent to 20 t fresh matter (FM) ha^{-1}) and straw (equivalent to 5 t C ha^{-1}) and incubated in plastic containers (width= 33 cm; length= 40 cm; filling height= 10 cm; bulk density= 1.3 g cm^{-3}). After filling the containers, distilled water was added to obtain a moisture content of 50% WFPS (water-filled pore space) and the containers were covered with a single layer of gas permeable parafilm to minimize water loss. The actual moisture content in de containers was 47% WFPS. The containers were incubated at constant temperature (15°C). The treatments were (1) unamended soil, (2) celery only (= soil + celery residues) and (3) straw (= soil + celery residues + straw), in duplicate. For the unamended soil and straw treatment, two extra containers were filled to which vinasses (equivalent to 1 t C ha^{-1}) was added after 84 days. At that time

the other containers received an equivalent amount of distilled water as contained in vinasses. To this end the containers were emptied, the soil mixture was thoroughly mixed with vinasses or water, and the containers were refilled and incubated as described earlier. From that time on, the treatments were: (1) unamended soil, (2) celery only (= soil + celery residues), (3) straw (= soil + celery residues + straw), (4) soil + vinasses (= unamended soil + vinasses) and (5) straw + vinasses (= soil + celery residues + straw + vinasses), in duplicate.

At the start of the second simulated period (198 days), the containers were emptied again, the soil mixtures were thoroughly mixed with non-labelled celery residues (equivalent to 20 t FM ha^{-1}) and straw (equivalent to 5 t C ha^{-1}) (in the corresponding treatments) and the containers were refilled and incubated as described earlier. After 316 days, vinasses (equivalent to 1 t C ha^{-1}) was added to the soil + vinasses and straw + vinasses treatment, while the other treatments received an equivalent amount of distilled water. The procedure and treatments were the same as described earlier.

During the experiment samples were taken with a small auger. Each container was sampled by taking four augerings and the soil was bulked into one collective sample per container. Samples were taken 21, 42, 84, 104, 142, 198, 230, 244, 258, 283, 316, 338, 358 and 380 days after the start of the incubation.

Chemical analyses

Total N and its ^{15}N atom% in soil were determined as for the celery residues. *Mineral N* was extracted with (*1N*) KCl (extraction ratio= 1:2) and the extracts were analysed for NH_4^+-N and NO_3^--N colorimetrically with a continuous flow auto-analyser (Chemlab System 4, Skalar, The Netherlands). The ^{15}N atom% of NO_3^--N was determined by converting NO_3^--N to N_2O-N (Stevens and Laughlin, 1994), and measuring ^{15}N atom% of N_2O using a trace gas module (ANCA-TGII, PDZ Europa, UK) connected to an IRMS (Model 20-20, SerCon, UK). Only the ^{15}N atom% of NO_3^- was determined since the NH_4^+

concentrations were very small compared to NO_3^-. *Microbial biomass C* was determined by fumigation-extraction according to Voroney et al. (1993) using a 24h fumigation time, a (*0.1N*) KCl extractant, a soil-to-extractant ratio of 1:2 (both for fumigated and non-fumigated samples) and a conversion factor k_{EC} of 0.25. Total organic C of the extracts was determined using a Total Organic Carbon analyser (TOC-V_{CPN}, Shimadzu, Japan). *Microbial biomass N* was not determined directly, but was calculated from microbial biomass C, due to problems with the TN module of the Total Organic Carbon analyser (TOC-V_{CPN}, Shimadzu, Japan), using a C:N ratio of 6.0, which can be considered as the average C:N ratio of microbial biomass (Powlson et al., 1987; Jensen et al., 1997; Jedidi et al., 2004). The use of a similar C:N ratio for the different treatments is supported by several studies that found no significant changes in C:N ratio after addition of organic materials (Ocio et al. 1991; Bremer and van Kessel, 1992; Joergensen et al., 1994; Jensen et al., 1997).

Calculations and statistics

In the so-called 'difference method', the *net N mineralization of the celery residues* was calculated as the difference between the mineral N content in the celery only treatment and the mineral N content in the unamended soil. The *N immobilization of straw* was calculated as the difference between the mineral N content in the straw treatment and the mineral N content in the celery only treatment. The *net N release from vinasses* was calculated as the difference between the mineral N content in the soil + vinasses treatment and the mineral N content in the unamended soil. The *priming effect (or remineralization of immobilized N) caused by vinasses* was calculated as the mineral N content in the straw + vinasses treatment (treatment 5) minus the mineral N content in the celery residues + straw treatment (treatment 3), minus the net N release of vinasses (calculated from treatments 1 and 4).

With the ^{15}N method, the total ^{15}N recovery (%) was the total amount of celery derived ^{15}N recovered in soil expressed as % of the total amount of ^{15}N added. The percentage of ^{15}N in mineral N was the amount of celery derived ^{15}N recovered in the mineral N pool expressed as % of the total amount of ^{15}N

added. To calculate the N immobilization of celery-N by straw and the remineralization of immobilized celery-^{15}N by vinasses, the percentage of ^{15}N in mineral N was converted to mg N kg^{-1} soil by multiplying by the total amount of celery-N added, and then the calculations were the same as for the difference method.

Analysis of variance and a Duncan post hoc test (One-way ANOVA in SPSS) was used to determine variances attributable to treatments.

5.3 Results and discussion

Total N and total ^{15}N recovery

Total N was quite constant during the complete incubation period, and no significant differences could be found between the different treatments. Total N was on average 1.97 ± 0.11 g N kg^{-1} soil in all treatments. The ^{15}N atom% in the unamended soil and soil + vinasses treatment was at natural abundance level (on average 0.3705%) during the complete experiment. The ^{15}N atom% in the celery only, straw and straw + vinasses treatments was significantly higher than in the unamended soil, but between these treatments no significant differences could be found (^{15}N atom% was on average 0.6367%). The ^{15}N recovery was on average 98.2% (± 6.0%), indicating that almost no celery-^{15}N losses occurred. The only possible N losses from the plastic containers were gaseous losses (N_2, NO, N_2O, NH_3), but they seemed to be insignificant due to the low WFPS (47%) and low pH (6.4).

Microbial and mineral N in unamended soil

At the start of the experiment, a rather low microbial biomass N was 17.7 ± 1.1 mg N kg^{-1} soil (0.9% of total N) was found in the unamended soil (Fig. 5.1). Directly after the start of the first period, the microbial biomass N increased in the unamended soil, probably due to some disturbance of the soil when the plastic containers were filled. After 84 days, the microbial biomass N remained more or less constant and varied around 23.4 mg N kg^{-1} soil (1.2 % of total N).

Also the N mineralization rate of the unamended soil was rather low, especially for a soil used for vegetable production, namely 0.09 mg N kg^{-1} soil day^{-1} (~ 0.4 kg N ha^{-1} day^{-1} for a depth of 30 cm) (Fig. 5.2). N mineralization rates between 0.9 and 1.6 kg N ha^{-1} day^{-1} have been found for soils used for intensive vegetable production in Flanders (Demyttenaere, 1991).

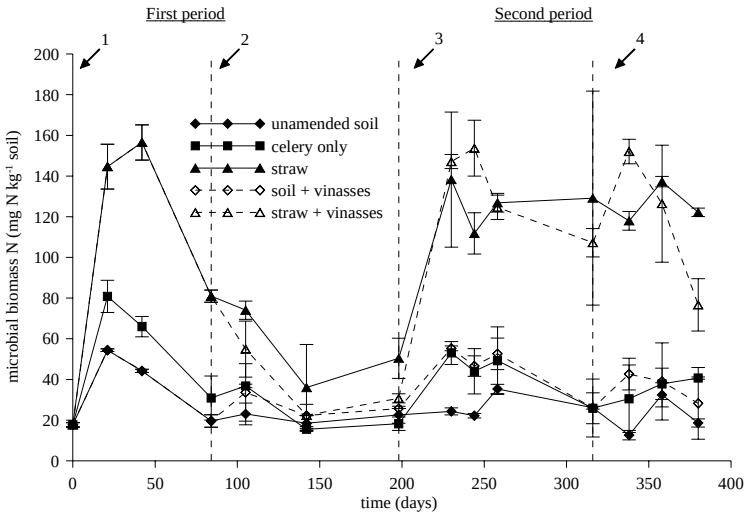

Fig. 5.1 Microbial biomass N in the different treatments; error bars are standard deviations; 1= incorporation of ^{15}N labelled celery residues and straw; 2= incorporation of vinasses; 3= incorporation of non-labelled celery residues and straw; 4= incorporation of vinasses

Microbial and mineral N in celery residues only treatment

At the start of the first period, microbial biomass N increased significantly ($P<$ 0.05) after incorporation of ^{15}N labelled celery residues compared to the unamended soil, and peaked at 21 days after incorporation when it was 26.4 mg N kg^{-1} soil (49% of added celery-N) higher than the unamended soil (Fig. 5.1). After 21 days, microbial biomass N decreased rapidly, and at day 84 no significant differences could be found between the celery only treatment and the unamended soil. Also, the mineral N content and the percentage of celery derived ^{15}N in the mineral N pool increased rapidly during the first days after incorporation of the ^{15}N labelled celery residues compared to the unamended

soil (Fig. 5.2 and 5.3). The percentage of celery derived ^{15}N recovered in the mineral N pool followed a similar pattern as the net mineral N content (difference method), indicating that the extra mineral N found in the celery only treatment was released effectively by the celery residues (data not shown). The net amount of mineral N released from the ^{15}N labelled celery residues by the end of the first period (198 days) was 16.3 mg N kg^{-1} soil (30% of added celery-N) as calculated by the difference method, or 36% of celery derived ^{15}N as calculated by the ^{15}N method. The N release did not differ significantly between both calculation methods. After 198 days, mineral ^{15}N remained more or less constant until the end of the experiment (36% of celery derived ^{15}N by day 380).

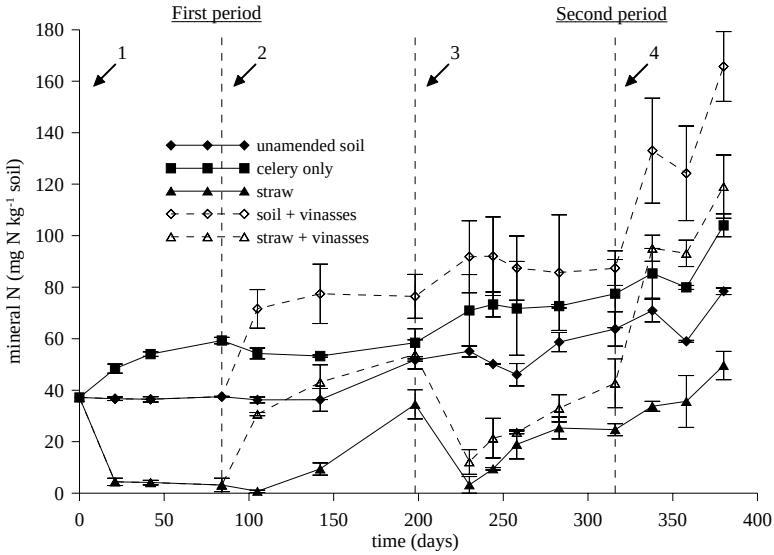

Fig. 5.2 Mineral N in the different treatments; error bars are standard deviations; explanation numbers see Fig. 5.1

At the start of the second period, the incorporation of non-labelled celery residues caused a new increase in microbial N, which peaked after 230 days and was 28.3 mg N kg^{-1} soil (67% of newly added celery-N) higher compared to the unamended soil (Fig. 5.1). The peak was again followed by a decrease, and by day 312, no more significant differences in microbial biomass N could

be found between the celery only treatment and the unamended soil. Again the mineral N content increased as well, and the net N release after the incorporation of the non-labelled celery residues was 14.4 mg N kg^{-1} (34% of added N) by day 380.

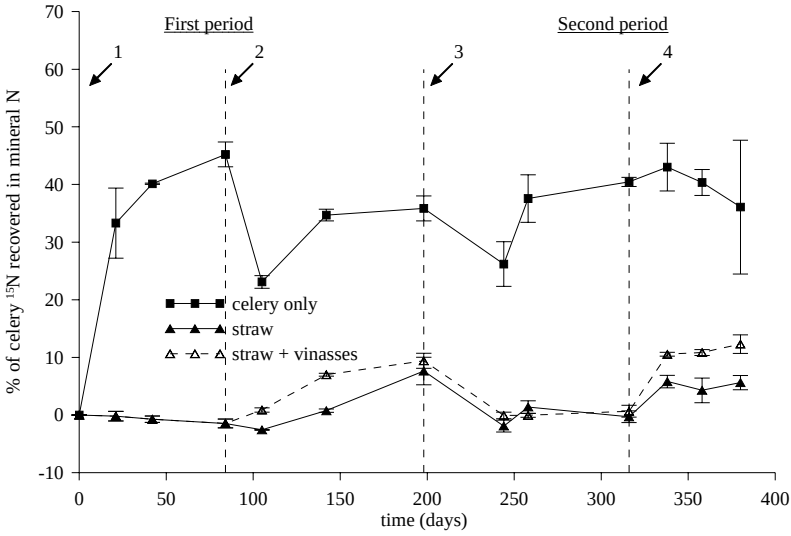

Fig. 5.3 Percentage of celery derived ^{15}N in mineral N pool; error bars are standard deviations; explanation numbers see Fig. 5.1

Celery residues are easily decomposable, containing a large amount of water-soluble compounds (30 to 40% on organic matter; Chaves et al., 2005a, 2005b). Since it is believed that bacteria are the main decomposers of soluble compounds (Mary et al., 1996), the celery residues were probably quickly colonized and decomposed by a bacterial population, leading to fast immobilization of nutrients into microbial biomass, explaining its increase in microbial biomass N after incorporation of celery residues. A fast increase (within 30 days) in microbial biomass N (between 14 and 27% of added N) after incorporation of N-rich crop residues has previously been found (Jensen, 1994a; Bending et al., 1998; Rahn et al., 2003). The larger immobilization of celery-N into microbial biomass N in this study may be due to use of dried and ground celery residues. Ground residue material is more accessible to micro-

organisms than intact plant parts, due to the increased surface area of the residues exposed to decomposition (Angers and Recous, 1997) and the lack of intact lignified barrier tissue (Summerell and Burgess, 1989). Therefore, the initial colonization rate of the residues is favoured. The increase in microbial biomass N in the second period, after the incorporation of the non-labelled celery residues, was extremely high compared to other studies (Jensen, 1994a; Bending et al., 1998; Rahn et al., 2003). This was probably due to some reimmobilization of celery-^{15}N into the microbial biomass, next to N immobilization of the new non-labelled celery-N, as suggested by a decrease in the percentage of celery derived ^{15}N in the mineral N pool a around day 244 (Fig. 5.3).

In both periods, after peaking, microbial N showed a fast decrease and after *ca.* 100 days no more significant differences between the celery only treatment and unamended soil could be found. This rapid decrease could be associated with the rapid decrease of the bacterial population decomposing the celery residues when the easily available C became limiting (Swift et al., 1979; Mary et al., 1993). This will have partially accounted for the increase in mineral N observed in the celery only treatment next to the release of excessive crop residue-N which was not needed for the biosynthesis of the micro-organisms. Under field conditions, the released celery-N would be at risk for N loss processes like leaching and denitrification. The potential N losses after the incorporation of celery residues would correspond with *ca*. 65 kg N ha^{-1} (~ 30 cm depth) under field conditions.

Microbial and mineral N in celery residues plus straw treatment

Mixing celery residues with straw led to an extra increase in microbial biomass N compared to the celery only treatment in both periods (Fig. 5.1), while the mineral N content decreased to levels below those in the unamended soil during the complete experiment (Fig. 5.2). During the first period, maximum microbial biomass N in the straw treatment occurred after 42 days, and was 112.3 mg N kg^{-1} soil higher compared to the unamended soil or 122% of total added N (= celery-N + straw-N), indicating that also soil-N was immobilized.

After 42 days, microbial biomass N showed a decrease (Fig. 5.1), but remained significantly ($P< 0.05$) higher than in the unamended soil or celery only treatment during the rest of the first period. During the second period, microbial biomass N also increased after incorporation of straw and at day 230 it was 68.8 mg N kg^{-1} soil (73% of total added N) higher than the microbial biomass N at the end of the first period in the straw treatment. Thereafter, microbial biomass N remained more or less at that level until the end of the experiment. After 380 days, microbial biomass N in the straw treatment was still significantly ($P< 0.01$) higher compared to the unamended soil and celery only treatment, and was around 118.9 mg N kg^{-1} soil (6.0% of total N).

In both immobilization periods, the mineral N content in soil decreased rapidly after incorporation of straw and was on average 3.1 mg N kg^{-1} soil during the first 105 days in the first period, and on average 6.3 mg N kg^{-1} soil during the first 46 days in the second period. Also the percentage of celery derived ^{15}N in the mineral N pool was very low and even negative during the first *ca*. 100 days (-1.2% of celery derived ^{15}N in first period; -0.3% of celery derived ^{15}N in second period) also indicating that next to celery-N some soil-N was immobilized by straw, and that during the second period, a portion of remineralized celery-^{15}N was again immobilized in the microbial biomass. Hence, during the first period, straw immobilized up to 56.1 mg N kg^{-1} soil (~ 219 kg N ha^{-1} for a 30 cm depth) of which 25.1 mg N kg^{-1} soil was celery-^{15}N, and during the second period, straw immobilized up to 45.9 mg N kg^{-1} soil (~ 179 kg N ha^{-1} for a 30 cm depth) of which 6.7 mg N kg^{-1} soil was celery-^{15}N (Fig. 5.4). Maximum N immobilization was followed by some remineralization in both periods, but the mineral N content remained significantly ($P< 0.05$) lower than in the celery only treatment and even than in the unamended soil during the complete experiment. By the end of the first period, 32.2 mg N kg^{-1} soil was remineralized, and by the end of the second period, a remineralization of 11.1 mg N kg^{-1} soil was observed. Remineralization in both periods was accompanied by an increase in the percentage of celery-^{15}N in the mineral N pool (7.6% of celery derived ^{15}N by day 198; 5.6% of celery derived ^{15}N at day 380) (Fig. 5.3). However, the

increase in total mineral N was larger than the percentage of celery derived ^{15}N in the mineral N pool, indicating that mainly remineralization of non-labelled N in stead of immobilized celery-^{15}N was responsible for this.

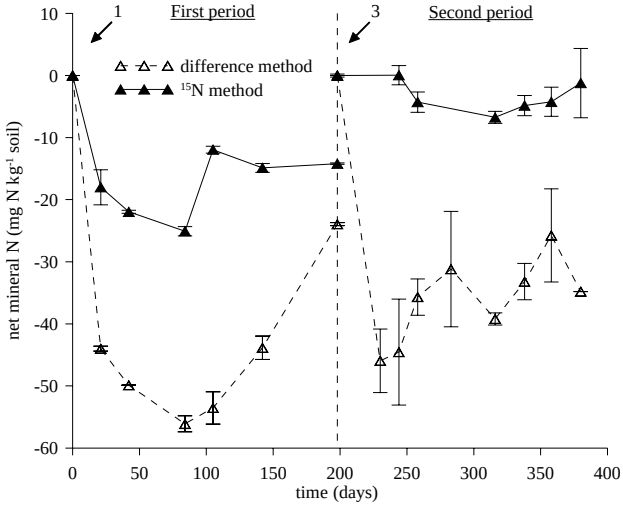

Fig. 5.4 N immobilization potential of straw; error bars are standard deviations; explanation numbers see Fig. 5.1

Straw has been proven to have an N immobilization potential, but to a lesser extent (maximum 30 mg N kg^{-1} soil) (De Neve et al., 2004; Chaves et al., 2005a). The high N immobilization potential of straw in this study can be attributed to the use of ground material of both celery residues and straw which were mixed intimately with soil. As previously mentioned, the initial colonization rate, and therefore the decomposition of dried and ground residues is favoured. Since an increased decomposition means a higher N release from the decomposing celery residues and a higher N demand by the micro-organisms during straw decomposition, N immobilization will increase.

The fast increase in microbial biomass N after incorporation of straw was probably due to a fast growing population of bacteria decomposing the water-soluble, non-N-limited compounds in straw (Cochran et al., 1988; Mary et al., 1996; Jensen et al., 1997). When the easily decomposable C became limiting,

the bacteria decayed releasing the immobilized non-labelled straw-N (Cochran et al., 1988) what explains the observed remineralization of especially non-labelled N in both periods. Fungi probably decomposed the slowly available, N limited components in straw, like cellulose and lignin, using celery-N for the build-up of their biomass (Mary et al., 1996). The degradation of these components is more difficult and slower, leading to the significantly higher microbial biomass N in the straw treatment compared to the celery only treatment until the end of the experiment (Cochran et al., 1988; Recous et al., 1995).

It has been found that straw incorporation reduces NO_3^- leaching during the first winter, but that it may lead to higher mineral N contents and higher nitrate leaching on the long term due to a higher microbial biomass and N release from this biomass (Nicholson et al., 1997; Catt et al., 1998; Silgram and Chambers, 2002). Remineralization was observed in this study, but the mineral N content in the straw treatment remained significantly lower than in the unamended soil and celery only treatment during the complete experiment. Hence, in the time span of this experiment there were no indications that incorporation of straw enhanced N mineralization or NO_3^- leaching. At day 380, microbial biomass N was still significantly higher in the straw treatment than in the unamended soil or celery only treatment. Part of this microbial N will eventually become part of the stable soil organic matter (SOM) pool whereas another part will be mineralized, and the partitioning between those two pools will determine the potential long term NO_3^- leaching loss.

Microbial and mineral N after application of vinasses

In the straw + vinasses treatment, vinasses was added after 84 days and after 312 days in order to enhance remineralization of immobilized celery-N (i.e. priming effect). Although it is widely accepted that micro-organisms play a crucial role in the process, the mechanisms leading to priming effects remain poorly understood. The most common explanation for priming effects is an increased soil organic matter decomposition as a result of a higher microbial population or activity due to the higher availability of energy and nutrients

from added organic materials (Sörensen, 1974; Kuzykov et al., 2000; Fontaine et al., 2003).

Compared to the straw treatment where no vinasses was added, microbial biomass N was not significantly influenced when vinasses was added in both periods in the straw + vinasses treatment (Fig. 5.1). The total amount of mineral N in soil increased significantly after vinasses addition, but this was mainly due to a large net N release from the vinasses itself, on average 36.9 mg N kg^{-1} soil during the first period and 47.9 mg N kg^{-1} during the second period, as was observed in the soil + vinasses treatment compared to the unamended soil. According to the difference method, vinasses was not able to remineralize immobilized N (i.e. no real priming effect) in both periods (Fig. 5.5). However, after 198 and 380 days, the percentage of celery-^{15}N recovered in the mineral N pool was higher when vinasses was added compared to the straw treatment (+1.7% of celery derived ^{15}N in mineral N pool at day 198 and +6.6% of celery derived ^{15}N at day 380 compared to straw treatment) (Fig. 5.3). The ^{15}N method indeed showed that vinasses did slightly stimulate the remineralization of immobilized celery-^{15}N, with a remineralization of 10.9 mg N kg^{-1} at the end of the first period, and of 8.6 mg N kg^{-1} at the end of the second period (Fig. 5.5). However, this was probably due to a pool substitution effect rather than a real priming effect. In the straw + vinasses treatment, the available amount of non-labelled mineral N in soil was much larger then in the straw treatment. Therefore, the fungi decomposing the slowly available components in straw, probably used relatively more non-labelled N than remineralized celery-^{15}N in the straw + vinasses treatment, leading to more celery derived mineral ^{15}N remaining in soil.

A possible reason for the low remineralization potential of vinasses may be an unsuitable biochemical composition, e.g. not enough readily available C, for inducing N priming effects, since most researchers found N priming effects after incorporation of low molecular weight compounds like glucose (Asmar et al., 1994; Falih and Wainwright, 1996; Wheatly et al., 2001).

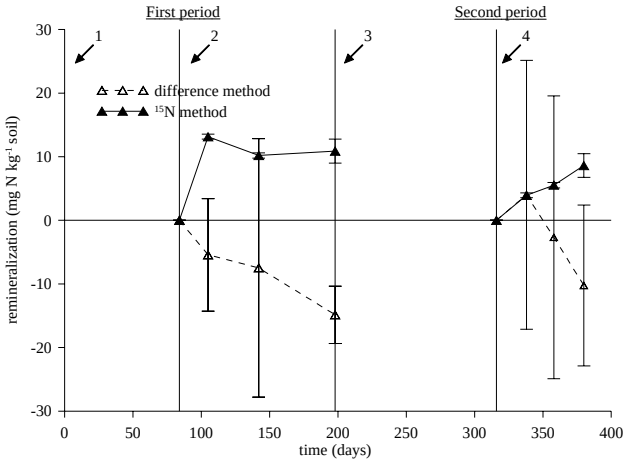

Fig. 5.5 Remineralization of immobilized N (real priming effect) induced by vinasses; error bars are standard deviations; explanation numbers see Fig. 5.1

Difference method and ^{15}N method

The main drawback of the difference method compared to the ^{15}N method is that it assumes that the basal N mineralization is the same in the presence and absence of residues (Watkins and Barraclough, 1996). Several studies have confirmed that this assumption is not always correct and that N release from soil organic matter may increase or decrease after incorporation of fresh organic matter (Cadisch et al., 1998; Wivstad, 1999; Kuzyakov et al., 2000). However, in this study, the amount of N released from the labelled celery residues did not differ significantly between both calculation methods, indicating that the incorporation of celery residues did not affect the N release from the soil organic matter. This may also indicate that the basal microbial biomass was not influenced by the incorporation of celery residues, and that the increase in microbial biomass N was solely due to the immobilization of celery-N into the microbial biomass.

Another difference between the two calculation methods, especially when examining the N immobilization by straw and the remineralization by vinasses, is that the difference method does not distinguish between the immobilization

or remineralization of soil-N and celery-N, while the ^{15}N method only shows the immobilization or remineralization of the celery derived ^{15}N. Straw immobilized both soil-N and celery-N which was not shown by the results of the ^{15}N method. Hence, the ^{15}N method underestimated the N immobilization potential of straw while immobilization of soil-N may also be valuable when aiming to reduce nitrate leaching. Also the remineralization of immobilized N by vinasses was quite different between the two calculation methods. A priming effect occurs when the N release is higher than the sum of the N release from the added material and the N release from the soil itself, meaning that an extra amount of mineral N is released from soil organic matter which can be both soil organic N or recently immobilized celery-N (Jenkinson et al., 1985; Powlson and Barraclough, 1993). According to the difference method a complete priming effect is calculated (i.e. the release of both soil-N as celery-N), while the ^{15}N method only gives the remineralization of celery derived ^{15}N. Hence, the difference method gives a better idea of the total amount of mineral N available for the subsequent crop, while the ^{15}N method only indicates whether the immobilized celery-N is remineralized or not.

5.4 Conclusions

Mixing celery residues with straw may be a management option to reduce NO_3^- leaching of celery-N during winter. Around 100 days after the incorporation of straw, some remineralization occurred, but the mineral N content remained lower than in the celery only treatment, and even than in the unamended soil, during the complete experimental time (380 days). However, the amount of microbial biomass N at the end of the experiment in the treatment with straw was significantly higher, indicating that there is potential for an enhanced N mineralization and NO_3^- leaching on the long term. Although vinasses slightly increased the remineralization of celery-^{15}N, it was not able to stimulate sufficient remineralization of immobilized N so that a crop would be able to profit from it. This study suggests that it is difficult to obtain remineralization of immobilized N in a consistent manner.

Chapter 6

Screening organic wastes for their potential to manipulate the N release from N-rich crop residues under field conditions

Chaves B, De Neve S, Boeckx P, Van Cleemput O, Hofman G (2006) Manipulating the N release from N-rich crop residues by using organic wastes under field conditions (submitted).

Abstract The potential to manipulate the N release from vegetable crop residues by using organic wastes was tested under field conditions. At the start of the experiment, cauliflower residues (~ ca. 73 t fresh matter ha^{-1}) together with an immobilizer waste (~ ca. 5 t C ha^{-1}: straw, green waste compost, saw dust, paper sludge) were incorporated into a silt loam soil. After 154 days, a remineralizer waste (~ ca. 1 t C ha^{-1}: vinasses, dairy sludge) was incorporated into the soil. During the field experiment, the mineral N content in soil was measured at regular time intervals, and net N release, NO_3^- leaching and denitrification were simulated using a N mineralization/immobilization model coupled to a NO_3^- leaching model. The immobilizer wastes showed a small N immobilization potential (maximally 62 kg N ha^{-1} for compost during 154 days) and a small reduction in nitrate leaching (maximally 37 kg N ha^{-1} for compost during 154 days). The immobilizer wastes reduced the denitrification losses, except for straw. Possible reasons for the low N immobilization potential of the immobilizer wastes are their high lignin content, the low temperatures and wet conditions during the experiment and a heterogeneous mixing between crop residues and organic wastes. There was no consistent remineralization of N after addition of vinasses or dairy sludge in any of the treatments. Reasons for low remineralization could be the low amount of initially immobilized N available for remineralization, and an unsuitable composition of the remineralizer wastes, e.g. not readily decomposable, to induce remineralization, especially for dairy sludge.

6.1 Introduction

Manipulating the N mineralization of N-rich crop residues may be an option to reduce NO_3^- residues in soil, and consequently reduce the risk of NO_3^- leaching. Immobilizer wastes like straw, paper waste, green waste compost, saw dust and polyphenol-rich legume tree prunings have already proved to possess a potential for N immobilization under controlled conditions (Handayanto et al., 1997; Rahn et al., 2003; De Neve et al., 2004; Chaves et al., 2005a). However, only a limited number of studies were conducted under field conditions. Vinten et al. (1998) found in a field study that ploughing in paper mill sludge together with N-rich crop residues reduced NO_3^- leaching by up to 90 kg N ha^{-1}. Vityakon et al. (2000) demonstrated that straw with a high C:N ratio (C:N 79) could delay N mineralization of groundnut or Sesbania by 4 weeks under field conditions.

Some laboratory studies also showed that it is possible to remineralize immobilized N by incorporating other organic wastes, like molasses (De Neve et al., 2004) and vinasses (Chaves et al., 2005a). However, to our knowledge, remineralization of immobilized N has not yet been tested under field conditions.

The aim of this study was to screen different organic wastes for their potential to immobilize N from crop residues or delay their N mineralization (*immobilizer wastes*) during the immobilization phase, and to test organic wastes for their potential to stimulate remineralization of immobilized N by the time a new crop is sown or planted (*remineralizer wastes*) during the remineralization phase.

6.2 Materials and methods

Crop residues and organic wastes

The crop residues used in the experiment included leaves, stems and roots of cauliflower (*Brassica oleracea* var. *botrytis*). As *immobilizer wastes* cereal

straw, green waste compost, saw dust and paper sludge were chosen, since these were shown to possess a potential for N immobilization under controlled conditions (Rahn et al., 2003; De Neve et al., 2004; Chaves et al., 2005a). Green waste compost was collected early in the composting process (i.e. before maturing) because we presumed that immature compost would have a higher N immobilization capacity. Paper sludge was waste of a paper factory producing magazine and newspaper paper. As *remineralizer wastes* vinasses, waste material from the sugar industry, and dairy sludge, waste from the dairy industry, were chosen, since these are widely available organic wastes of which it was expected that they would cause remineralization, because of their high content of easily decomposable C. It has been shown that addition of C in the form of sugars can lead to a marked increase in soil microbial activity (Falih and Wainwright, 1996).

Subsamples of the crop residues and organic wastes were dried at 55°C until constant weight for the determination of the dry matter content, and then ground for further analyse. Total C and N contents were determined using a CNS elemental analyser (Variomax CNS, Elementar, Germany). The Stevenson fractionation, modified by De Neve and Hofman (1996), and the CNS elemental analyser were used to determine the lignin fraction (+ structural proteins) and its C:N ratio, since these parameters were needed to determine the N mineralization or immobilization of the different materials. The results of the biochemical analysis are presented in Table 6.1.

Field site

The field experiment was set up on a silt loam soil in the vegetable growing region of East-Flanders (Poeke, Belgium). Some physical and chemical characteristics of the soil are presented in Table 6.2. The field experiment was laid out in a randomised complete block design with three replicates and plots of 5.5 m by 1.5 m. The individual plots were separated by small pathways of 0.5 m wide. Sampling was done while standing in the pathways in order to avoid compaction of soil in the plots under wet conditions.

Table 6.1 Amounts of fresh matter and N added with the cauliflower residues and organic wastes and some biochemical characteristics of the materials

	FM	N	C	DM	N_{tot}	C:N	$C:N_L$
	t ha^{-1}	kg ha^{-1}	t ha^{-1}	%	g kg^{-1} DM		
Cauliflower residues							
Leaves	50	162	1.9	10.7	30.2	11.6	35.4
Stems + roots	23	59	1.1	11.3	22.6	18.6	122.3
Leaves + stems + roots [a]	73	221	3.0	10.9	27.7	13.9	63.9
Immobilizer wastes							
Cereal straw	12	89	5.2	84.9	8.00	46.4	74.2
Green waste compost	21	107	5.1	55.0	9.08	44.3	32.9
Saw dust	10	12	5.1	91.2	1.02	420	987.6
Paper sludge	56	255	5.0	29.6	12.9	18.4	60.3
Remineralizer wastes							
Vinasses	5	191	1.3	67.0	5.93	6.90	- [b]
Dairy sludge	30	91	0.6	4.93	61.8	7.60	17.4

[a] ratio stems + roots : leaves = 1:2 on dry matter; sum or weighed average; [b] no lignin fraction in vinasses; FM: applied fresh matter; N: applied total N; N: applied total C; DM: dry matter; N_{tot}: organic N content; $C:N_L$: C:N ratio of the lignin fraction

Table 6.2 Some physical and chemical properties of the soil and input data for the Burns-α leaching model

Depth	Clay	Silt	Sand	pH_{KCl}	OM	BD	SAT	FC	WP
cm	%	%	%		%	g cm^{-3}	m%	m%	m%
0-30	10.4	55.6	34.0	6.4	1.83	1.32	29.1	17.7	8.6
30-60	14.8	54.6	30.6	6.1	0.77	1.58	25.8	18.6	12.3
60-90	12.3	42.8	44.9	6.1	0.55	1.65	24.3	18.0	11.5

OM: organic matter; BD: bulk density; SAT, FC, WP: gravimetric moisture content (m%) at saturation, field capacity and wilting point

The experiment consisted of an immobilization and remineralization phase. At the start of the immobilization phase (22 October 2002), each plot received cauliflower residues (at a rate of 50 t fresh matter (FM) leaves ha^{-1} and 23 t FM stems + roots ha^{-1}) and an immobilizer waste (equivalent to ca. 5 t C ha^{-1}, Table 6.1). The materials were incorporated with a rotary cultivator to a depth

of 20 cm. The treatments were: (1) unamended soil, (2) cauliflower only (= cauliflower residues), (3) straw (= cauliflower residues + straw), (4) compost (= cauliflower residues + green waste compost), (5) saw dust (= cauliflower residues + saw dust) and (6) paper sludge (= cauliflower residues + paper sludge). Each treatment was laid out in triplicate within each block during the immobilization phase (Fig. 6.1), except for the cauliflower only treatment (see further). Soil samples were taken with an auger to a depth of 90 cm in 3 layers: 0-30 cm, 30-60 cm and 60-90 cm. In each plot four augerings were taken, and bulked to give one composite sample per plot. Samples were taken at 13, 27, 42, 55, 90, 125 and 154 days after the incorporation of the materials. At each sampling occasion, the fresh soil was extracted with (*1N*) KCl (extraction ratio 1:2) and the extracts were analysed for NO_3^--N and NH_4^+-N with a continuous flow auto-analyser (Chemlab, System 4, Skalar, The Netherlands).

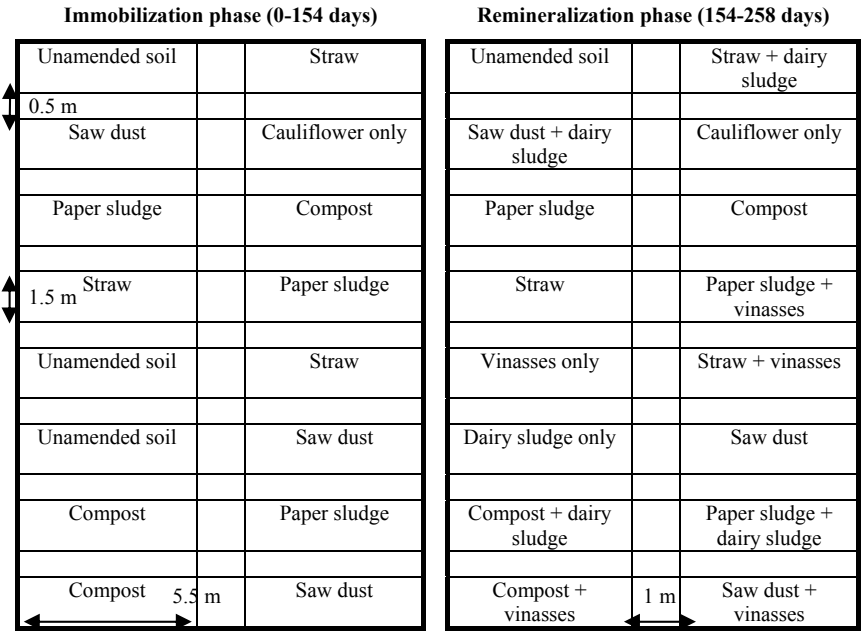

Fig. 6.1 The different treatments (amendments) and lay out of a block of the field experiment during the immobilization (left) and remineralization (right) phase

At the start of the remineralization phase (25 March 2003), two of three replicates per treatment in each block received a remineralizer waste (either vinasses or dairy sludge, equivalent to ca. 1 t C ha^{-1}, Table 6.1), which was incorporated with a rotary cultivator to a depth of 20 cm. In the third replicate no remineralizer was added, and it served as a control immobilizer treatment used to calculate the remineralization of immobilized N. In the cauliflower only treatment, which was only needed to calculate the net N release from the cauliflower residues, no remineralizer was added. The treatments during the remineralization phase included, next to the 6 treatments of the immobilization phase, (7) dairy sludge only, (8) vinasses only, (9) straw + vinasses (= cauliflower residues + straw + vinasses), (10) straw + dairy sludge (= cauliflower residues + straw + dairy sludge), (11) compost + vinasses (= cauliflower residues + green waste compost + vinasses), (12) compost + dairy sludge (= cauliflower residues + green waste compost + dairy sludge), (13) paper sludge + vinasses (= cauliflower residues + paper sludge + vinasses), (14) papers sludge + dairy sludge (= cauliflower residues + paper sludge + dairy sludge), (15) saw dust + dairy sludge (= cauliflower residues + saw dust + dairy sludge) and (16) saw dust + vinasses (= cauliflower residues + saw dust + vinasses). During the remineralization phase, each treatment occurred only once within each block (Fig. 6.1). Sampling was done similarly as in the immobilization phase and took place at 169, 183, 203, 231 and 258 days after the start of the experiment. The samples were analysed as described earlier for the immobilization phase.

Meteorological data

Meteorological data were obtained from the KMI (Royal Meteorological Institute of Belgium). For the purpose of this study, the data of the weather stations Eeklo and Kruishoutem, which are located at less than 20 km distance from the field site, were averaged. The average weather data are presented in Fig. 6.2.

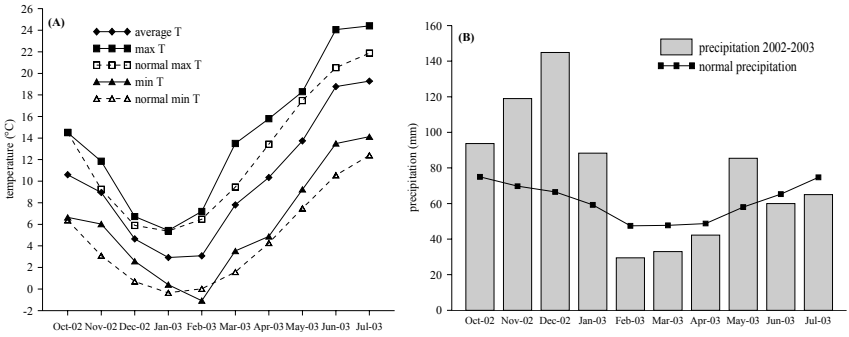

Fig. 6.2 Meteorological data from October 2002 till July 2003: (A) temperature and (B) precipitation

N mineralization, leaching and denitrification model

An N mineralization model coupled to a leaching and denitrification model was used to simulate N mineralization, NO_3^- leaching and denitrification during the immobilization phase (154 days).

The N mineralization model uses first-order kinetics to simulate the net N release from organic amendments. For the cauliflower residues, the parameters of the N mineralization model were obtained from laboratory incubations developed to predict net N mineralization of vegetable crop residues as a function of chemical composition (De Neve and Hofman, 1996), in which the percentage of organic N mineralized at time t is given by:

$$N_{org}(t) = N_{A,org}\,(1 - \exp(-k_{org}\,t)) \qquad (6.1)$$

with $$N_{A,org} = 76.6 - 0.653\ C{:}N_L \qquad (6.2)$$

$$k_{org} = 1.73 - 0.0144\ N_{org} \qquad (6.3)$$

In these equations $N_{A,org}$ is the amount of mineralizable organic N (% of organic N) and k_{org} is the rate constant for N mineralization of organic N (day^{-1}), $C{:}N_L$ is the C:N ratio of the lignin fraction and N_{org} is the organic N content

(in % of total N). The model assumes that the mineral N already present in the crop residues is released gradually within 30 days after incorporation.

Equations (6.2) and (6.3) were developed for vegetable crop residues with a low C:N ratio (8 - 30) (De Neve and Hofman, 1996), and can therefore not be used to predict the N release from the immobilizer wastes which had a high C:N ratio (18 - 420). The parameters describing N immobilization for the organic wastes were obtained from laboratory incubations where immobilizer wastes were mixed with crop residues (De Neve et al., 2004; Chaves et al., 2005a). Where possible, a first-order kinetics model was fitted to the N immobilization patterns of the organic wastes (C:N 16 - 864) used in these two studies. The total amount of immobilized N was then given by:

$$N_{tot}(t) = N_{A,tot}(1 - \exp(-k_{tot}\, t)) \qquad (6.4)$$

where $N_{A,tot}$ is the amount of immobilized N (% of total N) and k_{tot} is the rate constant for N immobilization (day^{-1}). In those wastes where the first-order kinetics model could not be fitted, the average amount of N immobilized during the incubation was taken as an estimate of $N_{A,tot}$ (Chaves et al, 2004). The model parameters were then related to the biochemical composition of the organic wastes:

$$N_{A,tot} = 20.2 - 1.68\ C{:}N \qquad (R^2_a = 0.968,\ P < 0.01) \qquad (6.5)$$

$$k_{tot} = 0.837 - 0.203 \ln(C_{tot}) \qquad (R^2_a = 0.512,\ P < 0.05) \qquad (6.6)$$

where C:N is the carbon to nitrogen ratio and C_{tot} is the total carbon content (% DM).

The N mineralization or immobilization models were combined with a model describing the temperature dependence of the N mineralization rate constant for both soil organic matter and crop residues (De Neve et al., 1996):

$$k_{org}(T) \text{ or } k_{tot}(T) = k_{opt} \exp(-\kappa (1 - T/T_{opt}))^2 \qquad (6.7)$$

with k (T) the N mineralization/immobilization rate constant as a function of temperature, T temperature (°C), T_{opt} the optimum temperature (°C), κ a temperature dependence parameter, and k_{opt} the rate constant at optimum temperature. Input data for the N mineralization/immobilization model is given in Tables 6.1, 6.2 and 6.3. The model was run in daily time steps. The net N mineralization at day i (ΔN_i) was calculated as:

$$\Delta N_i = N_A \, k_i \exp(-k_i \, t) \, \Delta t \qquad (6.8)$$

In this equation k_i is the mineralization/immobilization rate constant corrected for the temperature at day i and Δt is one day.

The N mineralization-immobilization model was coupled to the Burns-α model (Moreels et al., 2003), an adapted version of the Burns model (Burns, 1974), where parameter α, was added to enable the model to simulate moisture contents between field capacity and saturation. This model was chosen because it requires only easily available soil and meteorological data, and of the numerous leaching models published, it is one of the few that has been applied to actual field conditions, often with good results (Scotter et al., 1993). For the calibration of the α-parameter, PEST (Model-independent Parameter Estimation; Doherty, 2001) was used. The input data for the Burns leaching model are given in Table 6.2.

To simulate denitrification, the NEMIS model was used (Hénault and Germon, 2000; Moreels et al., 2003). Data of actual (N_2+N_2O) emission after incorporation of crop residues and organic wastes were obtained from Vanhecke (2004) (Table 6.3). In that study, potential denitrification rates over short periods (4 days) were measured under laboratory conditions after incorporation of crop residues and organic wastes to the same silt loam soil as in this study. The values are comparable to potential denitrification rates obtained by Aulakh and Rennie (1987) and Aulakh et al. (1991) during the first days after incorporation of straw or crop residues. The simulation of the water movement and denitrification are better explained in the appendix.

Statistical analysis

To determine significant differences among treatments, an analysis of variance with more than one independent variable ('General linear model - univariate' procedure in SPSS) and a Duncan post hoc test were used. A set of statistical parameters was used to evaluate the model: correlation coefficient (R), modelling efficiency (EF), coefficient of determination (CD) and coefficient of residual mass (CRM) (Smith et al., 1997; Moreels et al., 2003).

6.3 Results

N immobilization phase

Figure 6.3 shows the mineral N content in the different soil layers for the different treatments during the immobilization phase. In all treatments a redistribution of mineral N to deeper soil layers, and finally below the 0-90 cm layer, due to NO_3^- leaching, was observed. In order to evaluate the N immobilization potential of the immobilizer wastes, the amount of mineral N released in the cauliflower only treatment was subtracted from the amount of mineral N released in the treatments amended with a particular immobilizer waste. The N immobilization potential was calculated for the 0-30 cm layer, and for each sampling date during the first 55 days, since thereafter NO_3^- leaching started as can be seen by an increase in mineral N in the 30-60 cm and 60-90 cm layers (Fig. 6.3) and this interfered with the measurements of N immobilization. Only few significant differences were found between treatments with an immobilizer waste and the cauliflower only treatment due to large standard deviations, which are typical when fresh materials are incorporated in soil. Straw, green waste compost and saw dust showed a small N immobilization at the first sampling date (13 days). The used green waste compost had the largest N immobilization potential (51 kg N ha^{-1}), followed by straw (37 kg N ha^{-1}), and saw dust (33 kg N ha^{-1}). Paper sludge had no N immobilization potential and released an extra amount of mineral N up to 36 kg N ha^{-1} by day 42.

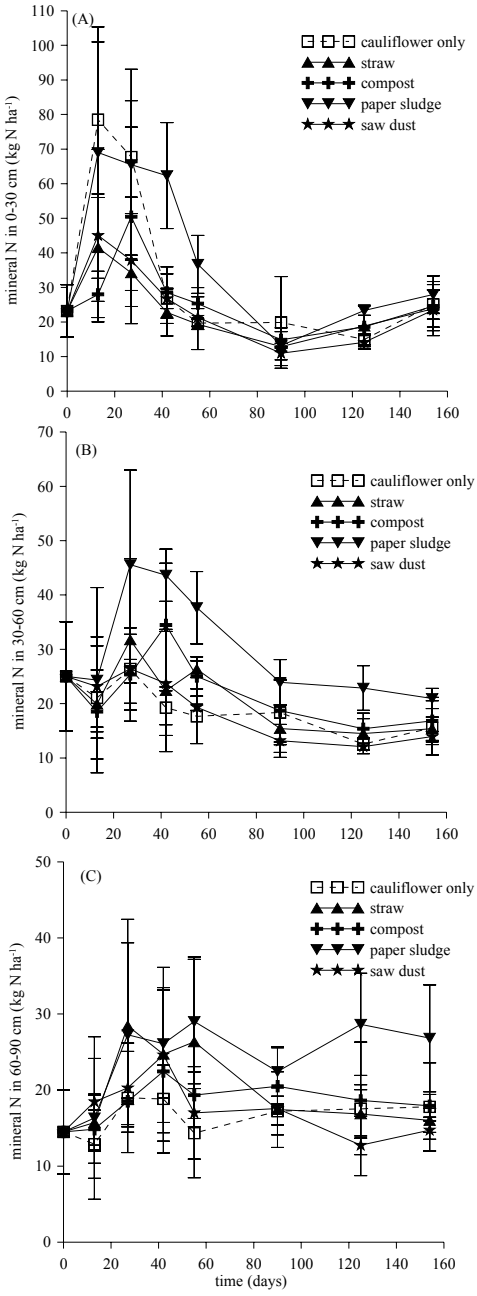

Fig. 6.3 The mineral N content in the different soil layers during the immobilization phase: (A) 0-30 cm, (B) 30-60 cm and (C) 60-90 cm; error bars are standard deviations

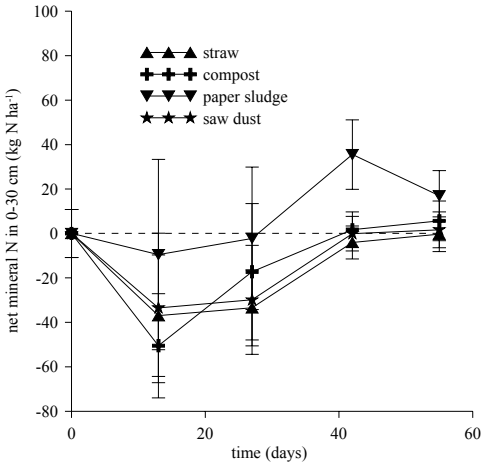

Fig. 6.4 Measured N immobilization potential of straw, green waste compost, paper sludge and saw dust during the first 55 days after incorporation in the top 30 cm layer; error bars are standard deviations; data were significantly different from zero ($P< 0.05$) at day 13 for straw, green waste compost and saw dust

Figure 6.5 shows the measured and simulated soil mineral N contents (using the N mineralization-immobilization model coupled to the Burns-α model and the denitrification model) for the 0-90 cm layer for all treatments during the immobilization phase. The model performance is given in Table 6.4. The model efficiency (EF) was positive in most treatments, except for paper sludge and compost, and the correlation coefficients were quite high. However, the positive CRM values for most treatments where an organic waste was added, indicate that the model in general underestimated the mineral N content in soil. The underestimation of soil mineral N in these treatments was probably due to an overestimation of the immobilization potential (i.e. N_A and k) of the organic wastes, since the model parameters N_A and k were based on laboratory incubations where N immobilization proceeded under more favourable conditions and where the wastes and crop residues were much more intensively mixed (use of ground materials which were more homogeneously mixed).

The N release, NO_3^- leaching and denitrification in the unamended soil simulated by the model were 40.5, 85.2 and 0.16 kg N ha^{-1}, respectively (Table 6.3). The simulated N mineralization in the cauliflower only treatment during the immobilization phase was 131.1 kg N ha^{-1}. To calculate the net N release from the cauliflower residues the mineral N released from the unamended soil was subtracted from the mineral N released in the cauliflower only treatment. This yielded 90.6 kg N ha^{-1} (41% of added N), which is in the range of values found in other field studies (De Neve and Hofman, 1998). The simulated amount of NO_3^--N leached below 90 cm and simulated denitrification in the cauliflower only treatment were 139.7 kg N ha^{-1} and 4.72 kg N ha^{-1}, respectively.

When straw, green waste compost, saw dust or paper sludge were incorporated together with cauliflower residues, the simulated N release and the amount of NO_3^--N leached below 90 cm was less that that of the cauliflower only treatment, but the differences were rather small (Table 6.3). The maximum reductions in mineral N contents (i.e. N immobilization) and NO_3^- leaching occurred after incorporation of green waste compost. They were 62 and 37 kg N ha^{-1}, respectively. These simulations confirm the limited N immobilization potential of the immobilizer wastes, as was already suggested by the measured data (Fig. 6.3), and that especially their capacity to reduce NO_3^- leaching is low.

While straw slightly increased denitrification as compared to the cauliflower only treatment, saw dust, green waste compost and paper sludge slightly decreased denitrification as compared to the cauliflower only treatment.

Table 6.3 Input and output data for the N mineralization-immobilization model coupled to the Burns-α model for 154 days (immobilization phase)

Treatment	DM	N_A	κ	k_{opt}	D_P	$N_{release.}$	$N_{leached}$	N_{den}
	t ha^{-1}	% of added N		day^{-1}	kg ha^{-1} day^{-1}	kg ha^{-1}		
Unamended soil	- [a]	- [a]	2.63	0.650 [b]	9.8	40.5	85.2	0.16
Cauliflower only	7.96	34.9 [c]	3.38	0.185	74.8	131.1	139.7	4.72
Straw	10.2	-57.6 [d]	5.36	0.572	105.5	75.9	105.9	5.01
Compost	11.6	-54.2 [d]	5.36	0.555	93.1	69.4	102.6	4.26
Saw dust	9.12	-683.8 [d]	5.36	0.222	64.7	82.2	114.7	3.34
Paper sludge	16.6	-10.7 [d]	5.36	1.054	61.7	95.3	116.9	3.26

[a] not applicable; [b] for unamended soil k_{opt} (in kg N ha^{-1} day^{-1}); [c] % of organic N; [d] % of total N; DM: dry matter content; N_A: amount of mineralizable N; κ: the temperature dependence parameter; k_{opt}: the optimum rate constant at optimum temperature; D_P: potential denitrification rate (~20 cm depth); $N_{release}$: simulated N mineralization for 154 days; $N_{leached}$: simulated N leached below 90 cm for 154 days; N_{den}: simulated denitrification for 154 days

Table 6.4 Statistical evaluation of the models' performance for simulation of the NO_3^--N content (0-90 cm and all sampling dates) during the immobilization phase (154 days)

Treatment	R [a]	EF [b]	CD [c]	CRM [d]
Optimum value	*1*	*1*	*>1*	*0*
Unamended soil	0.57	0.24	1.46	-0.04
Cauliflower only	0.65	0.30	3.27	-0.17
Straw	0.78	0.51	2.02	0.09
Compost	0.40	-0.05	1.95	0.15
Saw dust	0.74	0.53	2.31	-0.04
Paper sludge	0.50	-0.24	1.83	0.31

[a] Correlation coefficient; [b] Modelling efficiency; [c] Coefficient of determination; [d] Coefficient of residual mass

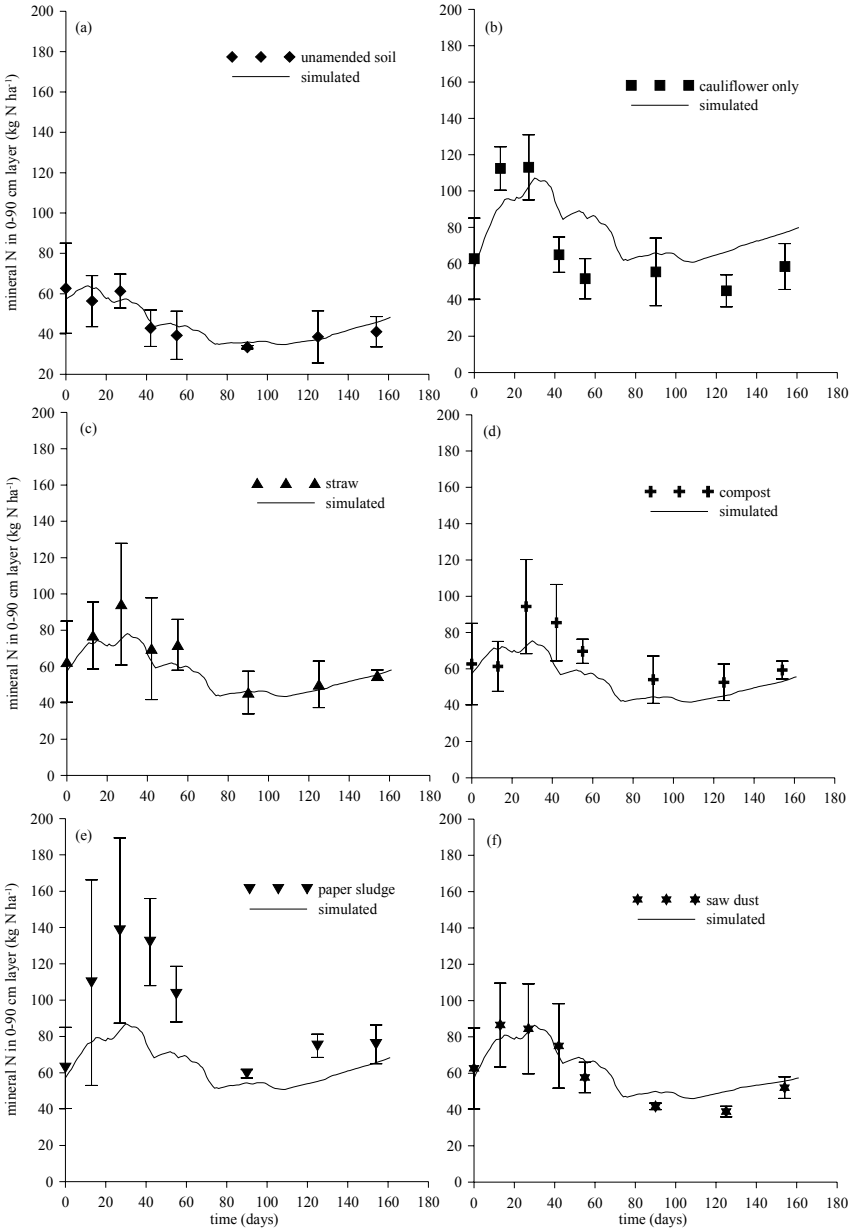

Fig. 6.5 Measured and simulated mineral N content over the whole soil profile (0-90 cm) during the immobilization phase (154 days); error bars are standard deviations of the measured data

Remineralization phase

The priming effect (or remineralization of immobilized N) caused by the remineralizer wastes was calculated as the soil mineral N content in the 'cauliflower residues + immobilizer + remineralizer' treatment minus the soil mineral N content in the 'cauliflower residues + immobilizer' treatment, minus the net N mineralization of vinasses and dairy sludge at each sampling date in the top 30 cm layer (Fig. 6.6). The standard deviations were very large due to the incorporation of a new fresh organic material (vinasses or dairy sludge), and therefore, few significant differences were found, i.e. priming effects were almost none existent. When vinasses or dairy sludge were added after green waste compost, saw dust or paper sludge, even a general trend of N immobilization was observed. Only in combination with straw, some remineralization occurred, both for vinasses and dairy sludge. This remineralization was only significant at 1 sampling date (at day 231), and was 96 and 34 kg N ha^{-1} in the straw + vinasses and straw + dairy sludge treatment, respectively. Remineralization never started immediately after the incorporation of vinasses or dairy sludge. The earliest remineralization occurred at about 30 days after incorporation.

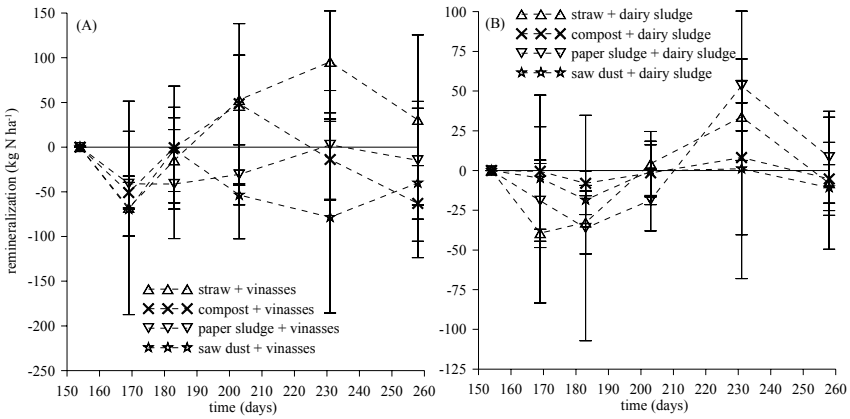

Fig. 6.6 Remineralization (priming effect) of vinasses (A) and dairy sludge (B) in the top 30 cm; error bars are standard deviations; data were only significantly higher than zero ($P < 0.05$) at day 231 for straw + vinases and straw + dairy sludge

6.4 Discussion

Immobilizer wastes

A high C:N ratio has been proven to be an indicator for N immobilization and, in general, the application of organic materials with a C:N ratio higher than 20-40 promotes net N immobilization (Iritani and Arnold, 1960; Fox et al., 1990; Vigil and Kissel, 1991). The C:N ratios of straw, green waste compost and saw dust were above 40, only the C:N ratio of paper sludge was much lower (C:N 18.4) than expected. However, from both measured and simulated data, we must conclude that the N immobilization potential of all immobilizer wastes was rather low, and that especially the reduction in NO_3^- leaching was limited.

The low N immobilization after incorporation of paper sludge was probably due to its relatively low C:N ratio (C:N 18.4; Eq. (6.5) yielded only -10.7% of added N) which is lower than the C:N ratios for paper sludge found in most studies: from C:N 24.6 (Vinten et al., 1998) over C:N 51 (Chaves et al., 2005a), C:N 86 (Aitken et al., 1998) up to C:N 520 (Rahn et al., 2003). However, for paper sludge, the C:N ratio may not always be a good indicator for N immobilization. Chaves et al. (2005a) used a paper sludge with a C:N ratio of 51, but found no N immobilization, while Vinten et al. (1998) used a paper sludge with a C:N of 24.6 that did reduce NO_3^- leaching when mixed with calabrese or lettuce residues. Vinten et al. (1998) assumed that the paper sludge used in their study contained different types of N, and that a large part of the N was difficult to decompose, e.g. polyacrylamides (coming from the polyacrylamide flocculant used in the waste treatment process) which are highly resistant to microbial degradation, leading to an 'effective' N content of only 0.65% on organic matter basis (instead of 2.36% on organic matter basis) and an 'effective' C:N ratio of 90 (instead of 24.6). Thus, it seems that for paper sludge, the bioavailability of N is variable. Since no polyacrylamide flocculant was used in the production process of the paper sludge in this study, the sludge probably contained a smaller portion of non-available N. The distribution of N over the different fractions in the Stevenson fractionation also

confirmed that only a small part of N was contained within the resistant lignin fraction (13.8% of total N).

In case of straw, saw dust and green waste compost, the C:N ratio was above the critical C:N ratio (20-40), and N immobilization was expected (equation (6.5) yielded $N_{A,tot}$ values for straw, saw dust and green waste compost of -57.6%, -683.8% and -54.2% of added N, respectively). Still these immobilizer wastes had a limited effect on the N mineralization/immobilization turnover of cauliflower residues. A reason for the low N immobilization potential of these wastes could be their lignin content. Chaves et al. (2005a) found that an immobilizer waste should not only have a high C:N ratio (> 20-40), but should also be easily decomposable (i.e. low lignin content < 43% on OM) to have a good potential to immobilize N. Both the green waste compost and the saw dust used in this study had a high lignin content (49% and 46% on OM, respectively). Hence, lignin may have slowed down the decomposition of the wastes, causing an asynchronization between the decomposition of crop residues and immobilizer waste, resulting in low N immobilization. However, the wheat straw used in this experiment had a rather low lignin content (26.9% on OM) compared to other studies (Rahn et al., 2003; De Neve et al., 2004; Chaves et al., 2005a), meaning that in the case of straw other factors, as further explained, must have had an influence.

An explanation for the low N immobilization under field conditions could be the heterogeneous mixing between crop residues and immobilizer wastes. Decomposer micro-organisms only have access to part of the mineral N pool if organic wastes are not homogeneously distributed (Wang and Bakken, 1997). Moritsuka et al. (2004) found that N immobilization extended to 10 mm from the soil-straw interface after 5 days of incubation. Although, the crop residues and immobilizer wastes were incorporated together in soil, it is be possible that the mixing was poor, so that the micro-organisms decomposing the immobilizer wastes could not fully use the mineral N released from the crop residues. Furthermore, the organic wastes used in laboratory experiments were mostly ground before incorporation (De Neve et al., 2004; Chaves et al.,

2005a, 2006a), while in this experiment the materials contained larger particles (10-15 cm) and were more coarse. This may have led to a less intimate mixing with the crop residues.

The specific experimental conditions could have lowered N immobilization. A first reason could be the low air temperatures during the field experiment. In October and November, the average temperatures were 10.6°C and 9.0°C, respectively, while in December, January and February temperatures dropped below 5°C. It has been found that decomposition and N mineralization from N-rich plant residues is still substantial during a low temperature period (T< 5°C) (Breland, 1994; Magid et al., 2004). However, decomposition of recalcitrant substances is more limited at low temperatures than that of more easily decomposable substances, leading to a low N immobilization at low temperatures while the N release from easily decomposable substances still continues (Nicolardot et al., 1994; Andersen and Jensen, 2001). Soil moisture conditions during the field experiment may also partially explain the low N immobilization. Directly after the start of the experiment, precipitation was much higher than normal (Fig. 6.2). Although there were corridors between the plots, the wet conditions made it difficult to sample, and it is possible that soil compaction did occur, leading to lower decomposition of both crop residues and immobilizer wastes, and higher denitrification than predicted by model calculations, and thus leading to lower N immobilization.

It is also important to mention that, according to the model simulations, straw increased denitrification (i.e. N_2 and N_2O emissions). N_2 emission means an economical loss of N, but has no impact on the environment. However, N_2O is a powerful greenhouse gas (Mosier and Schimel, 1991) and it affects the stratospheric ozone layer, resulting in a higher UV-B intensity reaching the earth surface (Cicerone, 1987). Chaves et al. (2005b) found that straw reduced N_2O emissions (by 60%) compared to the cauliflower only treatment during laboratory incubations. Hence, it is suggested that the simulated increase in denitrification should be attributed to extra N_2 emission.

Remineralizer wastes

In this study, a priming effect was observed only when vinasses or dairy sludge were incorporated after straw (96 and 34 kg N ha^{-1} for straw + vinasses and straw + dairy sludge, respectively) and the effect was only significant at one sampling date (day 231).

A possible reason of the low remineralization may be the initial low N immobilization of the immobilizer wastes. Indeed, it is mainly the recently immobilized N that can be readily remineralized (Jensen, 1994a), and hence, when N immobilization is low, the potential for remineralization will also be limited. Furthermore, in case of dairy sludge, the biochemical composition may have been unsuitable for inducing N priming effects, e.g. not enough readily available C, since most researchers found N priming effects after incorporation of low molecular weight compounds like glucose (Asmar et al., 1994; Falih and Wainwright, 1996; Wheatly et al., 2001).

However, obtaining timely N remineralization may be a crucial factor for success when trying to manipulate the N release from crop residues. It has been found that straw incorporation reduces NO_3^- leaching during the first winter, but that it may lead to higher mineral N contents and higher nitrate leaching on the long term compared to conditions when no straw was incorporated (Nicholson et al., 1997; Catt et al., 1998; Silgram and Chambers, 2002). Hence, if we are not able to stimulate remineralization before a new crop is sown or planted, the immobilized N may become gradually available and at a much later stage, and enhance the risk of NO_3^- leaching.

6.5 Conclusions

At this moment, manipulating the N release of N-rich crop residues using organic wastes is still quite difficult to achieve under field conditions. During the immobilization phase, the immobilizer wastes showed only limited N immobilization and a limited reduction in NO_3^- leaching, and during the

remineralization phase, very little remineralization of immobilized N was observed.

Chapter 7

Manipulating the N release from N-rich crop residues by using organic wastes on soils with different textures during two years

Chaves B, De Neve S, Mateu Piulats L, Boeckx P, Van Cleemput O, Hofman G (2006) Manipulating the N release from N-rich crop residues by using organic wastes on soils with different textures during two years (submitted).

Abstract The potential to manipulate the N release from vegetable crop residues by using organic wastes was tested under field conditions during two years and on three soil textures (silt loam, sandy loam, loamy sand). At the start of each year (autumn), crop residues (cauliflower or leek residues) were incorporated together with an immobilizer waste (green waste compost, saw dust or straw) in order to immobilize the released crop residue-N, followed by an incorporation of a remineralizer waste (malting sludge or vinasses) in spring aiming to remineralize the immobilized N. During the two years, mineral N and microbial biomass N in soil were measured at regular time intervals. During the immobilization phase, the net N release, NO_3^- leaching and denitrification were simulated using a N mineralization/immobilization model coupled to a NO_3^- leaching model. During the remineralization phase, a crop was grown on the field and the dry matter yield and N uptake of the crop was determined. During the first year, green waste compost and saw dust showed only a small N immobilization potential and a small reduction in NO_3^- leaching, probably due to a high lignin content and an insufficient mixing between organic wastes, crop residues and soil. During the second year, straw showed a good N immobilization potential in the loamy sand soil (89% of the released crop residue-N) and reduced NO_3^- leaching to levels similar as in the unamended soil. In the silt loam and sandy loam soil, the N immobilization potential of straw and the reduction in NO_3^- leaching were smaller (immobilization of 35% of the released crop residue-N, reduction in NO_3^- leaching between 35 and 50%), probably due to a more heterogeneous mixing between crop residues, organic wastes and soil compared to the loamy sand. There was no consistent remineralization of immobilized N after addition of malting sludge or vinasses in any of the treatments. Reasons for this low remineralization could be the small amount of initially immobilized N available for remineralization, and an unsuitable composition of the remineralizer wastes to induce remineralization.

7.1 Introduction

Manipulating the N mineralization of N-rich crop residues may be an option to reduce NO_3^- residues in soil, and reduce the risk of NO_3^- leaching after vegetables. Immobilizer wastes like straw, paper waste, green waste compost and saw dust have already proved to possess a N immobilization potential (Rahn et al., 2003; De Neve et al., 2004; Chaves et al., 2005a).

Straw incorporation may reduce NO_3^- leaching during the winter directly following application, but may lead to increased NO_3^- leaching on the long term due to enhanced N mineralization from an increased microbial biomass (Nicholson et al., 1997; Catt et al., 1998; Silgram and Chambers, 2002). Therefore, knowledge concerning the long-term effect of organic wastes is crucial before the use of organic wastes can be further stimulated.

Soil texture has a predominant effect on the activity of soil biota and the rate of organic matter decomposition and N mineralization (Hassink, 1993). It is generally accepted that there is more physical protection of soil organic matter, fresh organic material and micro-organisms in fine-textured than in coarse-textured soils, and surely texture will influence the N immobilization and remineralization potential of organic wastes.

Vinten et al. (1998) found a reduction in NO_3^- leaching after incorporation of paper waste, but found no significant effect on the N uptake of the following crop. Nevertheless, manipulating the N release from crop residues and incorporating organic wastes may affect the subsequent crop, and this needs to be further examined.

At this moment, manipulating the N release of N-rich crop residues using organic wastes is still quite difficult to achieve under field conditions (Chaves et al., 2006b). However, only limited studies on manipulating the N release from crop residues were conducted under field conditions and these were mostly short-term experiments (one year) located on one soil type (Vinten et

al., 1998; Vityakon et al., 2000; Chaves et al., 2006b). More extensive field experiments are needed to further evaluate the method under field conditions.

In this study, we investigated the effect of organic wastes on the N mineralization-immobilization turnover (NMIT) of N-rich crop residues during two years. The work had three aims: (1) to evaluate the effect of manipulating the N release from crop residues *in situ* on a longer time scale (two years); (2) to examine the effect of soil texture on the N immobilization or remineralization potential of organic wastes and (3) to determine the effect of manipulating the N release from crop residues on the following crop.

7.2 Materials and methods

Field experimental site, crop residues and organic wastes

The field experiments were located in the intensive vegetable growing region in Flanders (Belgium) on three locations with three different soil textures: a silt loam (Poeke), a sandy loam (Ardooie; only in the second year) and a loamy sand soil (Aalter). Some physical and chemical characteristics of the soils are shown in Table 7.1.

The crop residues used in the experiment included residues of cauliflower (*Brassica oleracea* var. *botrytis*) and leek (*Allium porrum*). As *immobilizer wastes* green waste compost, saw dust and cereal straw were chosen, since these are widely available organic wastes with a high C:N ratio, which have proved to cause N immobilization (De Neve et al., 2004; Chaves et al., 2005a). Green waste compost was collected early in the composting process (i.e. before maturing) because we presumed immature compost would have a higher N immobilization potential. As *remineralizer wastes* malting sludge, waste from the malting industry, and vinasses, waste from the sugar industry, were chosen, since these are widely available organic wastes of which was presumed that they would cause remineralization, because of their high content of easily decomposable C. It has been shown that addition of C in the form of

sugars can lead to a marked increase in soil microbial activity (Falih and Wainwright, 1996).

Table 7.1 Some physical and chemical properties of the soils and input data for the Burns-α model

Depth	Clay	Silt	Sand	OM	BD	SAT	FC	WP
cm	%	%	%	%	g cm^{-3}	m%	m%	m%
Silt loam								
0-30	13.6	51.8	34.6	1.5	1.38	30.5	17.9	11.3
30-60	17.0	54.0	29.0	0.7	1.44	29.5	18.4	14.2
60-90	16.7	57.2	26.1	0.4	1.55	25.2	15.4	9.8
Sandy loam								
0-30	8.5	26.4	65.1	1.2	1.62	24.0	15.2	8.6
30-60	8.5	27.6	63.9	0.8	1.60	23.1	17.6	10.3
60-90	8.4	26.2	65.4	0.6	1.52	25.0	18.8	8.9
Loamy sand								
0-30	7.8	13.6	78.6	2.0	1.51	24.8	16.5	11.5
30-60	9.3	11.8	78.9	1.0	1.50	26.9	21.4	14.9
60-90	15.9	6.9	77.2	0.5	1.31	29.5	25.0	20.6

BD: bulk density; OM: organic matter; SAT, FC, WP: gravimetric moisture content (m%) at saturation point, field capacity and wilting point

Subsamples of the crop residues and organic wastes were dried at 55°C until constant weight for the determination of the dry matter content, and then milled for further analysis. Total C and N contents were determined using a CNS elemental analyser (Variomax CNS, Elementar, Germany). The Stevenson fractionation as modified by De Neve and Hofman (1996) and the CNS elemental analyser were used to determine the C:N ratio of the lignin fraction, since this parameter was needed to determine the N mineralization or immobilization of the different materials (cfr. *Chapter 6*). The biochemical analysis of the crop residues and organic wastes is presented in Tables 7.2 and 7.3.

Table 7.2 Biochemical composition of the crop residues and organic wastes used in the first year

	FM	N	DM	N_{tot}	C:N	$C:N_L$
	t ha^{-1}	kg ha^{-1}	%	g kg^{-1} DM		
Cauliflower residues in silt loam						
Leaves	60.6	352	15.7	36.9	8.93	33.8
Stems	21.2	101	20.5	23.2	18.1	122.3
Leaves+stems [a]	81.8	453	17.2	34.0	11.8	52.2
Cauliflower residues in loamy sand						
Leaves	53.3	275	14.1	36.7	8.48	36.8
Immobilizer wastes						
Compost	22.7	124	82.4	6.6	69.4	76.7
Saw dust	10.1	6.3	88.8	0.7	710.6	1857
Remineralizer waste						
Malting sludge (silt loam)	44.4	88	5.54	35.6	8.08	21.5
Malting sludge (loamy sand)	44.4	62	5.02	27.5	10.4	21.9

[a] sum/weighed average; FM: applied fresh matter; N: applied total N; DM: dry matter; N_{tot}: total N content; $C:N_L$: C:N ratio of lignin fraction

Table 7.3 Biochemical composition of the crop residues and organic wastes used in the second year

	FM	N	DM	N_{tot}	C:N	$C:N_L$
	t ha^{-1}	kg ha^{-1}	%	g kg^{-1} DM		
Cauliflower leaves						
Silt loam	60.6	452	17.8	41.9	9.78	38.2
Sandy loam	60.6	381	18.6	33.8	10.3	48.2
Leek leaves						
Loamy sand	53.3	137	7.16	35.8	10.9	28.7
Immobilizer waste						
Cereal straw	12.8	50	90.8	4.4	105.4	177.4
Remineralizer waste						
Vinasses	6.1	216	60.0	55.4	7.38	-[a]

[a] no lignin fraction in vinasses; abbreviations see Table 7.2

Experimental set up

The experiment was laid out as a randomised complete block design with three replicates and plots of 5.5 m by 1.5 m. The individual plots were separated by small pathways of 0.5 m wide. Sampling was done while standing in the pathways in order to avoid compaction of soil under wet conditions.

The experiment lasted two years, and each year consisted of an immobilization and remineralization phase. At the start of the first year (20 October 2003 for silt loam, 4 November 2003 for loamy sand), each plot received cauliflower residues and an immobilizer waste (green waste compost or saw dust) which were incorporated with a rotary cultivator to a depth of 15 cm. The treatments are given in Fig. 7.1. The saw dust treatment was only established on the silt loam in order to limit the total amount of treatments and keep the experiment manageable. At several times samples were taken with an auger to a depth of 90 cm in four layers: 0-15 cm, 15-30 cm, 30-60 cm and 60-90 cm. The division in 0-15 cm and 15-30 cm during sampling was done since most effects on the N mineralization-immobilization turnover in soil were expected in the top 15 cm (i.e. depth of incorporation). During the first immobilization phase each treatment occurred twice within each block, and in each plot two augerings were taken and the soil of duplicate treatments within one block was bulked to give one composite sample per treatment per block (the duplicate treatment was needed to add a remineralizer waste during the following phases). Samples were taken 22, 41, 84, 97, 111, 139, 162 days after the start of the experiment on the silt loam and 27, 70, 83, 97, 125, 162 days after the start of the experiment on the loamy sand. After 162 days (31 March 2004 for silt loam, 14 April 2004 for loamy sand), one of the two replicates within one block received malting sludge, as remineralizer waste, which was incorporated with a rotary cultivator to a depth of 15 cm (Fig. 7.1). From this moment on, each treatment occurred once within one block, and four augerings were taken within each plot, and bulked to give one composite sample per plot. Samples were taken on day 175, 237, 259 and 363 on the silt loam and on day 183, 223, 237 and 343 on the loamy sand.

At the start of the second year (18 October 2003 for the silt loam, 12 October 2004 for the loamy sand, 5 October 2004 for the sandy loam), each plot received again vegetable crop residues (cauliflower residues on the silt loam and on the sandy loam; leek residues on the loamy sand) and an immobilizer waste (straw) which were incorporated as before (Fig. 7.1 and 7.2). Samples were taken on day 363, 392, 420, 454, 490 and 532 on the silt loam and on day 343, 378, 406, 441, 476 and 517 on the loamy sand. On the sandy loam, where the field experiment was set up only from the second year, each treatment occurred twice within one block during the second immobilization phase, and in each plot two augerings were taken and the soil of duplicate treatments within one block was bulked to give one composite sample per treatment per block. Samples were taken 29, 55, 92, 132 and 188 days after the start of the experiment on the sandy loam. On the 4th of April 2005 for the silt loam, 5th of April 2005 for the loamy sand and on 11th of April for the sandy loam, a remineralizer waste (vinasses) was added to half of the plots, and incorporated as before (Fig. 7.1 and 7.2). Samples were taken on day 566, 594, 645 for the silt loam, on day 545, 580 and 605 for the loamy sand and on day 224, 251 and 370 for the sandy loam.

Crop yield

During the first year, ryegrass (*Lolium perenne* L.) was sown on the silt loam on the 28th of May 2004 (day 220) and leek (*Allium porrum*) was planted on the loamy sand on the 15th of May 2004 (day 193). During the second year, lettuce was sown on the loamy sand on the 30th of May (day 573) and leek was planted on the sandy loam on the 14th of June (day 252). No crop was grown on the silt loam during the second year.

The crops received a reduced fertilization compared to conventional practices in order to make the remineralization potential of malting sludge and vinasses more visible and since it was presumed that the remineralizer wastes would release a minimum of 70 kg N ha^{-1} (Chaves et al., 2005a, 2006b). Ryegrass was fertilized on the 6th of July 2004 (day 259), 2nd of August 2004 (day 286) and 23rd of August 2004 (day 307) with in total 240 kg N ha^{-1}

(ammoniumnitrate), 78 kg P_2O_5 ha^{-1} (triplesuperphosphate) and 90 kg K_2O ha^{-1} (patentkali). Ryegrass was harvested on the 23rd of August 2004 (day 307; first cut) and on the 18th of October 2004 (day 363; second cut). During the first year, leek was fertilized on the 28th of June 2004 (day 237; loamy sand) and during the second year, on the 28th of July 2005 (day 296; sandy loam) with 140 kg N ha^{-1} (ammoniumnitrate), 73 kg P_2O_5 ha^{-1} (triplesuperphosphate) and 109 kg K_2O ha^{-1} (patentkali). Leek was harvested on the 7th of September 2004 during the first year (day 308; loamy sand) and harvested on 10th of October 2005 during the second year (day 370; sandy loam). Lettuce received no extra N fertilizer since the presumed N release from vinasses would be sufficient, only 100 kg K_2O ha^{-1} (patentkali) was supplied on the 20th of June 2005 (day 594) and lettuce was harvested on the 1st of July 2005 (day 605).

To determine the yield of the crops, a section of 1 m² was harvested within each plot, and the amount of plant material was weighed. A subsample of the plant material was brought to the lab for analysis of the dry matter content and N content as for the crop residues and organic wastes. The N uptake of each crop at a given sampling date and in a given treatment was determined using the N uptake-curve of that crop, where the total N uptake was set equal to the measured total N uptake of that crop at harvest in the specific treatment. The N uptake curves were adapted from studies where the growth conditions were similar as in our study (ryegrass, Spedding, 1971; leek, Geypens and Honnay, 1995; lettuce: Breimer, 1989).

Chemical analysis

Mineral N in 30 g of fresh soil was extracted with (*1N*) KCl (ratio 1:2) and the extract was analysed for NO_3^--N and NH_4^+-N with a continuous flow auto-analyser (Skalar, Chemlab System 4, The Netherlands). Microbial biomass C was determined by fumigation-extraction according to Voroney et al. (1993) using a 24h fumigation time, a (*0.1N*) KCl extractant, a soil-to-extractant ratio of 1:2 (both for fumigated and non-fumigated samples) and a conversion factor k_{EC} of 0.25. Total organic C of the extracts was determined using a Total Organic Carbon analyser (TOC-V_{CPN}, Shimadzu, Japan). Microbial biomass N

was not determined directly, but was calculated from microbial biomass C, due to problems with the TN module of the Total Organic Carbon analyser (TOC-V_{CPN}, Shimadzu, Japan), using a C:N ratio of 6.0, which can be considered as the average C:N ratio of microbial biomass (Powlson et al., 1987; Jensen et al., 1997; Jedidi et al., 2004). The use of a similar C:N ratio for the different treatments is supported by several studies that found no significant changes in C:N ratio after addition of organic materials (Ocio et al., 1991; Bremer and van Kessel, 1992; Joergensen et al., 1994; Jensen et al., 1997).

N mineralization, leaching and denitrification model

An N mineralization model coupled to a leaching and denitrification model was used to simulate N mineralization, NO_3^- leaching and denitrification during the immobilization phases in both years (Chapter 6).

Meteorological data

Meteorological data were obtained from the KMI (Royal Meteorological Institute of Belgium) (Fig. 7.3). For the field experiments in Poeke and Aalter averaged data from the weather stations in Eeklo and Kruishoutem (located less than 20 km away from the field sites) was used, and for the field experiment in Ardooie the data from the weather station in Beitem (located less then 10 km away from the field site) was used to run the model.

Statistical analysis

To determine significant differences between treatments an analysis of variance with more than one independent variable ('General linear model - univariate' procedure in SPSS) and a Duncan's post hoc test were used. A set of statistical parameters was used to evaluate the model: correlation coefficient (R), modelling efficiency (EF), coefficient of determination (CD) and coefficient of residual mass (CRM) (Smith at al., 1997; Moreels et al., 2003).

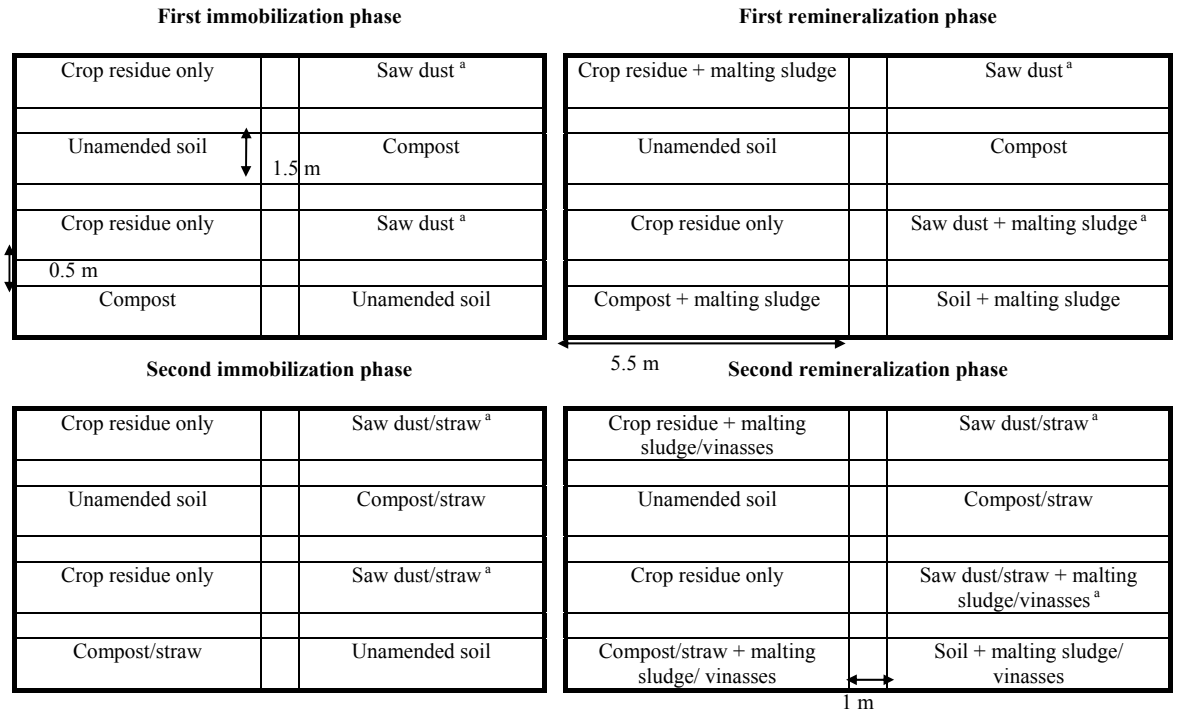

Fig. 7.1. Lay out of the field experiment (example for one block) on the silt loam and loamy sand ([a] the saw dust treatment did not occur on the loamy sand)

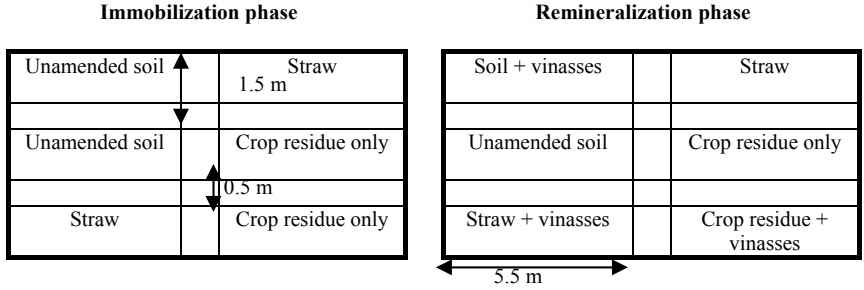

Fig. 7.2. Lay out of the field experiment (example for one block) on the sandy loam (second year)

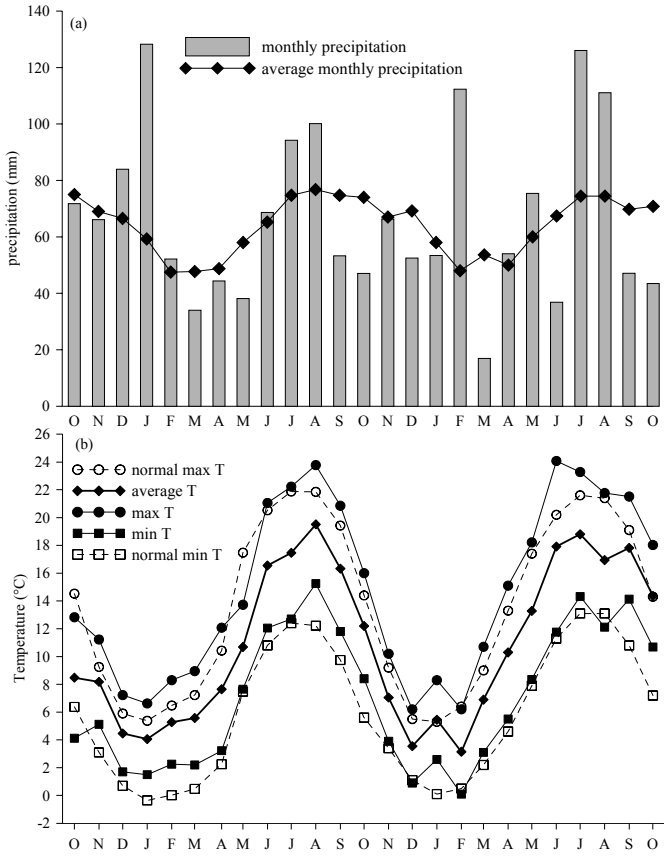

Fig. 7.3 Meteorological data from October 2003 till October 2005 (average of three weather stations: Beitem, Eeklo and Kruishoutem): (a) precipitation and (b) temperature

7.3 Results

N immobilization phase

Microbial biomass N

Microbial biomass N at the start of the experiment in the different textures was 93 kg N ha^{-1} (2.6% of total N), 60 kg N ha^{-1} (2.3% of total N) and 55 kg N ha^{-1} (1.6% of total N) in silt loam, sandy loam (second year) and loamy sand, respectively (Fig. 7.4).

Incorporating crop residues led to a significant ($P < 0.05$) increase in microbial biomass N in all textures in both years. During the first year, the maximum increase in microbial biomass N was between 41 kg N ha^{-1} (9% of added N) in the silt loam and 53 kg N ha^{-1} (19% of added N) in the loamy sand compared to the unamended soil. During the second year, the maximum increase in microbial biomass N was 69 kg N ha^{-1} (15% of added N) in silt loam, 37 kg N ha^{-1} (9% of added N) in sandy loam and 32 kg N ha^{-1} (24% of added N) in loamy sand compared to the unamended soil. This increase in microbial biomass N was visible only the first sampling date, and thereafter no more significant differences between the crop residue only treatment and unamended soil could be found.

During the first year, incorporating crop residues together with green waste compost or saw dust did not significantly influence microbial biomass N compared to the crop residue only treatment, although there was a trend that microbial biomass N was higher when an immobilizer waste was added, especially in the silt loam. During the second year, mixing crop residues with straw led to a significant ($P < 0.05$) increase in microbial biomass N compared to the crop residue only treatment in all textures. In the silt loam, the increase in microbial biomass N compared to the unamended soil was maximum 117 kg N ha^{-1} (23% of total added N (=crop residue-N + straw-N)) in the compost/straw treatment and maximum 83 kg N ha^{-1} (17% of total added N) in

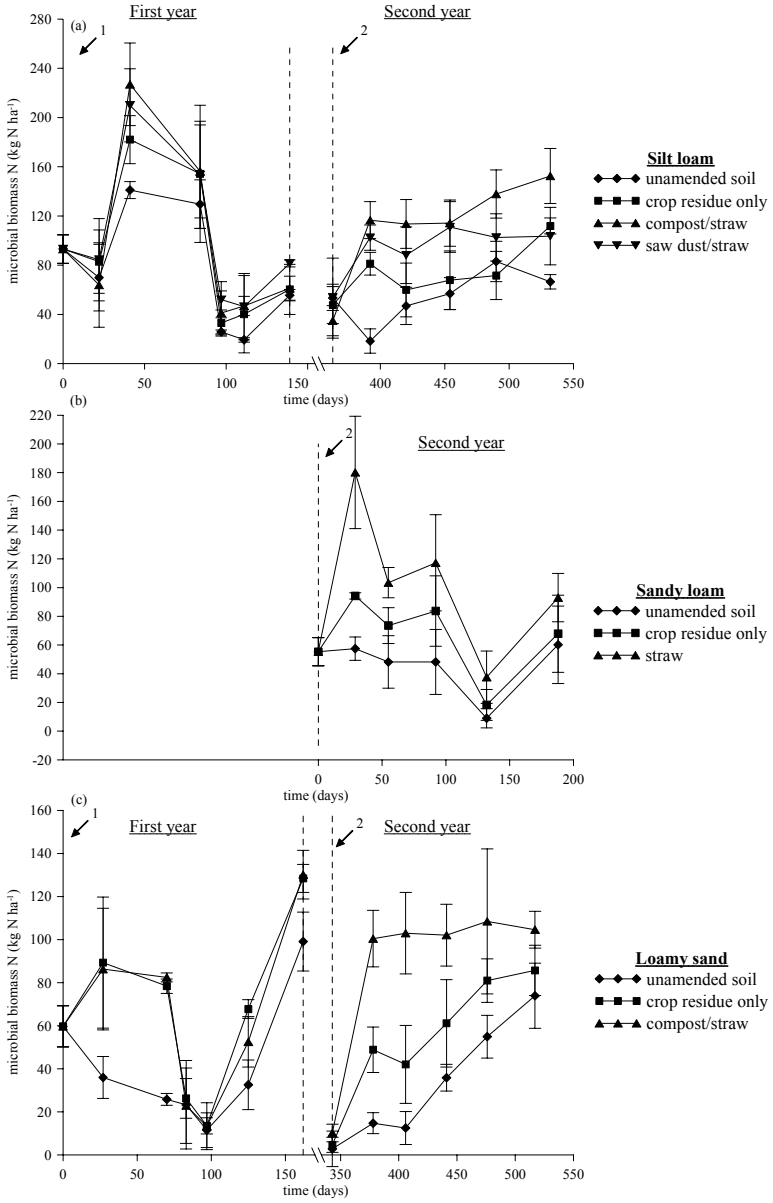

Fig. 7.4 Microbial biomass N in top 15 cm in silt loam (a), sandy loam (b) and loamy sand (c) in the immobilization phase of both years; error bars are standard deviation; 1= incorporation of crop residues and compost or saw dust; 2= incorporation of crop residues and straw

the saw dust/straw treatment. The increase in microbial biomass compared to the unamended soil was 123 kg N ha^{-1} (29% of total added N) in sandy loam and 83 kg ha^{-1} (44% of total N added) in loamy sand. In the silt loam and loamy sand, microbial biomass N was significantly higher than in the crop residue only treatment during ca. 100 days, while in the sandy loam, the increased microbial biomass N lasted only 55 days. At the end of the second immobilization phase, no significant differences in microbial biomass N between treatments could be found, although there was still a general trend that microbial biomass N was higher in the straw treatments compared to the unamended soil and crop residue only treatment.

Mineral N

The measured mineral N content in the top 15 cm during the immobilization phase of both years is shown in Fig. 7.5. Incorporating crop residues increased the mineral N content in both years and in all textures. During the first year, green waste compost and saw dust had a limited N immobilization effect both on the silt loam as on the loamy sand, while during the second year, straw significantly ($P < 0.05$) reduced the mineral N content in all textures compared to the crop residue only treatment.

During the immobilization phases of both years, a redistribution of mineral N to deeper soil layers, and finally out of the profile due to NO_3^- leaching occurred (data not shown). The N mineralization/immobilization model coupled to the Burns-α model (Chapter 6) was used to simulate the N release, NO_3^- leaching and denitrification in all treatments during the immobilization phase (i.e. until the moment when a remineralizer waste was added) in both years. The input data is given in Tables 7.1, 7.2, 7.3, 7.4 and 7.5, and the model performance is given in Table 7.6 and Fig. 7.6 and 7.7. The model efficiency (EF) and CD values were positive in most treatments, the correlation coefficients were quite high and CRM values were generally close to zero. Only the mineral N contents in the compost treatment during the first year were underestimated by the model, both in the silt loam as in the loamy

sand, as evidenced by the positive CRM values and low EF values (even negative in the silt loam). In the second year, the mineral N contents in the unamended sandy loam and loamy sand soils were overestimated.

The simulated N mineralization in the top 30 cm of the unamended soils at the end of the immobilization phase was 77 and 93 kg N ha^{-1} in the silt loam and loamy sand, respectively, during the first year (Table 7.4), and 86, 110 and 106 kg N ha^{-1} for the silt loam, sandy loam and loamy sand, respectively, during the second year (Table 7.5). In both years, the simulated NO_3^- leaching was the highest in the loamy sand and the lowest in the silt loam, and the simulated denitrification was in all unamended soils negligible compared to leaching losses (< 2 kg N ha^{-1}).

To calculate the simulated net N release from the crop residues, the simulated amount of mineral N from the unamended soil was subtracted from the simulated amount of mineral N in the crop residue only treatment. During both years, the simulated net N release from the crop residues was lower in the silt loam (on avg. 44% of added N for both years) than in the sandy loam (63% of added N) and loamy sand (on avg. 64% of added N for both years). Incorporating crop residues, led to an increase in simulated NO_3^- leaching in all textures (increase between 44 and 99 kg N ha^{-1} compared to unamended soils), and, in relation to the amount of N added with the crop residues, the highest leaching losses were simulated in the loamy sand (on avg. 34% of added N in loamy sand compared to 17% of added N in silt loam and sandy loam for both years). Simulated denitrification also increased after incorporation of crop residues and was between 19 (loamy sand) and 41 kg N ha^{-1} (silt loam) higher than in the unamended soils.

To calculate the simulated N immobilization potential of the immobilizer wastes, the simulated amount of mineral N in the crop residue only treatment was subtracted from the simulated amount of mineral N in the treatments amended with a particular immobilizer waste. During the first year, compost immobilized 72% of the N released by the crop residues (111 kg N ha^{-1}) in the

silt loam and 59% (106 kg N ha^{-1}) in the loamy sand, while saw dust (in silt loam) immobilized 36% of the released crop residue-N (55 kg N ha^{-1}). Hence, according to the model calculations green waste compost had a good potential to immobilize N both in the silt loam as in the loamy sand. However, the model underestimated the mineral N content in the compost treatment in both textures (Table 7.6), and the actual N immobilization potential of green waste compost was probably much lower, as was suggested by the measured mineral N (Fig. 7.5). During the second year, straw immobilized 31% of the released crop residue-N (74 kg N ha^{-1}) in the silt loam, 35% (85 kg N ha^{-1}) in the sandy loam and 89% (75 kg N ha^{-1}) in the loamy sand. In most cases, NO_3^- leaching was reduced by the organic wastes and the reduction varied between 31 and 65 kg N ha^{-1}. During the second year in the loamy sand, straw reduced NO_3^- leaching to levels comparable to the unamended soil. However, in the saw dust/straw treatment in the silt loam, NO_3^- leaching was slightly higher than in the crop residue only treatment probably due to the higher mineral N content in soil at the start of the second year (204 kg N ha^{-1} in the saw dust/straw treatment compared to on avg. 155 kg N ha^{-1} in the other three treatments). When cauliflower leaves were mixed with straw, simulated denitrification losses increased in the silt loam (with 7.6 kg N ha^{-1}) and sandy loam (with 15 kg N ha^{-1}) during the second year compared to the crop residue only treatment. In all other treatments, small reductions in simulated denitrification compared to the crop residue only treatment were found (reductions between 1.1 and 4.9 kg N ha^{-1}).

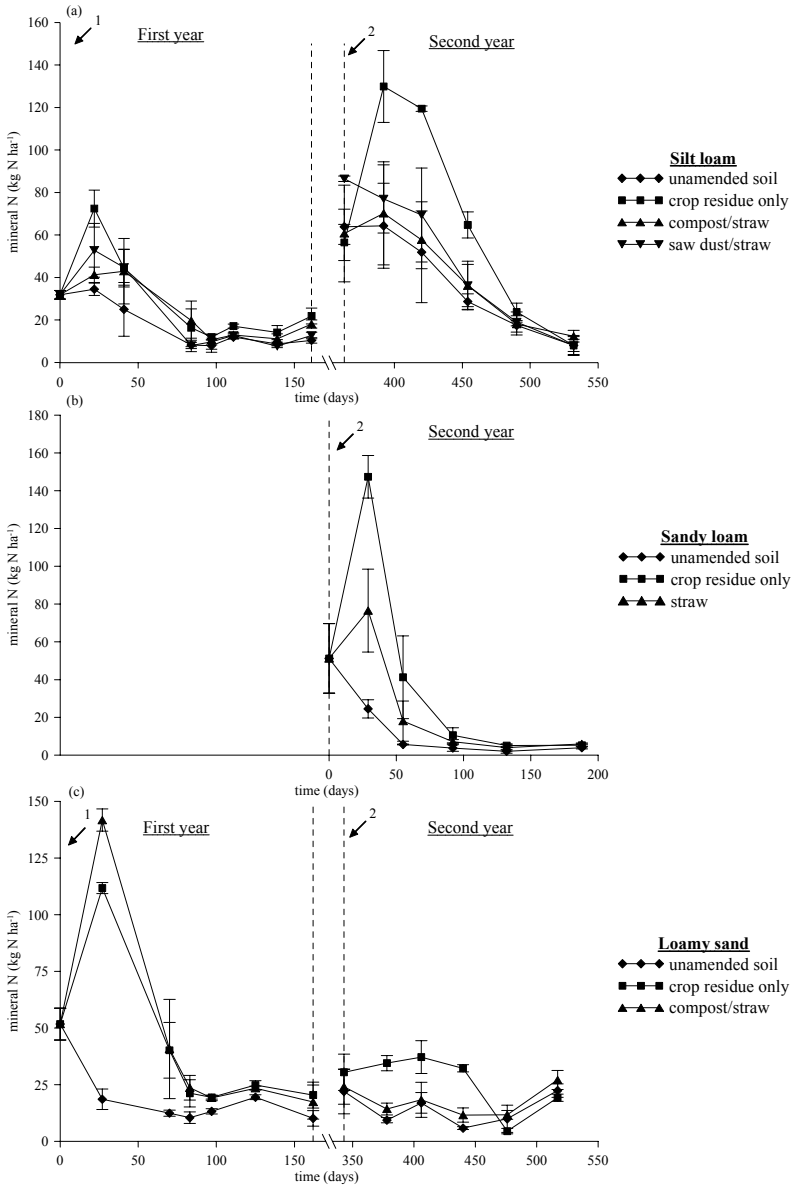

Fig. 7.5 Measured mineral N in the top 15 cm in silt loam (a), sandy loam (b) and loamy sand (c) during the immobilization phases of both years; error bars represent standard deviations; 1= incorporation of crop residues and compost or saw dust; 2= incorporation of crop residues and straw

Table 7.4.Input and output data for the N mineralization/immobilization model coupled to the Burns-α model during the immobilization phase of the first year

Treatment	DM	N_A [c]	k_{opt}	D_P	$N_{release}$	$N_{leached}$	N_{den}	net N [e]
	t ha^{-1}	% of added N	day^{-1}	kg ha^{-1} day^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Silt loam (162 days [a])								
Unamended soil	- [b]	- [b]	0.650 [d]	4.07	76.9	170.1	0.67	-†
Crop residue only	10.4	42.5	0.149	56.1	229.1	261.6	15.3	152.2
Compost	18.7	-98.5	0.400	69.8	118.4	195.9	13.6	-110.7
Saw dust	8.97	-1176.3	0.360	48.5	173.7	230.9	11.6	-55.4
Loamy sand (162 days [a])								
Unamended soil	- [b]	- [b]	0.760 [d]	1.97	92.7	204.9	0.86	- [b]
Crop residue only	7.49	52.5	0.243	32.9	272.3	304.2	21.0	179.6
Compost	18.7	-98.5	0.400	40.9	166.3	254.3	19.9	-106.0

[a] simulation days; [b] not applicable; [c] % of organic N for crop residue only and % of total N for organic wastes; [d] for unamended soil k_{opt} (in kg N ha^{-1} day^{-1}); [e] negative values indicate N immobilization; DM: dry matter content; N_A: amount of mineralizable N; κ: the temperature dependence parameter; k_{opt}: the optimum rate constant at optimum temperature; D_P: potential denitrification rate (~15 cm depth); $N_{release}$: simulated N mineralization in the top 30 cm; $N_{leached}$: simulated N leached below 90 cm; N_{den}: simulated denitrification; net N: net N release for crop residues or N immobilization for organic wastes

Table 7.5 Input and output data for the N mineralization/immobilization model coupled to the Burns-α model during the immobilization phase of the second year

Treatment	DM	N_A [c]	k_{opt}	D_P	$N_{release}$	$N_{leached}$	N_{den}	net N [e]
	t ha^{-1}	% of added N	day^{-1}	kg ha^{-1} day^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Silt loam (169 days [a])								
Unamended soil	- [b]	- [b]	0.650 [d]	4.07	86.2	133.4	1.94	- [b]
Crop residue only	10.8	51.6	0.132	56.1	328.6	183.5	41.3	242.4
Compost/straw	11.0	-156.7	0.398	79.1	254.6	163.3	61.9	-74.0
Saw dust/straw	11.0	-156.7	0.398	79.1	254.6	188.6	61.5	-74.0
Sandy loam (188 days [a])								
Unamended soil	- [b]	- [b]	0.690 [c]	2.34	110.4	146.7	1.69	- [b]
Crop residue only	3.8	57.9	0.129	36.7	351.6	233.4	39.2	241.2
Straw	11.0	-156.7	0.398	51.7	266.8	193.9	47.8	-84.8
Loamy sand (174 days [a])								
Unamended soil	- [b]	- [b]	0.760 [c]	1.97	106.4	159.7	0.84	- [b]
Crop residue only	11.8	45.2	0.185	32.9	191.0	203.8	19.8	84.7
Compost/straw	11.0	-156.7	0.398	46.4	115.8	157.0	17.1	-75.2

[a] simulation days; [b] not applicable; [c] % of organic N for crop residue only and % of total N for organic wastes; [d] for unamended soil k_{opt} (in kg N ha^{-1} day^{-1}); [e] negative values indicate N immobilization; abbreviations see Table 7.4

Table 7.6 Statistical evaluation of the models' performance for simulating the NO_3^--N content in all soil layers during the immobilization phase of both years

	First immobilization phase				Second immobilization phase			
	R [a]	EF [b]	CD [c]	CRM [d]	R [a]	EF [b]	CD [c]	CRM [d]
Optimum value	1	1	>1	0	1	1	>1	0
Silt loam								
Unamended soil	0.65	0.24	1.49	0.26	0.76	0.55	1.37	0.06
Crop residue only	0.77	0.55	1.20	0.08	0.79	0.61	1.57	0.06
Compost/straw	0.53	-0.24	1.00	0.37	0.80	0.34	0.71	-0.18
Saw dust/straw	0.66	0.34	1.23	0.12	0.70	0.20	0.78	-0.18
Sandy loam								
Unamended soil	-	-	-	-	0.82	0.48	0.95	-0.30
Crop residue only	-	-	-	-	0.83	0.61	3.04	-0.07
Straw	-	-	-	-	0.81	0.34	0.93	-0.39
Loamy sand								
Unamended soil	0.92	0.83	1.42	0.08	0.80	0.33	0.78	-0.42
Crop residue only	0.79	0.62	1.48	0.03	0.86	0.52	0.71	-0.23
Compost/straw	0.91	0.26	1.34	0.47	0.90	0.61	0.63	-0.17

[a] Correlation coefficient; [b] Modelling efficiency; [c] Coefficient of determination; [d] Coefficient of residual mass; - not applicable

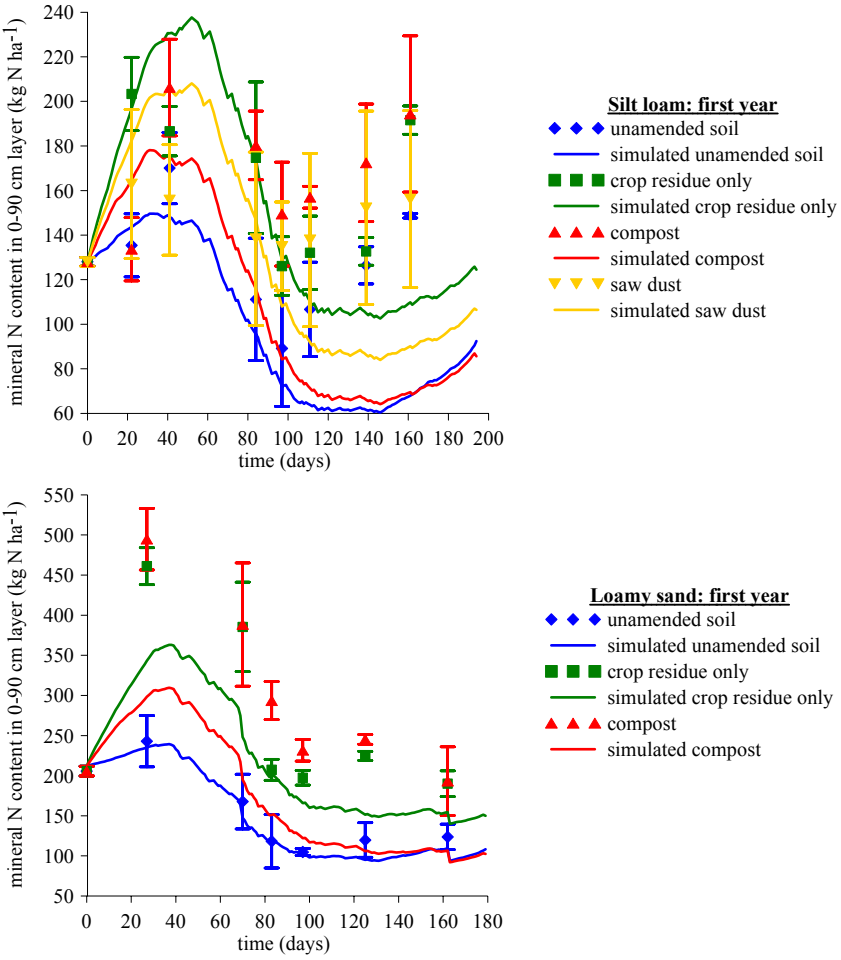

Fig. 7.6 Measured and simulated mineral N content over the whole soil profile (0-90 cm) during the immobilization phase of the first year; error bars are standard deviations of measured data

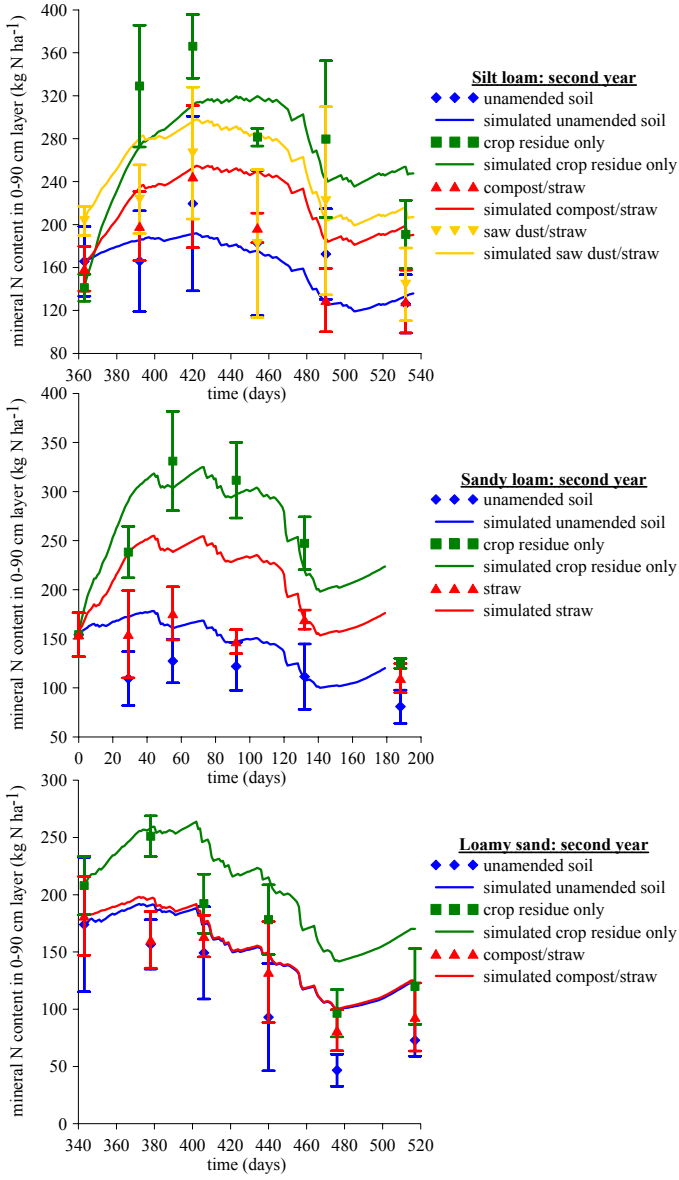

Fig. 7.7 Measured and simulated mineral N content over the whole soil profile (0-90 cm) during the immobilization phase of the second year; error bars are standard deviations of measured data

Remineralization phase

Microbial biomass

The general trend found in the immobilization phase, namely that microbial biomass N was generally higher in the treatments where an immobilizer waste was added (without remineralizer waste) compared to the unamended soil or crop residue only treatment, was also visible during the remineralization phase of both years (Fig. 7.8).

When malting sludge was incorporated in the compost/(straw) or saw dust/(straw) treatments at the start of the remineralization phase, there was a small, short-lived trend (but non-significant) towards a higher microbial biomass N compared to the respective treatments where no malting sludge was added (Fig. 7.8). The increase was 21 en 32 kg N ha^{-1} in the silt loam when malting sludge was added after compost and saw dust, respectively, while in the loamy sand, the increase was only 14 kg N ha^{-1} when malting sludge was added after compost. The increase in microbial biomass N lasted only one sampling date (20-30 days), except after saw dust where it lasted two sampling dates (98 days).

During the second remineralization phase, adding vinasses to the (compost)/straw treatments had no effect on microbial N in the loamy sand and sandy loam (Fig. 7.8). In the silt loam, there was again a small trend (but non-significant) towards a higher microbial biomass N when vinasses was added compared to the respective treatments where no vinasses was added. Microbial biomass N increased up to 33 kg N ha^{-1} in the compost/straw + malting sludge/vinasses treatment (during 62 days) and increased up to 63 kg N ha^{-1} in the saw dust/straw + malting sludge/vinasses treatment (during 113 days) in the silt loam.

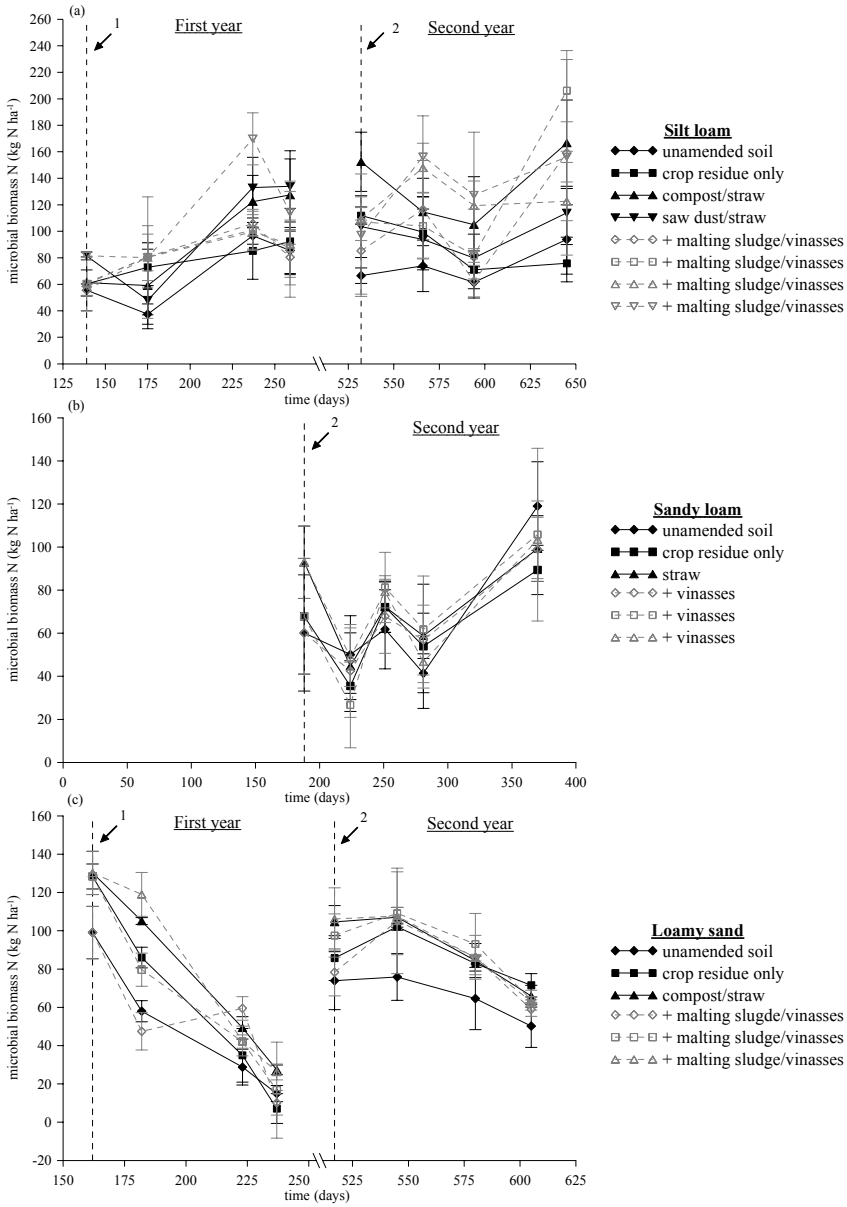

Fig. 7.8 Microbial biomass N in top 15 cm in the silt loam (a), sandy loam (b) and loamy sand (c) during the remineralization phases of both years; error bars are standard deviations; 1= incorporation of malting sludge; 2= incorporation of vinasses

Dry matter yield and N uptake of the subsequent crop

The significant differences between the treatments for the dry matter yield and N uptake were limited, and therefore, only general trends observed during both years are described.

Leek, ryegrass (first cut) and lettuce generally had the lowest dry matter yield when grown on the unamended soil (Tables 7.7, 7.8 and 7.9). The second cut of ryegrass on the silt loam (first year) showed absolutely no significant differences in dry matter yield or N uptake between the treatments. When crop residues had been added to the soil in autumn, the dry matter yield of the subsequent crop was mostly significantly ($P < 0.05$) higher compared to the unamended soil. When the crop residues had been mixed with an immobilizer waste in autumn, the dry matter yield of the subsequent crop was mostly higher compared to the unamended soil, but generally lower than in the crop residue only treatment. Adding a remineralizer waste (malting sludge, vinasses) in spring mostly decreased the dry matter yield of the subsequent crop compared to the respective treatment where no remineralizer waste was added, except when the remineralizer waste was added to an unamended soil.

The N uptake showed similar trends as the dry matter yield, with the lowest N uptake in the unamended soil, followed by the treatments where crop residues had been mixed with an immobilizer waste and followed by the crop residue only treatment. In the first year, the highest N uptake was found when malting sludge had been added to an unamended soil, both in the silt loam as in the loamy sand. However, when malting sludge was added in combination with crop residues or an immobilizer waste, the N uptake was mostly lower than in the respective treatment where no malting sludge had been added. In the second year, adding vinasses mostly led to a lower N uptake compared to the respective treatments without vinasses, except in the sandy loam where the crop residue/vinasses treatment gave the highest N uptake.

Table 7.7 Dry matter yield (DM) and N uptake of ryegrass cropped on the silt loam (first year); between brackets standard deviations; different letters indicate significant differences (*P*< 0.05) in a column

Treatment	*First cut*		*Second cut*	
	DM	N_{uptake}	DM	N_{uptake}
	t ha^{-1}	kg N ha^{-1}	t ha^{-1}	kg N ha^{-1}
Unamended soil	2.8 (0.5) [a]	104.0 (11.2) [a]	6.1 (0.8) [a]	214.8 (36.4) [a]
Crop residue only	3.7 (0.3) [ab]	149.0 (11.0) [b]	6.2 (0.4) [a]	209.6 (10.8) [a]
Compost	4.3 (0.7) [b]	162.9 (24.7) [b]	6.1 (0.4) [a]	199.1 (35.9) [a]
Saw dust	3.5 (0.2) [ab]	141.4 (2.9) [ab]	5.7 (0.7) [a]	198.6 (24.5) [a]
Soil + malting sludge	5.4 (0.7) [c]	221.3 (25.9) [c]	5.5 (0.8) [a]	172.0 (16.2) [a]
Cauliflower + malting sludge	3.3 (0.8) [ab]	124.2 (28.9) [ab]	6.5 (0.2) [a]	228.8 (14.5) [a]
Compost + malting sludge	3.7 (0.6) [ab]	145.7 (23.1) [b]	5.9 (1.3) [a]	199.1 (41.4) [a]
Saw dust + malting sludge	3.3 (0.2) [ab]	130.8 (1.2) [ab]	6.0 (0.0) [a]	210.6 (12.6) [a]

Table 7.8 Dry matter yield (DM) and N uptake of leek (first year) and lettuce (second year) cropped on the loamy sand; between brackets standard deviations; different letters indicate significant differences (*P*< 0.05) in a column

Treatment	First year: leek		Second year: lettuce	
	DM *	N_{uptake}	DM	N_{uptake}
	t ha^{-1}	kg N ha^{-1}	t ha^{-1}	kg N ha^{-1}
Unamended soil	5.3 (0.6) [a]	181.1 (18.1) [ab]	4.1 (0.4) [ab]	156.6 (1.4) [a]
Crop residue only	8.1 (0.9) [b]	218.6 (25.3) [b]	5.2 (0.4) [b]	195.8 (20.2) [a]
Compost/straw	6.5 (0.5) [a]	194.2 (2.6) [ab]	4.5 (0.5) [ab]	184.1 (33.4) [a]
Soil + malting sludge/vinasses	6.3 (0.6) [a]	230.9 (46.0) [b]	3.8 (0.4) [a]	153.2 (18.9) [a]
Cauliflower + malting sludge/vinasses	6.6 (0.6) [a]	189.0 (10.8) [ab]	4.4 (0.7) [ab]	174.3 (19.7) [a]
Compost/straw + malting sludge/vinasses	6.3 (3.5) [a]	162.4 (22.8) [a]	5.0 (0.4) [b]	187.4 (14.2) [a]

* leek leaves were not removed before weighing the plants

Table 7.9 Dry matter yield (DM) and N uptake of leek (second year) cropped on the sandy loam; between brackets standard deviations; different letters indicate significant differences (*P*< 0.05) in a column

Treatment	DM *	N_{uptake}
	t ha^{-1}	kg N ha^{-1}
Unamended soil	7.9 (0.1) [a]	212.8 (28.2) [ab]
Cauliflower only	8.0 (0.2) [a]	211.5 (15.9) [ab]
Straw	8.5 (1.9) [a]	204.1 (28.3) [ab]
Soil + vinasses	8.6 (1.3) [a]	177.4 (19.7) [a]
Cauliflower + vinasses	10.2 (1.6) [a]	247.5 (28.4) [b]
Straw + vinasses	8.2 (2.2) [a]	190.8 (40.4) [ab]

* leek leaves were not removed before weighing the plants

Mineral N

Due to the N fertilization and N uptake by the growing crop, the measured mineral N content in soil during the remineralization phases of both years could not give a definite conclusion concerning the remineralization of immobilized N by malting sludge or vinasses (data not shown). Therefore, the remineralization of immobilized N (priming effect) at a certain sampling date was determined by taking into account the amount of N fertilizer added and an estimate of the N uptake of the crop at that sampling date and calculated as the mineral N content in the 'immobilizer waste + remineralizer waste' treatment minus the mineral N content in the 'immobilizer' treatment, minus the net N mineralization of malting sludge and vinasses at each sampling date in the top 15 cm layer (Fig. 7.9). In the silt loam, during the second year, no crop was sown on the field, hence, the measured mineral N data could be used directly to calculate the remineralization of immobilized N by vinasses.

No significant remineralization of immobilized N could be observed in the two years. In the first year, there was a general trend towards N immobilization (e.g. negative priming effect) after incorporation of malting sludge in both textures (silt loam and loamy sand). The negative priming effect of malting sludge was maximum 119 kg N ha^{-1} in the silt loam and maximum 36 kg N ha^{-1} in the loamy sand.

During the second year, there was a trend for a positive priming effect (however not significant) after incorporation of vinasses. In the silt loam, vinasses increased the mineral N content maximum 54 kg N ha^{-1}, in the sandy loam maximum 84 kg N ha^{-1} and in the loamy sand maximum 97 kg N ha^{-1}.

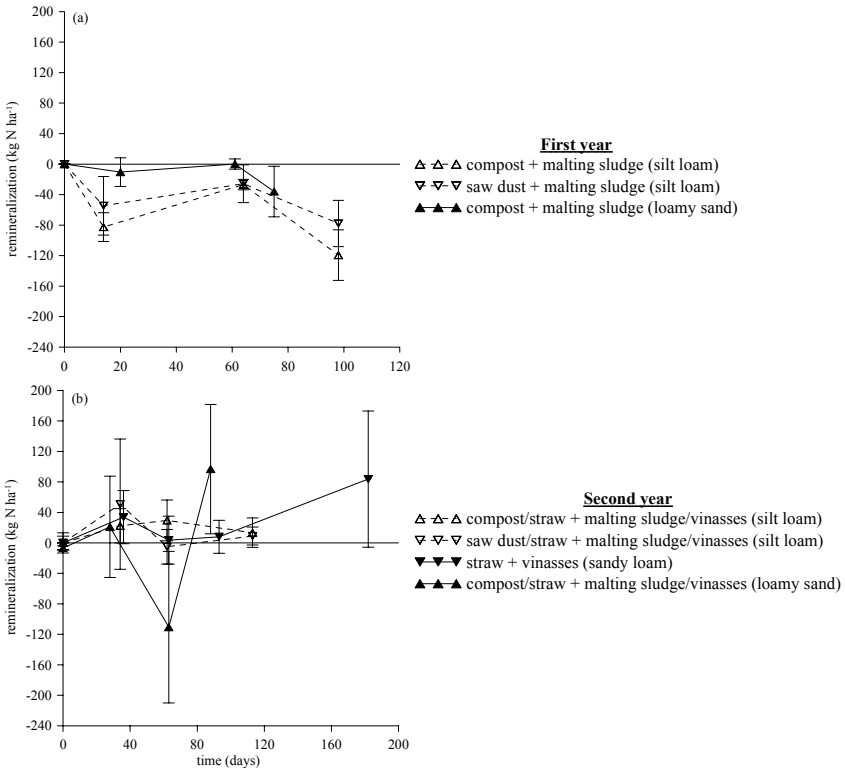

Fig. 7.9 Remineralization by malting sludge in the first year (a) and vinasses in the second year (b) in the different soil textures; error bars are standard deviations

7.4 Discussion

Immobilizer wastes

The actual N immobilization potential of green waste compost during the first year was low as was shown by the measured mineral N content and microbial biomass N (Fig. 7.4 and 7.5) and was overestimated by the model. The

overestimation of the N immobilization was probably partly due to the fact that the model parameters N_A and *k* were based on laboratory incubations where N immobilization proceeded under much more favourable conditions and where the organic wastes and crop residues were much more intimately mixed. Furthermore, the model used only the C:N ratio and the total C content of the organic wastes to estimate N_A and *k*, while it has been shown that other characteristics like the lignin content also influence the N immobilization potential of organic wastes (Chaves et al., 2005a). The high lignin content (48% on OM) of the green waste compost possibly slowed down the decomposition and N demand of micro-organisms, thus reducing the N immobilization potential, what was not simulated by the model. The model better simulated the N immobilization by saw dust which also had a high lignin content (43% on OM). In this case, the better model performance was probably due to the fact that saw dust is a much finer material that is more easily incorporated in soil and mixed with the crop residues.

Straw (lignin content of 37% on OM) showed the best N immobilization potential, especially in the loamy sand soil. The increase in microbial biomass N was proportional to the N immobilization potential (largest increase in loamy sand), indicating that crop residue-N was tied up in the microbial biomass, where it was protected from leaching.

Texture clearly had an influence on the N immobilization potential of straw, with the largest reductions in NO_3^- leaching in the loamy sand. In most studies, a lower N mineralization and higher N immobilization of both soil organic matter as fresh organic material and a higher microbial biomass is found in fine-textured soils which is attributed to a greater physical protection of organic matter and microbial biomass in fine-textured soils by sorption to mineral particles or incorporation into soil aggregates whereby plant residues may be rendered physically inaccessible to microbial turnover (Hassink, 1993; Rasiah, 1999; Thomsen et al., 2001). Texture also influences soil structure, e.g. pore size distribution, gas exchange, mobility of cells, diffusion of substrates, which are all more favourable in coarse-textured soils what leads to more N

mineralization in sandy soils (Schjonning et al., 1999). In contrast to other studies (Thomsen et al., 1996; Egelkraut et al., 2000), we found that the increase in microbial biomass N and the N immobilization potential were the highest in the loamy sand. These results may be explained by a better mixing between soil, crop residues and organic wastes in the loamy sand (easier incorporation) compared to the silt loam and sandy loam leading to an increased surface area of the crop residues and immobilizer wastes exposed to micro-organisms, a higher initial colonization rate of the materials and a better decomposition and N immobilization. Furthermore, when the mixing between crop residues and organic wastes is better, micro-organisms have more access to the N released from crop residues, and they can better use (immobilize) the released N (Wang and Bakken, 1997). Vinten et al. (1998) also showed that when crop residues and paper waste were left close to the soil surface, NO_3^- leaching (measured by suction cups) was less reduced than when they were incorporated (conventional ploughing).

Straw incorporation may reduce NO_3^- leaching during the first winter, but can lead to higher mineral N contents and higher NO_3^- leaching on the long term due to N mineralization from microbial biomass (Nicholson et al., 1997; Catt et al., 1998; Silgram and Chambers, 2002). A higher N mineralization during the growing season can be beneficial, since it can mean a reduction of N fertilizer recommendations. However, out-of-season N mineralization must be prevented, e.g. by a new incorporation of immobilizer wastes after harvest of the crop in autumn. In this study, there was a general trend that microbial biomass N remained higher when immobilizer wastes were added compared to the unamended soil and crop residue only treatment. Part of this microbial N will eventually become part of the stable soil organic matter (SOM) pool whereas another part will be mineralized, and the partitioning between those two pools will determine the potential long term NO_3^- leaching loss.

Remineralization of immobilized N and effects on the subsequent crop

Adding crop residues to soil had a positive effect on the dry matter yield and N uptake of the subsequent crop, especially in the loamy sand. Since there was

no correlation between the dry matter yield and N uptake of the crop and the mineral N content in soil in any of the treatments, the incorporation of crop residues may have increased the dry matter yield and N uptake due to a positive effect on soil structure, water holding capacity, etc. (Blevins and Frye, 1993). However, co-incorporation of an immobilizer waste in autumn mostly did not lead to an extra increase of the dry matter yield or N uptake of the crop compared to the crop residue only treatment. Vinten et al. (1998) also found no effect of paper waste which was incorporated together with crop residues in autumn on the dry matter yield or N uptake of the subsequent crop. This indicates that mixing crop residues with immobilizer wastes either does not lead to an extra positive effect on the soil structure or water holding capacity, or that the positive effects had disappeared by the time that the subsequent crop was planted. On the other hand, N immobilization after incorporation of organic wastes in autumn may occur at the expense of the N supply to the subsequent crop (Wivstad et al., 1996) and in this case stimulation of remineralization may be a crucial factor within the method of manipulating the N release from crop residues to limit any negative effects of immobilizer wastes on the subsequent crop.

The most common explanation for a priming effect (i.e. remineralization of immobilized N) is an increased soil organic matter decomposition as a result of a higher microbial population and activity due to the higher availability of energy and nutrients from added organic materials (Kuzyakov et al., 2000; Fontaine et al., 2003). The effects of malting sludge and vinasses on microbial biomass N were limited, and no significant remineralization of immobilized N could be observed, especially after addition of malting sludge.

Incorporating malting sludge or vinasses led in general to a decrease of the dry matter yield or N uptake of the subsequent crop compared to the respective treatments where no remineralizer waste was added. The negative effect of malting sludge and vinasses on the subsequent crop may have been caused by a limited supply of nutrients, negative impacts on soil structure, water holding capacity or toxic components in the wastes. For example vinasses has a large

salt content, and may have negative effects on the germination of seeds (Murillo et al., 1998).

Possible reasons of the low remineralization of malting sludge and vinasses may be the low N immobilization of the immobilizer wastes or an unsuitable biochemical composition for inducing N priming effects, i.e. not-easily decomposable, complex molecules (Chaves et al., 2006b).

7.5 Conclusions

This study confirms that the N immobilization potential of immobilizer wastes does not only depend on their C:N ratio, but that the general decomposability of the immobilizer waste is important (i.e. low lignin content, small enough particle size) as well as high soil temperatures, homogeneous mixing between crop residues and organic wastes, incorporation in soil under favourable conditions. Especially in the fine-textured soils in this study, the conditions for decomposition were rarely at optimum due to an insufficient mixing between the immobilizer wastes and the crop residues, low temperatures, wet conditions. Under more favourable conditions as in the loamy sand soil, N immobilization by straw reached 89% of the released crop residue-N with a reduction in NO_3^- leaching to levels comparable as in the unamended soil, but, especially in fine-textured soils, manipulating the N release from crop residues may not always be adequate to reduce NO_3^- leaching after vegetables.

The remineralizer wastes malting sludge or vinasses were not able to stimulate remineralization to an extent that a subsequent crop could benefit from it. Synchronization between the N release from crop residues and the N demand by a subsequent crop including the efficient re-use of immobilized residue-N, therefore remains difficult to achieve by the addition of organic wastes to the soil.

Chapter 8

Manipulating the N release from ^{15}N labelled crop residues by using straw under field conditions

Chaves B, De Neve S, Boeckx P, Dupont R, Van Cleemput O, Hofman G (2006) Manipulating the N release from ^{15}N labelled celery residues by using straw and vinasses under field conditions. (submitted)

Abstract The effect of straw and vinasses on the N mineralization-immobilization turnover of celery residues was investigated in a field experiment on a loamy sand. At the start of the experiment, ^{15}N labelled celery residues were mixed with straw in order to immobilize the released celery-N followed by an incorporation of vinasses after 200 days aiming to remineralize the immobilized N. Total N, mineral N and their ^{15}N enrichments as well as microbial biomass N were determined at regular time intervals. During the first 62 days after the incorporation, straw immobilized the celery derived ^{15}N in the microbial biomass and reduced the total celery-^{15}N losses from the top 25 cm by 40%. However, after 62 days, the N immobilization of straw ceased, probably due to lower temperatures (< 10°C), and was followed by remineralization of immobilized celery-^{15}N at a moment when the risk of NO_3^- leaching was still high. Hence, at the start of the next spring, the N losses from the straw treatment were similar as those from the celery only treatment. During the remineralization phase, vinasses caused no real priming effect, although it did slightly increase the amount of remineralized celery-^{15}N (3% of celery derived ^{15}N) compared to the straw treatment without vinasses (1.5% of celery derived ^{15}N) probably due to a pool substitution effect.

8.1 Introduction

At this moment, manipulating the N release from crop residues by using organic wastes is still quite difficult to achieve under field conditions and is not always adequate to reduce NO_3^- leaching after the harvest of vegetables (Chaves et al., 2006b).

Incorporating ^{15}N labelled crop residues in soil allows to follow the released N into the different N fractions (Müller and Sundman, 1988; Thomsen, 1993; Jensen et al., 1997; Wivstad, 1999). Therefore, incorporating ^{15}N labelled crop residues together with organic wastes under field conditions may reveal some of the mechanisms that take place in soil during the decomposition and may help to explain some unexpected phenomena observed.

The aim of this study was to examine the effect of straw (as an immobilizer waste) and vinasses (as a remineralizer waste) on the N mineralization-immobilization turnover (NMIT) of ^{15}N labelled crop residues under field conditions.

8.2 Materials and methods

Crop residues and organic wastes

^{15}N labelled celery residues (*Apium graveolens* L.) were chosen as crop residues because of their high N mineralization potential (Scharpf, 1991; De Neve and Hofman, 1996). The celery residues were separated into leaves and stems, dried at 55°C and added in a known ratio (leaves:stems = 1:1.5 on dry matter) in order to achieve a more homogeneous N mineralization. Cereal straw was chosen as *immobilizer waste* since it has a high C:N ratio and was already shown to possess a N immobilization potential (Rahn et al., 2003; De Neve et al., 2004; Chaves et al., 2005a, 2006a). Vinasses from the sugar industry, was chosen as *remineralizer waste*, because of its high content of easily decomposable C. It has been shown that addition of C in the form of

sugars can lead to a marked increase in soil microbial activity (Falih and Wainwright, 1996).

Subsamples of the crop residues and organic wastes were dried at 55°C until constant weight for the determination of the dry matter content, and then milled for further analysis. Total C and N contents were determined using a CNS elemental analyser (Variomax CNS, Elementar, Germany). The ^{15}N atom% of total N was determined using an elemental analyser (ANCA-SL, PDZ Europe) connected to an Isotope Ratio Mass Spectrometer (IRMS; Model 20-20, PDZ Europe). The Stevenson fractionation as modified by De Neve and Hofman (1996) and the CNS elemental analyser were used to determine the C:N ratio of the lignin fraction since, this parameter was needed to determine the N mineralization or immobilization of the different materials (cfr. *Chapter 6*). The biochemical composition of celery residues and organic wastes is presented in Table 8.1.

Table 8.1 Biochemical composition of celery residues, straw and vinasses

	DM t ha^{-1}	N kg ha^{-1}	DM %	N_{tot} g kg^{-1} DM	^{15}N atom%	C:N	C:N_L
Celery residues							
Leaves	1.59	71.0	17.8	40.1	8.02	10.0	26.3
Stems	2.35	59.0	9.07	25.9	9.27	14.2	99.6
Leaves + stems [a]	3.94	130.0	12.6	32.2	8.76	12.5	67.3
Immobilizer waste							
Straw	12.0	52.7	86.2	4.4	-[b]	105.4	177.4
Remineralizer waste							
Vinasses	3.66	215.5	60.0	55.4	-[b]	7.38	-[c]

[a] sum/weighed average; [b] not applicable; [c] no lignin fraction in vinasses; DM: applied dry matter; N: applied total N; DM: dry matter; N_{tot}: total N content; C:N_L: C:N ratio of lignin fraction

Experimental set up

The field experiment, a randomised complete block design with 3 replicates and plots of 1 m by 1 m, was set up on a loamy sand in the vegetable growing region in East-Flanders (Kruishoutem, Belgium). Some physical and chemical

characteristics of the soil are presented in Table 8.2. Field was laid out in beds. The individual plots were separated by pathways of 1 m wide. Sampling was done while standing in the pathways in order to avoid compaction of soil under wet conditions.

The experiment lasted from late summer until the end of spring, and consisted of an immobilization and remineralization phase. At the start of the experiment (25 August 2004), each plot received dried ^{15}N labelled celery residues (at a rate of 35 t FM ha^{-1}; ratio leaves:stems = 1:1.5 on dry matter) and straw, as immobilizer waste (equivalent to 5 t C ha^{-1}). The materials were incorporated with a rotary cultivator to a depth of 25 cm. The treatments were: (1) unamended soil, (2) celery only and (3) straw (= celery residues + straw). Each treatment was replicated twice within each block during the immobilization phase, except the celery only treatment (see further). Samples were taken with an auger to a depth of 75 cm in 3 layers: 0-25 cm, 25-50 cm and 50-75 cm. It was impossible to sample deeper soil layers in this experiment because of a compact layer below 75 cm. In each plot, 2 augerings were taken and the soil of duplicate treatments within one block was bulked into one composite sample per treatment per block. Samples were taken 13, 35, 62, 96, 133, 173 and 200 days after the incorporation of the organic materials.

Table 8.2 Some physical and chemical properties of the soil and input data for the Burns-α leaching model

Depth	Clay	Silt	Sand	OM	BD	SAT	FC	WP	pH_{KCl}
cm	%	%	%	%	g cm^{-3}	m%	m%	m%	
0-25	6.5	8.1	85.4	1.89	1.42	28.0	16.9	6.52	6.14
25-50	5.5	9.2	85.2	1.57	1.39	29.4	17.4	6.98	5.31
50-75	6.6	8.5	84.9	1.09	1.40	21.2	15.8	6.11	4.60

BD: bulk density; OM: organic matter; SAT, FC, WP: gravimetric moisture content (m%) at saturation point, field capacity and wilting point

At the start of the remineralization phase (14 March 2005; day 200), one of two replicates of the unamended soil and straw treatment within one block received vinasses, as remineralizer waste, (equivalent to 1.5 t C ha^{-1}) which

was incorporated with a rotary cultivator to a depth of 25 cm. The celery only treatment, which was only needed to calculate the net N release from the celery residues, received no vinasses. This resulted in two additional treatments during the remineralization phase, namely (4) soil + vinasses and (5) (celery residues +) straw + vinasses. During the remineralization phase, each treatment occurred once within each block. Four samples were taken within each plot, and the samples were bulked into one composite sample per plot. Samples were taken 229, 258 and 292 days after the start of the experiment

Chemical analysis

Total N and ^{15}N atom% in soil was determined as for the celery residues. *Mineral N* (until a depth of 75 cm) was extracted with (*1N*) KCl (extraction ratio = 1:2) and the extracts were analysed for NH_4^+-N and NO_3^--N colorimetrically with a continuous flow auto-analyser (Chemlab System 4, Skalar, The Netherlands). The ^{15}N atom% of NO_3^--N was determined by converting NO_3^--N to N_2O-N (Stevens and Laughlin, 1994), and measuring ^{15}N atom% of N_2O using a trace gas module (ANCA-TGII, PDZ Europe) connected to the IRMS (Model 20-20, SerCon, UK). Only the ^{15}N atom% of NO_3^--N was determined since the NH_4^+-N concentrations were very small compared to NO_3^--N. *Microbial biomass C* was determined by fumigation-extraction according to Voroney et al. (1993) using a 24h fumigation time, a (*0.1N*) KCl extractant, a soil-to-extractant ratio of 1:2 (both for fumigated and non-fumigated samples) and a conversion factor k_{EC} of 0.25. Total organic C of the extracts was determined using a Total Organic Carbon analyser (TOC-V_{CPN}, Shimadzu, Japan). *Microbial biomass N* was not determined directly, but was calculated from microbial biomass C, due to problems with the TN module of the Total Organic Carbon analyser (TOC-V_{CPN}, Shimadzu, Japan), using a C:N ratio of 6.0, which can be considered as the average C:N ratio of microbial biomass (Powlson et al., 1987; Jensen et al., 1997; Jedidi et al., 2004). The use of a similar C:N ratio for the different treatments is supported by several studies that found no significant changes in C:N ratio of the

microbial biomass after addition of organic materials (Ocio et al. 1991; Bremer and van Kessel, 1992; Joergensen et al., 1994; Jensen et al., 1997).

Calculations

In the so-called 'difference method', the *net N mineralization of the celery residues* was calculated as the difference between the mineral N content in the celery only treatment minus the mineral N content in the unamended soil. The *N immobilization of straw* was calculated as the difference between the mineral N content in the straw treatment minus the mineral N content in the celery only treatment. The *net N release from vinasses* was calculated as the difference between the mineral N content in the soil + vinasses treatment minus the mineral N content in the unamended soil. The *priming effect (or remineralization of immobilized N) caused by vinasses* was calculated as the mineral N content in the straw + vinasses treatment (treatment 5) minus the mineral N content in the celery residues + straw treatment (treatment 3), minus the net N release of vinasses (calculated from treatments 1 and 4).

In the ^{15}N method, the *total ^{15}N recovery* (%) was the total amount of celery derived ^{15}N recovered in soil expressed as % of the total amount of ^{15}N added. The *percentage of ^{15}N in mineral N* was the amount of celery derived ^{15}N recovered in the mineral N pool expressed as % of the total amount of ^{15}N added.

Meteorological data

The meteorological data were retrieved from the Provincial Experimental Station for Vegetable Production in Kruishoutem (Fig. 8.1).

N mineralization, leaching and denitrification model

A N mineralization model coupled to a NO_3^- leaching and denitrification model was used to simulate N mineralization, NO_3^- leaching and denitrification during the immobilization phase (200 days) (Chapter 6).

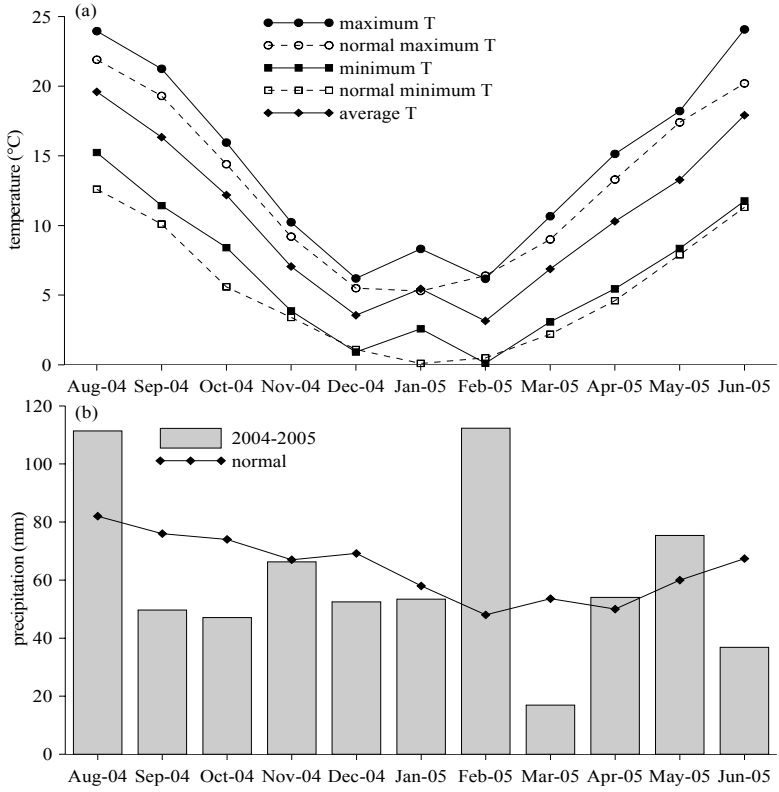

Fig. 8.1 Temperatures (a) and precipitation (b) during the field experiment

Statistical analysis

To determine significant differences between treatments, an analysis of variance with more than one independent variable ('General linear model - univariate' procedure in SPSS) and a Duncan post hoc test were used.

A set of statistical parameters was used to evaluate the model simulations: correlation coefficient (R), modelling efficiency (EF), coefficient of determination (CD) and coefficient of residual mass (CRM) (Smith at al., 1997; Moreels et al., 2003).

8.3 Results and discussion

N immobilization by straw

Total ^{15}N recovery

As expected, no significant differences in total N content between the different treatments could be found, and total N was on average 4.88 ± 0.33 t N ha^{-1} for the top 25 cm. The total ^{15}N recovery of celery derived ^{15}N during the experiment is given in Fig. 8.2 and Table 8.3.

Thirteen days after the incorporation of the celery residues, the total ^{15}N recovery was only 50% in the top 25 cm layer of the celery only treatment. In the straw treatment, the total ^{15}N recovery was even lower by day 13 (27% of total ^{15}N added), and thereafter the total ^{15}N recovery unexpectedly increased from day 13 till day 62, and reached a value exceeding that in the celery only treatment. The low ^{15}N recovery in both treatments and the strange pattern in the straw treatment were probably caused by the heavy rainfall (21.6 mm) on the first day of the experiment. This heavy rainfall probably led to losses of the dried ^{15}N labelled celery residues from the soil due to sideward leaching from the beds, on one hand. On the other hand, (gaseous) N losses may have occurred during the first days after the experiment was set up, since *Chapter 4* proved that denitrification can be rather high after incorporation of celery residues. Another reason was probably a heterogeneous distribution of the celery-^{15}N in soil, which is indicated by the large standard deviations of the total ^{15}N recovery. The total ^{15}N recovery in the straw treatment, from day 0 until day 62, must have been higher compared to the celery only treatment, as an increase of the ^{15}N recovery is theoretically impossible.

At day 62, the total ^{15}N recovery in the 0-75 cm layer was 51% in the celery only treatment and 74% in the straw treatment, of which 35% and 62% was recovered in the top 25 cm layer, respectively (Table 8.3). The total celery-^{15}N losses from the 25 cm layer at day 62 correspond to 85 and 50 kg N ha^{-1} for the celery only and straw treatment, respectively.

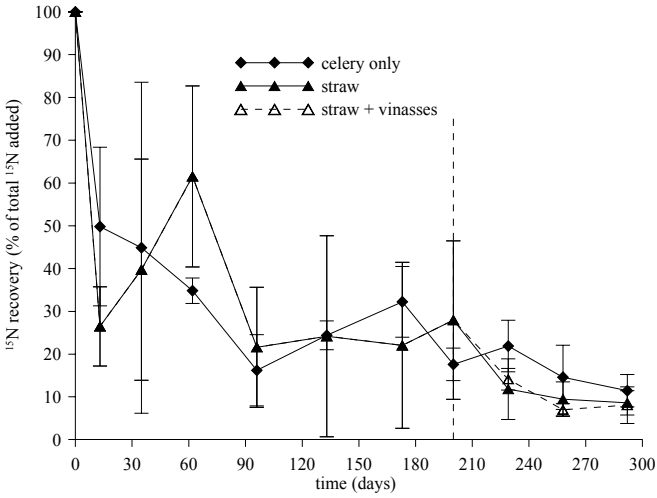

Fig. 8.2 Total ^{15}N recovery (% of total ^{15}N added) in top 25 cm in the celery only, straw and straw + vinasses treatment; error bars are standard deviations

Table 8.3 Total ^{15}N recovery (% of total ^{15}N added) and the percentage of ^{15}N in the mineral N pool (% of total ^{15}N added) in the top 25 cm and 75 cm; between brackets total N losses in kg N ha^{-1}

Time		0-25 cm			0-75 cm		
days		Celery	Straw	Straw + vinasses	Celery	Straw	Straw + vinasses
62	Total ^{15}N	34.8 (84.7)	61.5 (50.0)	- [a]	51.2 (63.5)	74.3 (33.5)	- [a]
	Mineral ^{15}N	5.2	2.8	- [a]	10.5	6.1	- [a]
200	Total ^{15}N	17.6 (107.1)	27.9 (93.7)	- [a]	23.3 (99.7)	31.4 (89.2)	- [a]
	Mineral ^{15}N	0.3	0.4	- [a]	0.6	0.5	- [a]
292	Total ^{15}N	11.4 (115.1)	8.60 (118.8)	8.01 (119.5)	16.3 (108.9)	15.8 (109.4)	12.5 (113.7)
	Mineral ^{15}N	0.0	0.0	0.1	0.0	0.0	0.0

[a] not applicable

After 62 days, the ^{15}N recovery rapidly decreased in the straw treatment, and became similar as in the celery only treatment at day 96. By the end of the

immobilization phase (200 days), N losses were more or less similar in the celery only and straw treatment, namely 107 kg N ha^{-1} and 94 kg N ha^{-1} from top 25 cm, respectively. Straw reduced the celery-N losses during the first 62 days after the incorporation, but could not conserve the celery-N in soil until the next spring. This is probably due to the relatively short period of N immobilization by straw followed by a remineralization of celery-^{15}N when the risk of NO_3^- leaching and denitrification was still high. A reason for a remineralization of celery-^{15}N could be a decrease in temperature from on average 14.9°C during the first 62 days to temperatures frequently below 10°C thereafter (on average 4.8°C between 62 and 200 days). It has been found that gross N immobilization is more sensitive to low temperatures than gross N mineralization (Andersen and Jensen, 2001) probably due to a lower affinity to both organic and inorganic substrate of micro-organisms (Nedwell, 1999), leading to a net N release at low temperatures.

Mineral N and ^{15}N recovery in mineral N

During the immobilization phase, a redistribution of mineral N to deeper soil layers and finally out of the profile, due to NO_3^- leaching, could be seen (Fig. 8.3). The N mineralization/immobilization model coupled to the Burns-α model was used to simulate the N release, NO_3^- leaching and denitrification until vinasses was added (day 200) (Tables 8.4 and 8.5, Fig. 8.4).

Table 8.4 Input and output data of the N mineralization/immobilization model coupled to the Burns-α model for a simulation period of 200 days (N immobilization phase)

	DM	N_A	κ	k_{opt}	D_P	$N_{release}$	$N_{leached}$	N_{den}
	t ha^{-1}	% of added N		day^{-1}	kg ha^{-1} day^{-1}	kg ha^{-1}		
Unamended soil	-[a]	-[a]	2.63	0.630 [b]	2.40	26.0	153.4	0.07
Celery only	3.95	32.7 [c]	3.38	0.231	43.7	96.0	200.7	2.36
Straw	12.0	-156.7 [d]	5.36	0.363	61.7	19.9	149.6	1.77

[a] not applicable; [b] for blank soil k_{opt} (in kg N ha^{-1} day^{-1}); [c] % of organic N; [d] % of total N; DM: dry matter content; N_A: amount of mineralizable N; κ: the temperature dependence parameter; k_{opt}: the optimum rate constant at optimum temperature; D_P: potential denitrification rate (~25 cm depth); $N_{release}$: simulated N mineralization in top 25 cm for the first 200 days; $N_{leached}$: simulated N leached below 75 cm for 200 days; N_{den}: simulated denitrification for the first 200 days)

Table 8.5. Statistical evaluation of the models' performance for simulating the NO_3^--N content in the 0-75 cm layer and at all sampling dates during the N immobilization phase (200 days)

	R [a]	EF [b]	CD [c]	CRM [d]
Optimum value	1	1	>1	0
Unamended soil	0.77	0.57	2.44	-0.07
Celery only	0.62	0.34	1.75	-0.14
Straw	0.69	0.45	1.89	-0.13

[a] Correlation coefficient; [b] Modelling efficiency; [c] Coefficient of determination; [d] Coefficient of residual mass

The model efficiency (EF) was positive in all treatments, CD values were higher than 1, and the correlation coefficients were quite high. However, the negative CRM values indicate that the model generally overestimated the mineral N contents in soil, probably due to the failure of the model to simulate (gaseous) N losses on the first days after the experiment was set up.

In the unamended soil, the simulated N release, NO_3^- leaching and denitrification were 26, 153 and 0.1 kg N ha^{-1}, respectively. Simulated denitrification from the unamended soil was very low, probably due to a low %WFPS (water-filled pore space; on average 53%) of the soil during the winter. NO_3^- leaching was quite high due to the high mineral N content at the start of the experiment (144 kg N ha^{-1}) and the sandy texture of the soil.

The simulated N mineralization, NO_3^- leaching and denitrification in the celery only treatment were 96, 201 and 2.4 kg N ha^{-1}, respectively (Table 8.4). Hence, the simulated net N release from the celery residues was 70 kg N ha^{-1} (54% of added N), and celery residues increased the N losses by 47 and 2.3 kg N ha^{-1} for NO_3^- leaching and denitrification, respectively. The high net N release from the celery residues compared to the potential N mineralization (N_A) used as model input (N_A: 33% of added N) was probably due to a higher N mineralization in the sandy soil compared to a silt loam soil, on which this model parameter was based (Hassink, 1993; Thomson et al., 1996; Rasiah, 1999; Egelkraut et al., 2000). The N losses simulated by the model were lower

than those derived from total ^{15}N recovery (Table 8.3, Fig. 8.2), what could be due to failure of the model to simulate (gaseous) N losses during the first days, and to the heterogeneous distribution of ^{15}N in soil. During the first 35 days, only 10% of celery derived ^{15}N was recovered in the mineral N pool in the celery only treatment (Fig. 8.5). After 35 days, the % of celery-^{15}N in mineral N decreased and around 96 days almost no celery-^{15}N was found in the mineral pool due to NO_3^- leaching. The reasons for the low net N release found by the ^{15}N method are probably the same as those for the low total ^{15}N recovery, namely the high precipitation on the first day of the experiment (leading to gaseous N losses) and a heterogeneous distribution of celery-^{15}N in soil.

In the straw treatment, the simulated N release, NO_3^- leaching and denitrification were 20, 150 and 1.1 kg N ha^{-1}, respectively, and straw had a N immobilization potential of 76 kg N ha^{-1}. The N losses in the straw treatment were similar to those in the unamended soil, and the model suggests that incorporating crop residues together with straw may be a good management option to reduce NO_3^- leaching from crop residues during the winter. The percentage of celery derived ^{15}N in the mineral N pool was only 2% during the first 62 days (Fig. 8.5) indicating that the celery-N was indeed not released as mineral N when straw was co-incorporated. However, total ^{15}N recovery showed that the N losses in the straw treatment at the end of the immobilization phase were similar as those in the celery only treatment, and that straw only temporary immobilized the celery-N. The disagreement between the results of the ^{15}N recovery and the model simulations were probably caused by large (gaseous) N losses on the first days after the setup of the experiment and the heterogeneous mixing of celery-^{15}N in soil.

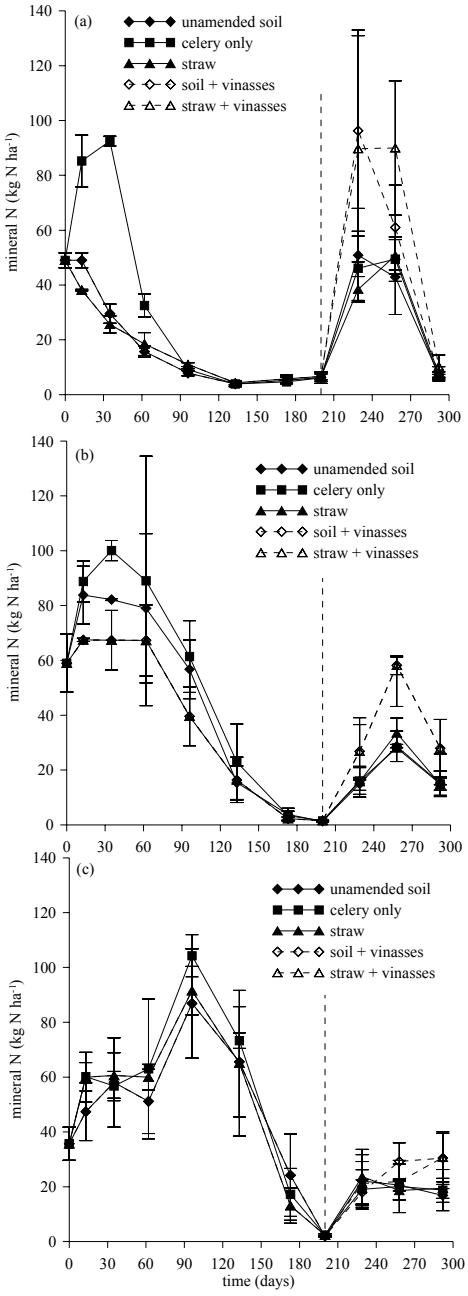

Fig. 8.3 Mineral N content in the different soil layers: (a) 0-25 cm, (b) 25-50 cm and (c) 50-75 cm; error bars are standard deviations

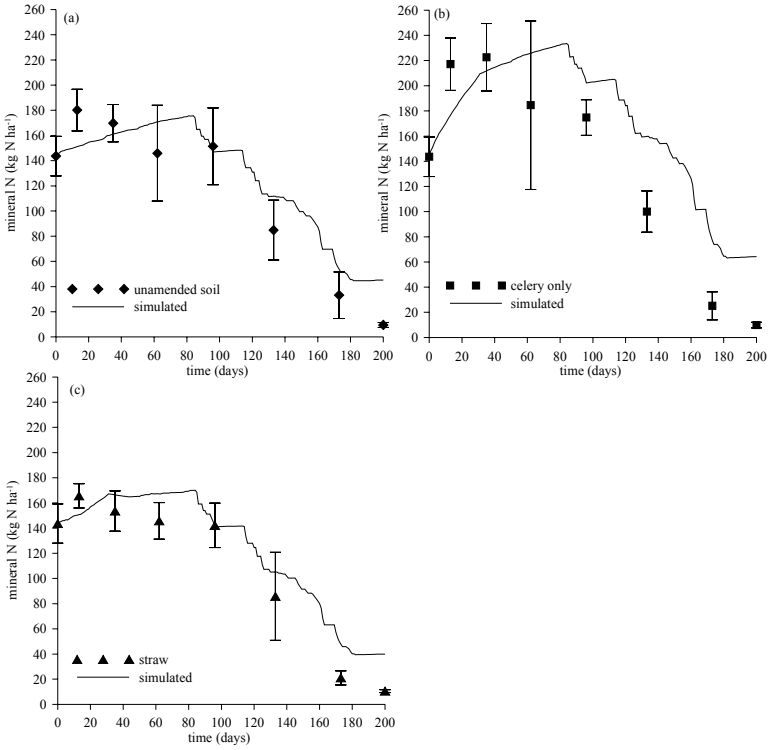

Fig. 8.4 Measured and simulated mineral N content over the whole soil profile (0-75 cm) during the immobilization phase (200 days) in the unamended soil (a), celery only treatment (b) and straw treatment (c); error bars are standard deviations on the measured data.

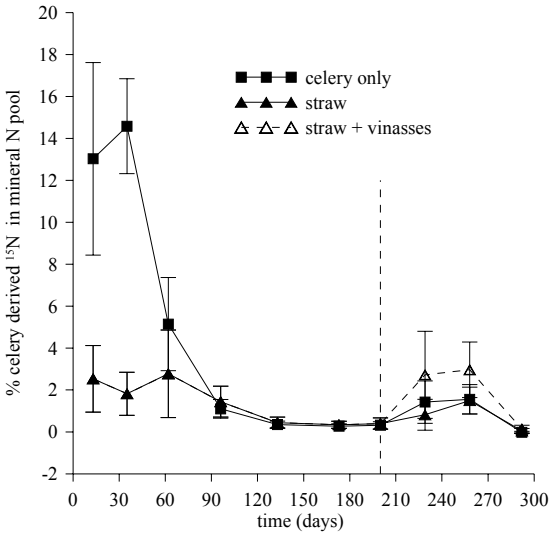

Fig. 8.5 Percentage of celery derived ^{15}N in the mineral N pool in the top 25 cm in the celery only, straw and straw + vinasses treatment; error bars are standard deviations

Microbial biomass N

Before the incorporation of celery residues and straw, the microbial biomass N accounted for 1.2% of total organic N in soil (55 kg N ha^{-1}) (Fig. 8.6). Similar values were found for sandy soils under continuous arable cropping (Powlson et al., 1987; Jensen, 1994a; Jensen et al., 1997). The incorporation of the ^{15}N labelled celery residues led to a significant increase in microbial biomass N which peaked after 13 days and was 48 kg N ha^{-1} (37% of total added celery-N) higher than in the unamended soil. A fast increase (within 30 days) in microbial biomass N (between 14 and 27% of total added N) after incorporation of N-rich crop residues has previously been found (Jensen, 1994a; Bending et al., 1998; Rahn et al., 2003). The large immobilization of celery-N into microbial biomass N in this study compared to the other studies was probably due to use of dried and ground celery residues (Chaves et al., 2006a).

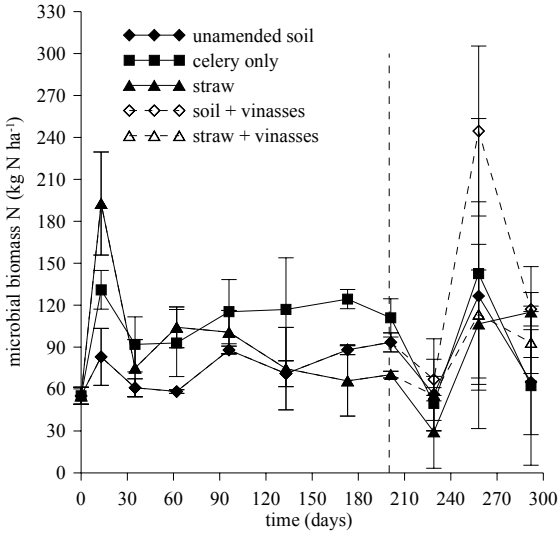

Fig. 8.6 Microbial biomass N in the top 25 cm in the different treatments; error bars are standard deviations

Mixing the ^{15}N labelled celery residues with straw led to an extra increase in microbial biomass N compared to the celery only treatment. Maximum microbial biomass N in the straw treatment occurred also after 13 days, and was 110 kg N ha^{-1} larger compared to the unamended soil or 60% of total added N (= celery + straw-N). From day 35, microbial biomass N, both in the celery only treatment and in the straw treatment, became similar as in the unamended soil, and no significant differences could be found between the treatments during the rest of the immobilization phase. In a laboratory study (Chaves et al., 2006a) microbial biomass N in the straw treatment remained significantly higher compared to the celery only treatment during the complete experiment (380 days). The shorter time span of increased microbial biomass N in this study may be due to the lower temperatures compared to the laboratory study (Pietikäinen et al., 2005), and also corresponds with the relatively short-lived N immobilization phase as found by the total ^{15}N recovery.

It has to be taken into account that the field was laid out in beds, meaning that the soil was much more sensitive to changes in air temperature. When the air temperature was high, a microclimate will have occurred in the top 25 cm of the soil leading to more microbial activity and to more N mineralization, what also explains the rather high N mineralization found in the celery only treatment. On the other hand, soil temperature in the beds will have decreased much faster when the air temperature was low, what also proves that low temperatures led to a temporal N immobilization by straw followed by remineralization.

Remineralization by vinasses

In the straw + vinasses treatment, vinasses was added at the start of the remineralization phase (day 200), with a view to enhance remineralization of immobilized celery-N (i.e. priming effect). It is widely accepted that micro-organisms play a crucial role in the process. However, the mechanisms leading to priming effects remain poorly understood. The most common explanation for priming effects is increased soil organic matter decomposition as a result of a higher microbial population or activity due to the higher availability of energy and nutrients from added organic materials (Sörensen, 1974; Kuzyakov et al., 2000; Fontaine et al., 2003).

No significant differences in the total ^{15}N recovery between the celery only, straw and straw + vinasses treatments could be found (Fig. 8.2, Table 8.3) and additional ^{15}N losses were small during the remineralization phase (on average 20 kg N ha^{-1} for the three treatments).

At the start of the remineralization phase, the mineral N content increased in all treatments as a result of higher temperatures, higher microbial activity and smaller N losses. When vinasses was added, the total mineral N content tended to increase compared to the treatments without vinasses, but this effect was not significant. The increase was mainly due to a net N release from vinasses itself (up to 45 kg N ha^{-1}) as was observed in the soil + vinasses treatment, and no real priming effect or remineralization of immobilized N could be found in the

top 25 cm (Fig. 8.7). Microbial biomass N was not significantly influenced either when vinasses was added in the straw + vinasses treatment compared to the straw treatment (Fig. 8.6). However, the percentage of celery-^{15}N remineralized was higher during the first 58 days after vinasses application (3% of celery derived ^{15}N) compared to when no vinasses was added (around 1.5% of celery derived ^{15}N in straw treatment). Chaves et al. (2006a) also found a slightly higher remineralization of celery derived ^{15}N after adding vinasses, but no real priming effect or effect on microbial biomass N, and suggested that the remineralization could be due to a pool substitution effect, since newly grown micro-organisms probably used proportionally more non-labelled N than remineralized celery derived ^{15}N in the straw + vinasses treatment, leading to more celery derived ^{15}N remaining in soil.

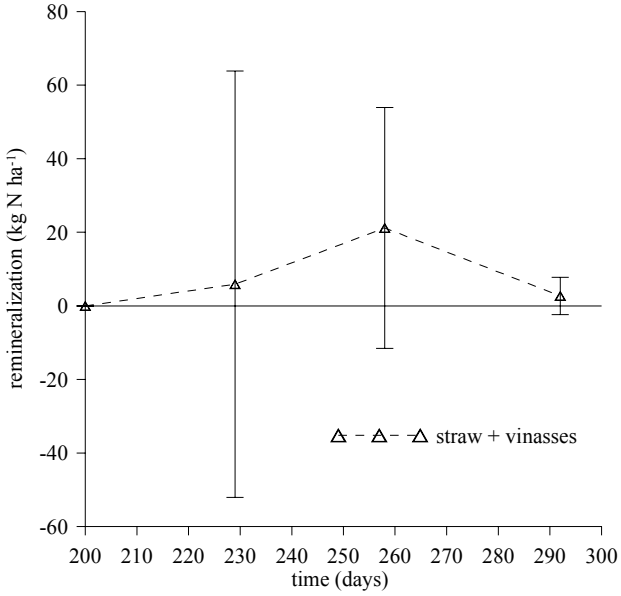

Fig. 8.7 Priming effect of vinasses in top 25 cm calculated by the difference method; error bars are standard deviations

An explanation for the low remineralization induced by vinasses could be the low amount of N immobilized by the time of addition of vinasses since it is mainly recently immobilized N that can be readily remineralized (Jensen,

1994a). Another possible reason for the low remineralization potential of vinasses may be an unsuitable biochemical composition, e.g. high molecular weight, not readily decomposable, for inducing N priming effects, since most researchers found N priming effects after incorporation of low molecular weight compounds like glucose (Asmar et al., 1994; Falih and Wainwright, 1996; Wheatly et al., 2001).

8.4 Conclusions

Straw has a potential to immobilize N released from crop residues and reduce NO_3^- leaching. However, under field conditions, low temperatures may shorten or even prevent N immobilization by organic wastes and lead to a remineralization of immobilized N, and hence make it impossible to retain the crop residue-N in soil until the following spring. Therefore, manipulating the N release from crop residues by using organic wastes may not always be adequate to reduce NO_3^- leaching after the harvest of vegetables. This study also confirms that remineralization of immobilized N occurs coincidentally and is difficult to obtain in a consistent manner.

Chapter 9

General discussion

9.1 Extrapolation of results from laboratory experiments to field experiments

In order to evaluate the method of manipulating the N release from vegetable crop residues as a tool to reduce NO_3^- leaching, in a first phase, experiments were setup in the laboratory where the effects could be quantified under controlled conditions, i.e. no N leaching losses, minimal gaseous N losses and constant temperatures and moisture contents. To validate the results found under laboratory conditions, in a second phase also field experiments were setup. However, it was not simple to extrapolate the results obtained under controlled laboratory conditions to the actual field conditions. In what follows, possible reasons for the difficulties with this extrapolation are discussed.

For the setup of the laboratory experiments, a compromise had to be found between conditions that allowed for a realistic simulation of the field conditions at the one hand, and conditions that would ensure a maximum effect of the different treatments and a reasonable variability at the other hand. The possible influence of these experimental laboratory conditions in relation to field conditions will be discussed in the following paragraphs.

All soils used in the laboratory and field experiments were selected for their low initial mineral N content (by analyzing a soil sample from each field for mineral N before the start of the experiment), to ensure that N mineralization and immobilization from the added organic materials would be more accurately measurable (i.e. against a low background mineral N in unamended soils). The low initial mineral N content of the soils can be linked to the low N mineralization rate of the unamended soils observed in most experiments. This contrasts with N mineralization rates between 0.9 and 1.6 kg N ha^{-1} day^{-1} found in soils used for intensive vegetable production in Flanders (Demyttenaere, 1991). The high input of animal manure in these soils used is one of the main reasons for their high N mineralization, especially in West-Flanders. However, our soils originated from fields in East-Flanders (Poeke,

Aalter) and from research stations (Beitem, Kruishoutem) where the input of animal manure was much lower, leading to the lower soil N mineralization rates found in this thesis. Therefore, the N losses measured here may still be a conservative estimate of the losses that can be expected in fields with average or high mineralization rates of native soil organic matter.

Throughout this research, different types of crop residues have been used because of two reasons. First, this allowed us to test a variation of important vegetable crops, both *Brassica* sp. as leafy crops, rather than just one species. Secondly, in the different experiments, different types of crop residues were sometimes used from a practical point of view such as availability. In case of celery, all the plant material can be used as crop residue, which is a practical and financial advantage for the laboratory experiments. In *Chapter 5* and *8*, the ^{15}N labelled residues were cropped in the greenhouse, using $K^{15}NO_3$ (20% ^{15}N atom%) as fertilizer. Celery residues were chosen because they are easy to cultivate and the entire plant can be used as crop residue, which is a great financial advantage. It was intended to add all organic materials as fresh materials to allow a closer simulation of field conditions. One drawback of using fresh materials is that it can lead to large standard deviations (De Neve and Hofman, 1996) as was frequently encountered in this thesis, both in laboratory and field experiments. The large standard deviations were also the result of incorporating several organic materials into the same soil either simultaneously or consecutively. E.g. during the remineralization phase up to three different organic materials were added to the same soil. Managing the variability in both laboratory and field experiments was one of the most important challenges of this project. In many instances the variability was too large to detect any significant effects of treatments, and in these cases only trends could be indicated.

Because of practical reasons, however, it was not always possible to use fresh materials. For example in *Chapter 4*, there were eight treatments in total, and each week, the N_2O emission of two treatments was measured (total time span of the experiment= 1 month). In each treatment, except the unamended soil,

the same celery residues had to be added (mixed with an immobilizer waste) in order to compare the different treatments. Therefore, the crop residues had to be dried to allow conservation of the celery residues during the complete experiment (1 month). In *Chapter 5* and *8*, where ^{15}N labelled celery residues were used, the celery residues were dried to obtain a homogeneous mixing and a large N mineralization/immobilization to ensure that the effects would be more accurately measurable and to minimum variability between replicates. Ground material is more accessible to micro-organisms than intact plant parts, due to the increased surface area of the material exposed to decomposition (Angers and Recous, 1997) and the lack of intact lignified barrier tissue (Summerell and Burgess, 1989). Therefore, the initial colonization rate and the decomposition of dried and ground residues are favoured. An increased decomposition means a higher N release from the decomposing crop residues and a higher N immobilization by the micro-organisms. Dried and ground material can also be more homogeneously mixed than fresh and chopped material and since decomposer micro-organisms only have access to part of the mineral N pool if organic wastes are not homogeneously distributed (Wang and Bakken, 1997), the use of dried and ground material will lead to a larger N immobilization.

The setup of the laboratory experiments was always chosen to ensure that all effects would be accurately measurable, and under these optimized laboratory conditions (15°C, 60% WFPS, homogenous mixing) immobilizer wastes like straw, saw dust and green waste compost were able to immobilize the N released from crop residues and to reduce the N_2O emission. However, these results could not always be transferred to actual field conditions due to suboptimal conditions such as lower temperatures during autumn and winter (< 10°C), wet conditions and a less homogenous mixing between crop residues and organic wastes. Nevertheless, in some occasions the results obtained in field conditions were comparable to those obtained under 'optimal laboratory conditions'. For example in *Chapter 7* on the loamy sand, straw was able to immobilize 89% of the released leek-N and to reduce the NO_3^- leaching to

levels comparable with those in the unamended soil, due to the easier and more homogeneous mixing in this soil.

9.2 Other important considerations when manipulating N release from crop residues

When manipulating the N release of crop residues, large amounts of organic C and N are incorporated in soil. In the experiments in this thesis, *ca*. 9000 kg C ha^{-1} and *ca*. 450 kg N ha^{-1} were incorporated to soil in one year when the crop residues were mixed with an immobilizer waste in autumn and a remineralizer waste was added in spring. If we assume that these organic materials are applied to the soil each year, with a humification coefficient of the crop residues, immobilizer waste and remineralizer wastes of 0.15, 0.22 and 0.10, respectively (Hénin and Dupuis, 1945; Kortleven, 1963; De Neve et al., 2003), and an annual decomposition rate of soil organic matter of 0.02 (Kortleven, 1963), than this would lead to an increase of the soil organic C and N of 50% and 4%, respectively, over a period of 50 years. This may increase problems like NO_3^- leaching on the long term. In the past it was already observed that long term applications of large amounts of animal manure to soil led to large soil N mineralization rates and an increased risk of NO_3^- leaching (Demyttenaere, 1991; VMM, 1998). Also straw incorporation, which reduced NO_3^- leaching during the first winter, can lead to higher mineral N contents and higher NO_3^- leaching on the long term (Nicholson et al., 1997; Catt et al., 1998; Silgram and Chambers, 2002). On the other hand, applying organic matter to soil contributes to the build up of soil organic matter and helps to maintain or improve the soil quality. Therefore, manipulating the N release from vegetable crop by using organic wastes can be expected to have several positive effects on soil physical, chemical and biological characteristics. Recently, the interest in soil organic matter is increasing again, since it is considered as one of the most important indicators for soil quality and sustainable agriculture (Kirchmann and Andersson, 2001).

A practical problem that we encountered during the experiments was that it was not always obvious to obtain organic wastes with a high C:N ratio. Overall, straw had the best N immobilization potential. However, straw is already used for other agricultural purposes, like litter in stables, farm manure, and therefore not really considered as a waste. The green waste composts used in this thesis were always immature composts containing large woody particles since they were collected early in the composting process and sieved over a mesh (10-15 cm). However, these composts were tailored to the needs of experiments in this thesis and are not commercially available at this moment. Good results were expected from paper waste, since in several previous studies paper waste had clearly shown a large N immobilization potential (Vinten et al., 1998; Rahn et al., 2003). However, it appeared that it is quite difficult to obtain paper waste with a sufficiently high C:N ratio in Belgium. The paper wastes tested in this thesis were all from factories producing magazine and newspaper paper, had C:N ratios lower than 51 and gave poor results. Literature showed that cardboard paper waste has higher C:N ratios (C:N 520; Rahn et al., 2003), and cardboard factories in Belgium do produce paper sludge with a high C:N ratio. However, the dry matter content of that sludge is very low (around 2% of dry matter), and the sludge is mostly recycled within the factory itself, and therefore not useable as an immobilizer waste. In Belgium no organic wastes with a high polyphenol content are available. Therefore, in this thesis, tannic acid was used as a model compound for organic wastes containing important amounts of water soluble polyphenols. In Mediterranean countries, olive oil mill waste is a typical waste with a high polyphenol content which could be applied as immobilizer waste.

Clearly incorporating organic wastes also means a cost for the farmer such as the cost of the organic wastes itself, the cost of the transport and the labour cost or the cost for the agricultural contractor for incorporating the material. The cost of materials like straw and green waste compost is quite high. For example straw costs *ca*. 50 euro per ton, and since the amount of straw added in this research was 12 t ha^{-1}, this results in a cost of 600 euro per ha^{-1} (personal communication with farmers). Compost distributed by SEDE

Benelux costs *ca*. 1 euro for 40 kg of fresh material, what would mean a cost of over 500 euro for 23 t FM ha^{-1} as used in this research. The cost of organic wastes coming from the industry such as malting sludge, paper sludge and dairy sludge can vary between 10 and 50 euro per ton dry material. The price strongly depends on the dry matter content, the quality of the waste and the amount of material needed (Steenhoudt and Gurdebeke, 2003). The transport cost of organic wastes increases as the dry matter content of the waste decreases and the transport distance increases, and can so strongly increase the total cost of the organic waste. Compared to the current practices (incorporating the crop residues with a cultivator; *ca*. 60 euro ha^{-1}), incorporating the crop residues together with organic wastes by using a rotary cultivator would cost twice as much (*ca*. 120 euro ha^{-1}) and incorporating a remineralizer waste in spring would again cost *ca*. 120 euro ha^{-1}. This indicates that the total cost of manipulating the N release from crop residues is certainly not negligible.

Since manipulating the N release from crop residues by using organic wastes is not always adequate to reduce NO_3^- leaching in the vegetable region in Flanders, the problem of NO_3^- leaching is not yet solved. Possible other methods to reduce NO_3^- leaching such as sowing catch crops and removal of crop residues have some drawbacks which were already discussed in the introduction. Summarizing again briefly, the N uptake of catch crops is not always sufficient when they are sown later than the beginning of September. The removal of crop residues means extra labour and machinery cost, an increased risk of soil compaction in wet conditions and a reduction of soil fertility. Furthermore, the current Flemish legislation is inadequate in relation to crop residues (Penninckx, 2005). Collecting crop residues and spreading these again is allowed solely on the same farm. Once crop residues have been removed from the field and stored, they become a waste and have to comply with the Vlarem legislation. When crop residues leave the farm and/or will be composted, they become a secondary raw material and have to comply with Vlarea, manure legislation or other legislation. This means that the farmer needs a license for composting crop residues which is coupled to a yearly

analysis and an extra cost. Hence, until the Flemish legislation is adapted, the removal of crop residues is not a realistic management option yet. Finally, farmers are not really supporters of the 'removal of crop residues'-idea. A farmer which co-operated in the experiments of this thesis stated "Applying organic material to soil in combination with crop residues seems more interesting to me than the removal of crop residues, because the N in the roots could also be immobilized, it would mean less labour (removal of crop residues and return them to the field is double work) and it is said that incorporation of straw can increase the soil quality for example by increasing the number of beneficial soil fungi".

A final possible method to reduce NO_3^- leaching after vegetables is treating the crop residues with nitrification inhibitors such as dicyandiamide (DCD) or 3,4 dimethylpyrazole phosphate (DMPP). This method gave some good results under laboratory conditions (Chaves et al., 2006), but it remains to be seen how well it works under field conditions.

9.3 Scope for further research

The N immobilization potential of organic wastes and the reduction in NO_3^- leaching was generally low under field conditions, due to an insufficient degradability of the immobilizer wastes (e.g. high lignin content) and suboptimum conditions for decomposition under field conditions like an insufficient mixing between the immobilizer wastes and crop residues, a poor incorporation in soil, low temperatures, wet conditions, etc. Hence, future research could focus on creating environmental conditions which are more favourable for manipulating the N release from crop residues. For example a specific screening of organic wastes with a high C:N ratio and a low lignin content (i.e. easily degradable) may provide organic wastes with a good N immobilization potential under field conditions. Since a good homogeneous mixing between crop residues and organic wastes is important to obtain sufficient N immobilization, further research may reveal whether for example lowering the particle size of the organic wastes or using a different application

method (e.g. no-till where the crop residues and organic waste are mixed but not incorporated) may enhance the N immobilization potential. From this thesis it seems that temperatures in Flanders during autumn and winter are suboptimal to obtain high N immobilization rates, but under warmer climate conditions manipulating the N release from crop residues in the sense of this thesis can be adequate to reduce NO_3^- leaching in the field to a much larger extent, for example in the Mediterranean. Further research is necessary to confirm this.

On the other hand, although the organic wastes used in this thesis had a low effect of on the NMIT in soil, these organic wastes may be useful to increase soil organic matter, improve soil structure or improve soil microbial activity. Further research to test the effect of immobilizer wastes like green waste compost and paper sludge on properties like general nutrient availability, C sequestration, disease suppression, soil microbial biomass and soil fauna etc. can therefore be interesting.

In this thesis priming effects occurred rather coincidentally, and the mechanisms leading to real positive priming effects remain poorly understood. On one hand, priming effects are an extra mineralization of C or N which may lead to an increase in NO_3^- leaching and gaseous N losses or lead to the decomposition of soil organic matter, while on the other hand the increased N release may be an extra nutrient source for the crop. Hence, future fundamental study of N priming effects in soil after incorporation of fresh organic materials is needed in order to identify the mechanisms behind a priming effect, the sources of priming effects (soil microbial biomass or soil organic matter) and the factors influencing a priming effect, like availability of organic C and mineral N in soil, moisture, temperature, microbial population (ratio fungi: bacteria) etc. Knowledge on the mechanisms leading to priming effects can be very useful in order to understand in which conditions priming effects occur and how they can be controlled.

Chapter 10

Summary and conclusions

10.1 Introduction

The general objective of this work was to examine whether manipulating the N release from crop residues by mixing them with organic wastes is a possible management option to reduce NO_3^- leaching during autumn and winter in intensive vegetable production. Manipulating the N release from crop residues passes through two phases - an immobilization and remineralization phase - and at the start of each phase an organic waste is incorporated. Organic wastes include e.g. straw, urban waste composts, composts from on-farm organic residues or green waste (hedge trimmings, grass clippings, …) or wastes from agricultural industries, mostly food industries, which can be used as amendments in agriculture on the condition that they contain no or small tolerable amounts of toxic compounds.

At the start of the immobilization phase (autumn-winter), crop residues are simultaneously mixed with organic wastes with a view to either immobilize the N released from crop residues or delay the N mineralization. In this way the released crop residue-N would be protected from leaching. By incorporating organic wastes at the start of the spring (remineralization phase), an additional advantage may be achieved if remineralization of immobilized N can be stimulated by the time a new crop is sown or planted.

Manipulating the N release from crop residues would have several advantages: further enhance synchronization between N release and crop N demand, reduction of NO_3^- leaching, reduced fertilizer use and an alternative route for waste disposal.

10.2 N release from vegetable root residues

In order to be able to manipulate the N release from crop residues, a reliable quantitative knowledge of the N mineralization of crop residues is needed. For most vegetables, a good knowledge is available about the amounts of above

ground crop residues left on the field and their N mineralization (Iritani and Arnold, 1960; De Neve et al., 1994; De Neve and Hofman, 1996). However, little is known concerning the N mineralization/immobilization from vegetable root residues in soil.

In the first chapter of this thesis a predictive relationship was established (under fixed environmental conditions) between the N mineralization of vegetable root residues and green manures and their (bio)chemical composition. The amount of mineralized N, $N_{A,tot}$, was best correlated with the C:N ratio, while the mineralization rate constant k_{tot} was best correlated with the lignin:N ratio. The approach taken here has two strengths. First, the relationship is independent of the length of the incubation because a first-order kinetics model was fit to the N mineralization data first, and then the (bio)chemical composition was linked to the model parameters. Secondly, the vegetable root residues and green manures differed largely in their (bio)chemical composition and their N mineralization patterns. Hence, we expect that the relationship may have some predictive value for other crop residues not included in this study.

The incubation study also showed that after a sufficient time (four months), roots from vegetables release N although in a much lesser extent than above ground crop residues. The overall N mineralization of cabbage roots (large + fine roots) was on average 15% of total N, which was lower than for the roots of other annual plants, like leek roots (50% of total N) and green manures roots (16 to 28% of total N). Compared to above ground residues, the N release from vegetable root residues will be almost negligible under actual field conditions, since the N release from recalcitrant residues (e.g. roots) is more limited by low temperatures than easily decomposable residues (e.g. leaves) (De Neve et al., 1996; Andersen and Jensen, 2001). Therefore, the actual contribution of vegetable root residues to NO_3^- leaching after the harvest of vegetables is probably limited.

10.3 Manipulating the N release from crop residues

Biochemical characteristics, like a high C:N ratio and a high lignin and/or polyphenol content, have proved to be indicators for N immobilization or decreased N mineralization. Therefore, it was suspected that organic wastes with these characteristics would have a potential to immobilize N released from crop residues, and that the immobilization phase would be easy to realize. More problems were expected during the remineralization phase, since reports of real positive priming effects are scarce up till now (Kuzyakov et al., 2000).

N immobilization of N released from crop residues

Under optimized laboratory conditions immobilizer wastes like straw, saw dust and green waste compost showed to possess a N immobilization potential, but biochemical characteristics of the immobilizer wastes seemed to have an important influence on the amount of N immobilized. C*hapter 3* showed that in order to have a good N immobilization potential, an immobilizer waste should have a high C:N ratio (> 40) and a low lignin content (<40% on OM), i.e. easily degradable. On one hand, a high lignin content may slow down the decomposition of the organic wastes, and hence, decrease the N demand of the micro-organisms, and N immobilization. On the other hand, micro-organisms degrade first easily decomposable material in soil (i.e. crop residues), and thereafter recalcitrant material (i.e. immobilizer wastes) (Iglesias-Jiménez, 2001; Nendel et al., 2004) leading to a separation in time of the N release from the crop residues and N immobilization by organic wastes, decreasing the probability for immobilizers to immobilize N released from crop residues.

Compared to the laboratory conditions, the N immobilization potential of organic wastes and the reduction in NO_3^- leaching was generally low under field conditions. Only in a loamy sand, straw was able to immobilize 89% of the released leek-N and to reduce the NO_3^- leaching to levels comparable with the unamended soil (*Chapter 7*). The disappointing results for N immobilization of crop residue-N seemed strongly linked to an insufficient degradability of the immobilizer wastes (e.g. high lignin content) and

suboptimum conditions for decomposition under field conditions like an insufficient mixing between the immobilizer wastes and crop residues, a poor incorporation in soil, low temperatures, wet conditions, etc.

A homogeneous mixing between crop residues and immobilizer wastes and good soil conditions during incorporation are important factors to obtain sufficient N immobilization, since decomposer micro-organisms only have access to part of the mineral N pool when the organic waste is not homogeneously distributed (Wang and Bakken, 1997). Hence, if mixing is poor, the depletion of mineral N form soil by the micro-organisms decomposing the immobilizer wastes will be less. In the laboratory experiment with ^{15}N labelled celery residues and straw (*Chapter 5*), both the crop residues and straw were added dried and ground leading to a very homogeneous mixing between soil, crop residues and straw and a very high N immobilization of straw which immobilized both crop residue-N as soil-N (~ up to 219 kg N ha^{-1}). The larger N immobilization by organic wastes in coarse-textured soils found in this study (*Chapter 7*), which was in contraction to other studies (Thomsen et al., 1996; Egelkraut et al., 2000), was probably due to the easier incorporation in soil and the better mixing between crop residues, organic wastes and soil in coarse-textured soils compared to fine-textured soils.

Temperatures must be sufficiently high to ensure that N immobilization takes place since the decomposition of recalcitrant substances is more limited by low temperatures than that of more easily decomposable substances, leading to a low N immobilization at low temperatures while the N release from easily decomposable substances still continues (Breland, 1994; Nicolardot et al., 1994; Andersen and Jensen, 2001). Furthermore, gross N immobilization is much more sensitive to low temperatures than gross N mineralization (Nicolardot, et al., 1994; Andersen and Jensen, 2001), leading to a net N release at low temperatures. *Chapter 8* indeed showed that a decrease in temperature during the N immobilization phase may lead to a remineralization of crop residue-N, which was previously immobilized, and hence, lead to losses of crop residue-N.

Furthermore, high precipitation and wet conditions, may lead to a lower decomposition of both crop residues and immobilizer wastes, more denitrification and hence to less N immobilization.

Under optimum (laboratory) conditions, organic wastes were also able to reduce the N_2O emission after incorporation of N-rich crop residues (reduction between 53 and 60% compared to the treatment with only crop residues under optimised lab conditions; *Chapter 4*). A reduction of N_2O emission is either due to the N immobilization potential of the organic wastes or to an increased N_2/N_2O ratio. Although N_2 emission means an economical loss of N, it has no direct negative impact on the environment. Therefore, even if N_2 emission was the major process, incorporating organic wastes together with N-rich crop residues would still be beneficial for the environment, since losses of reactive N to the environment (N_2O emission and NO_3^- leaching) would be reduced.

The incubations under laboratory conditions showed that also tannic acid has a good N immobilization potential. However, as in the study of De Neve et al. (2004), the N immobilization by tannic acid lasted during the complete experiment (190 days) and no remineralization of immobilized N could be observed, indicating that N immobilized by tannic acid may be more resistant to remineralization than N immobilized by organic wastes which were selected for their high C:N ratio. A reason may be that tannic acid does not cause biological immobilization, but reacts chemically with the N in the residues. The different mode of action of tannic acid compared to the other wastes also became clear in the experiment on N_2O emission (*Chapter 4*). Tannic acid was able to reduce the N_2O emission after incorporation of crop residues, but the N_2O fluxes showed a different pattern and the N_2O reduction was lower than for the other organic wastes (only 40% compared to 53-60% for the organic wastes like straw, saw dust and green waste compost).

Remineralization of immobilized N (i.e. priming effect)

Most remineralizer wastes tested in this thesis caused no or very short-lived positive priming effects and a subsequent crop was not be able to profit from

it. Therefore, it must be concluded that remineralization of immobilized N after incorporation of organic wastes occurs coincidentally, and is difficult to control or obtain in a consistent manner.

A first reason for the lack of consistent remineralization is probably the low N immobilization of the organic wastes during the immobilization phase. Hence, no immobilized N was available for remineralization, while it is mainly recently immobilized N that can be readily remineralized (Jensen, 1994a). Another reason is probably an unsuitable biochemical composition of the organic wastes for inducing N priming effects, since most researches found N priming effects after incorporation of low molecular compounds like glucose (Asmar et al., 1994; Falih and Wainwright, 1996; Wheatly et al., 2001). Therefore, the molecular weight of organic wastes may have been too high or these wastes may be not readily decomposable.

However, remineralization may be a crucial factor within the method of manipulating the N release from crop residues. If we are not able to stimulate remineralization before a new crop is sown or planted, the immobilized N may become gradually available as a result of N mineralization from microbial biomass during the subsequent period. If this N release occurs in spring or summer a crop will still be able to benefit from it and this could lead to reduction in N fertilizer recommendations, but if the mineralization occurs in autumn or winter, the risk of NO_3^- leaching may be increased. In this thesis there were no indications that incorporation of immobilizer wastes would enhance N mineralization on the long term, but the maximum time span of the experiments was only two years, and therefore not long enough to make definite conclusions on this matter. Nevertheless, out-of year mineralization could be prevented by e.g. a new incorporation of immobilizer wastes after the harvest of the crop.

Although only part of the questions in this research has been resolved, I hope that this thesis will further stimulate research concerning N transformations in soil and N losses from soil with or without incorporation of organic materials.

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Samenvatting en besluiten

Inleiding

Het algemene objectief van deze thesis was nagaan of het beïnvloeden van de N vrijstelling van oogstresten door aanwending van organische reststoffen een mogelijke management optie is om de NO_3^- uitspoeling in de intensieve vollegrondsgroenteteelt tijdens de winter te reduceren. Het beïnvloeden van de N vrijstelling uit oogstresten verloopt in twee fases - een immobilisatie fase en een hermineralisatie fase - en bij de start van iedere fase wordt een organische reststof in de bodem ingewerkt. Organische reststoffen omvatten o.a. compost van stadsafval, compost van organische afvalstoffen vanop het landbouwbedrijf, groencompost (van bv. snoeisel van hagen en gras) en afvalstoffen uit de landbouwindustrie, vnl. de voedingsindustrie, welke geen of weinig toxische componenten bevatten en kunnen toegediend worden aan landbouwbodems.

Bij de start van de immobilisatie fase (herfst-winter) worden oogstresten ingewerkt samen met organische reststoffen met de bedoeling om ofwel de vrijgestelde N te immobiliseren ofwel de N vrijstelling van de oogstresten te vertragen. Zo kan de N uit de oogstresten beschermd worden tegen N uitspoeling. Door het inwerken van organische reststoffen bij de start van de hermineralisatie fase kan een bijkomend voordeel bekomen worden door de geïmmobiliseerde N terug vrij te stellen op het moment dat een nieuw gewas aanwezig is.

Het beïnvloeden van de N vrijstelling van oogstresten kan verschillende voordelen hebben, zoals het bevorderen van de synchronizatie tussen N vrijstelling en N opname, een gereduceerde N uitspoeling, een gereduceerde N bemesting en een alternatieve afzet voor organische afvalstoffen.

N vrijstelling uit wortelresten van groenten

Om de N vrijstelling uit oogstresten van groenten te kunnen beïnvloeden is een betrouwbare en kwantitatieve kennis nodig van de N mineralisatie van oogstresten. Voor de meeste groenten is een goede kennis beschikbaar over de hoeveelheid bovensgrondse oogstresten die achterblijven op het veld en hun N vrijstelling (Iritani and Arnold, 1960; De Neve et al., 1994; De Neve and Hofman, 1996), maar over de N mineralisatie/immobilisatie van wortelresten van groenten is weinig gekend.

In het eerste hoofdstuk van deze thesis werd een voorspellende relatie opgesteld (onder specifieke omgevingsomstandigheden) tussen de N mineralisatie van wortelresten van groenten en bladeren, stengels en wortels van groenbemesters en hun (bio)chemische samenstelling. De hoeveelheid N die kan vrijgesteld worden, $N_{A,tot}$, was het best gecorreleerd met de C:N verhouding, terwijl de mineralisatie snelheidsconstante k_{tot} het best gecorreleerd was met de lignin:N verhouding. Deze benadering heeft twee voordelen. Ten eerste is de relatie onafhankelijk van de lengte van de incubatie aangezien eerst een eerste-orde model gefit werd aan de N mineralisatie data en pas dan de (bio)chemische parameters gelinkt werden aan de modelparameters. Ten tweede was de (bio)chemische samenstelling van de wortelresten van groenten en groenbemesters heel gevarieerd zodat de relatie wellicht ook een voorspelllende waarde kan hebben voor andere oogstresten die niet in de studie betrokken werden.

Deze incubatiestudie toonde ook aan dat, na een voldoende lange periode (vier maanden), wortelresten van groenten N vrijstellen, echter wel in mindere mate dan bovengrondse oogstresten. De N mineralisatie uit de volledige wortels van kolen (grove + fijne wortels) was gemiddeld 15% van de totale N wat lager is dan de N vrijstelling uit wortels van andere eenjarige planten zoals preiwortels (50% van de totale N) en groenbemesters (16 tot 28% van de totale N). In vergelijking met bovengrondse oogstresten zal de N vrijstelling uit wortelresten van groenten bijna verwaarloosbaar zijn onder

veldomstandigheden aangezien de N vrijstelling van moeilijk afbreekbare materialen (zoals wortels) meer gelimiteerd wordt door lage temperaturen dan de N vrijstelling uit makkelijk afbreekbare materialen (De Neve et al., 1996; Andersen and Jensen, 2001). Daardoor zal de werkelijke bijdrage van wortelresten van groenten aan de N uitspoeling tijdens de winter beperkt zijn.

Beïnvloeding van de N vrijstelling uit oogstresten

Biochemische parameters zoals een hoge C:N verhouding, een hoge lignine inhoud en een hoge polyphenol inhoud zijn indicatoren voor N immobilisatie of voor een vertraagde N mineralisatie. Daardoor werd verwacht dat het inwerken van organische reststoffen met deze karakteristieken zou leiden tot een immobilisatie van N vrijgesteld uit oogstresten, en dat de immobilisatie fase makkelijk realiseerbaar zou zijn. Meer problemen werden verwacht gedurende de hermineralisatie fase aangezien rapporteringen van echte positieve priming effecten schaars zijn (Kuzyakov et al., 2000).

N immobilisatie van N vrijgesteld uit oogstresten van groenten

Onder optimale laboratorium omstandigheden zorgden stro, houtsnippers en groencompost voor een immobilisatie van N vrijgesteld uit oogstresten, maar de biochemische eigenschappen van deze afvalstoffen bleken een sterke invloed te hebben op de hoeveelheid geïmmobilizeerde N. *Hoofdstuk 3* toonde aan dat een organische reststof over een hoge C:N verhouding (> 40) en een lage lignine inhoud (< 40% op organisch materiaal) (i.e. makkelijk afbreekbaar) moet beschikken om tot voldoende N immobilisatie te komen. Langs de ene kant kan een hoge lignine inhoud de afbraak van de organische reststoffen vertragen en zo de vraag naar N door de micro-organismen vertragen en de N immobilisatie verminderen. Langs de andere kant breken micro-organismen in de bodem eerst makkelijk afbreekbare marterialen af (i.e. oogstresten) en pas daarna de moeilijk afbreekbare materialen (i.e. organische reststoffen) (Iglesias-Jiménez, 2001; Nendel et al., 2004) wat leidt

tot een scheiding in tijd tussen de N vrijstelling uit de oogstresten en de N immobilisatie door de organische reststoffen.

In tegenstelling tot de laboratoriumomstandigheden was de N immobilisatie door de organische reststoffen en de reductie van N uitspoeling onder veldomstandigheden meestal beperkt. Enkel in de lemige zandbodem immobiliseerde stro 89% van de vrijgestelde N uit de preibladeren en zorgde stro voor een reductie van de N uitspoeling tot waarden vergelijkbaar met de onbehandelde bodem (*Hoofdstuk 7*). De tegenvallende resultaten onder veldomstandigheden blijken sterk gelinkt te zijn aan de lage afbreekbaarheid van de organische reststoffen (e.g. hoge lignine inhoud) en de suboptimale omstandigheden voor afbraak in het veld zoals een onvoldoende menging tussen de organische reststoffen en de oogstresten, een slechte inwerking in de bodem, lage temperaturen, natte omstandigheden, enz.

Een homogene menging tussen oogstresten en organische reststoffen en goede omstandigheden gedurende de inwerking in de bodem zijn belangrijke factoren voor het verkrijgen van voldoende N immobilisatie aangezien de afbraak-micro-organismen enkel toegang hebben tot een bepaald deel van de minerale N pool wanneer de organische reststoffen niet homogeen verdeeld zijn over de bodem (Wang and Bakken, 1997). Daarom is de uitputting van minerale N uit de bodem door de organische reststoffen beperkt als er geen goede menging is. In het laboratoriumexperiment met ^{15}N aangerijkte selderresten en stro (*Hoofdstuk 5*) werden zowel de selderresten als het stro gedroogd en gemalen toegediend wat zorgde voor een goede homogene menging tussen bodem, oogstresten en stro en een heel hoge N immobilisatie door het stro van zowel selder-N als bodem-N (~ tot 219 kg N ha^{-1}). De hogere N immobilisatie door organische reststoffen in bodems met grovere textuur t.o.v. bodems met een fijnere textuur gevonden in deze studie, in tegenstelling tot andere studies (Thomsen et al., 1996; Egelkraut et al., 2000), was wellicht te wijten aan de makkelijkere inwerking van de materialen en een betere menging tussen de oogstresten en de organische reststoffen in de grovere texturen t.o.v. de fijnere texturen.

De temperatuur moet voldoende hoog zijn om te verzekeren dat N immobilisatie zal plaatsvinden aangezien de afbraak van moeilijk afbreekbare materialen meer gelimiteerd wordt door lagere temperaturen dan deze van makkelijk afbreekbare materialen, wat leidt tot een lage N immobilisatie terwijl de N vrijstelling uit makkelijk afbreekbare oogstresten nog steeds doorgaat (Breland, 1994; Nicolardot et al., 1994; Andersen and Jensen, 2001). Verder is bruto N immobilisatie meer gevoelig voor lage temperaturen dan bruto N mineralisatie (Nicolardot et al., 1994; Andersen and Jensen, 2001), wat leidt tot een netto N vrijstelling bij lage temperaturen. *Hoofdstuk 8* toonde inderdaad aan dat een daling in temperaturen gedurende de N immobilisatie fase kan leiden tot een hermineralisatie van voordien geïmmobilizeerde oogstrest-N, en zo toch tot uitspoeling van N uit oogstresten.

Verder kunnen hoge neerslag en natte omstandigheden leiden tot een lagere afbraak van zowel oogstresten als organische reststoffen en tot meer denitrificatie en minder N immobilisatie.

Onder optimale (laboratorium) omstandigheden zorgde het inwerken van organische reststoffen ook voor een reductie in N_2O emissie na het inwerken van N-rijke oogstresten (reductie tussen 53 en 60% t.o.v. de behandeling met enkel oogstresten onder optimale laboratorium omstandigheden; *Hoofdstuk 4*). Een reductie in N_2O emissie is ofwel te wijten aan de N immobilisatie van de organische reststoffen ofwel aan een verhoogde N_2/N_2O verhouding. Alhoewel N_2 emissie leidt tot een economisch verlies van N, heeft N_2 geen direct negatieve impact op het milieu. Daarom is het inwerken van organische reststoffen positief voor het milieu, ook al is de N_2 emissie de belangrijkste oorzaak, aangezien de verliezen van reactieve N naar het milieu (N_2O emissie en N uitspoeling) verminderd worden.

De incubaties onder laboratorium omstandigheden toonden ook aan dat looizuur in staat is om de minerale N inhoud in de bodem te reduceren. Maar net zoals in de studie van De Neve et al. (2004) duurde de N immobilisatie door looizuur gedurende het volledige experiment (190 dagen) en kon geen

hermineralisatie vastgesteld worden. Dit toont aan dat de N vastgelegd door looizuur meer resistent is tegen hermineralisatie dan bij N immobilisatie door organische reststoffen geselecterd voor hun hoge C:N verhouding. Een mogelijke reden is dat looizuur geen biologische immobilisatie veroorzaakt, maar chemisch reageert met de N uit de oogstresten. Het verschillend werkingsmechanisme van looizuur t.o.v. de andere organische reststoffen kwam ook tot uiting bij het experiment over N_2O emissie (*Hoofdstuk 4*). Looizuur reduceerde de N_2O emissie na het inwerken van oogstresten, maar de N_2O fluxen vertoonden een verschillend patroon en de reductie in N_2O emissie was lager dan voor de andere organische reststoffen (40% t.o.v. 53-60% voor de andere organische reststoffen zoals stro, houtsnippers en groencompost).

Hermineralisatie van geïmmobilizeerde N (i.e. priming effect)

De meeste organische reststoffen getest in deze thesis vertoonde geen priming effect of enkel een effect gedurende een korte periode waardoor een volgend gewas niet in staat was om ervan te profiteren. Daarom moet er geconcludeerd worden dat hermineralisatie van geïmmobilizeerde N na het inwerken van organische reststoffen eerder toevallig voorkomt en moeilijk te controleren en te verkrijgen is op een consistente wijze.

Een eerste reden voor het ontbreken van consistente hermineralisatie is wellicht de lage N immobilisatie door de organische reststoffen gedurende de immobilisatie fase. Daardoor was er geen geïmmobilizeerde N beschikbaar voor hermineralisatie terwijl het juist voornamelijk de recent geïmmobilizeerde N is die makkelijk beschikbaar is voor hermineralisatie (Jensen, 1994a). Een andere reden kan zijn dat de biochemische samenstelling van de organische reststoffen niet geschikt was om een priming effect te induceren, aangezien de meeste onderzoekers een priming effect vonden na het inwerken van laag moleculaire componenten zoals glucose (Asmar et al., 1994; Falih and Wainwright, 1996; Wheatly et al., 2001). Het moleculaire gewicht van de organische reststoffen kan te hoog zijn of deze stoffen kunnen te complex zijn en onvoldoende afbreekbaar voor de micro-organismen. Nochtans kan de hermineralisatie een cruciale factor zijn binnen de methode

van het beïnvloeden van de N vrijstelling van oogstresten. Wanneer we niet in staat zijn om de geïmmobilizeerde N te hermineraliseren vooraleer een nieuw gewas aanwezig is, zal de geïmmobilizeerde N geleidelijk vrijkomen in de daaropvolgende periode als gevolg van N mineralisatie uit de microbiële biomassa. Wanneer deze N mineralisatie plaatsvindt gedurende de lente of zomer wanneer een gewas aanwezig is, kan dit leiden tot een gereduceerde N bemesting, maar wanneer deze N vrijkomt in herfst of winter kan het risico op N uitspoeling toenemen. In deze thesis waren geen aanwijzingen dat het inwerken van organische reststoffen de N mineralisatie op lange termijn zou doen toenemen, maar de maximale duur van de experimenten was slechts twee jaar en dus niet lang genoeg om definitieve concusies te trekken. Een verhoogde N vrijstelling buiten het seizoen kan eventueel wel verkomen worden door het opieuw inwerken van organische reststoffen na de oogst van het gewas.

Verder onderzoek

De N immobilisatie door de organische reststoffen en de reductie in N uitspoeling waren algemeen laag onder veldomstandigheden als gevolg van een onvoldoende afbreekbaarheid van de organische reststoffen (i.e. hoge lignine inhoud) en suboptimale omstandigheden voor afbraak in het veld zoals onvoldoende menging tussen de organische reststoffen en de oogstresten, een onvoldoende inwerking in de bodem, lage temperaturen en natte omstandigheden. Toekomstig onderzoek kan daarom focussen op het creëeren van optimale omstandigheden voor de beïnvloeding van de N vrijstelling uit oogstresten. Een specifieke screening van organische reststsoffen met een hoge C:N verhouding en een lage lignine inhoud (i.e. makkelijk afbreekbaar) kan bijvoorbeeld organische reststoffen opleveren die wel leiden tot een goede N immobilisatie onder veldomstandigheden. Aangezien een goede menging tussen organische reststoffen en oogstresten belangrijk is voor een goede N immobilisatie, kan verder onderzoek uitwijzen of bijvoorbeeld het verkleinen van de partikelgrootte van de organische reststoffen of een andere wijze van

inwerken (e.g. diepploegen, no-till) de N immobilisatie van organische reststoffen verbetert.

Anderzijds, aangezien de organische reststoffen gebruikt in deze thesis een klein effect hadden op de NMIT in de bodem, kunnen deze organische reststoffen eventueel wel bijdragen tot een verhoogd organisch stofgehalte, een verbeterde bodemstructuur en een verhoogde microbiële activiteit. Toekomstig onderzoek waarbij het effect van organische reststoffen zoals groencompost en papierslib getest wordt op eigenschappen zoals de beschikbaarheid van nutriënten, de C sequestratie, de bodem microbiële biomassa en de bodemfauna, kan daarom interessant zijn.

In deze thesis kwamen priming effecten eerder toevalling voor en de mechanismen die leiden tot een priming effect zijn nog niet gekend. Daarom blijft het moeilijk om een priming effect te verkrijgen op een consistente wijze. Toch kunnen priming effecten heel interessant zijn aangezien ze het mogelijk zouden maken om de vrijstelling van geïmmobilizeerde N te controleren en zo zouden ze kunnen leiden tot een verlaagde N bemesting. Verder onderzoek naar de werkingsmechanismen van priming effects kan daarom interessant zijn om op te helderen in welke omstandigheden priming effects voorkomen en hoe ze kunnen beïnvloed worden.

Ondanks dat slechts een deel van de onderzoeksvragen beantwoord werd, hoop ik dat deze thesis het verdere onderzoek inzake N transformaties in de bodem en N verliezen uit de bodem zowel met als zonder het inwerken van organische reststoffen, zal stimuleren.

Apendix

Burns-α model

Simulation of the water movement

De net amount of water (d) which is added or removed from the soil (i.e. effective precipitation) can be determined by the daily precipitation or irrigation (p) and the evapotranspiration (et):

$$d = p - et \quad (1)$$

If d > 0 (subroutine surplus) than the soil water will percolate to deeper soil layers. If d < 0 than capillary rise of the soil water will occur. When d = 0 no redistribution of nitrates takes place. The moisture content of the particular soil layer (moist), recovered after the addition with the amount d, will be compared to the moisture content at field capacity. If the field capacity is exceeded than a part of the water (watloss) will percolate to the underlying soil layer:

$$watloss = d + moist - fc \quad (2)$$

The moisture content of the particular soil layer will be set equal to the field capacity, meaning that all water above field capacity (fc) will drain to deeper soil layers. Since the NO_3^- concentration in the soil is uniformly distributed, the percentage of NO_3^- that migrates to the following layer (saltloss) will be equal to the percentage of water that disappears from the layer:

$$Saltloss = salt \times watloss / (d + moist) \quad (3)$$

If d < 0 (subroutine shortage) than water and NO_3^- will capillary rise from the underlying soil layer. The original evaporation module was adapted by the

suggestions of Mary et al. (1999)[1] in order to spread the evaporation of several soil layers compared to the original Burns model where each soil layer was first exhausted until wilting point (wp) before the underlying soil layer was used. The relative contribution of each soil layer to the total evaporation is determined as follows:

$$e/e_{tot}\ ak\ (1 - z/z_e)^2 \qquad (4)$$

in which e is the evaporation of the particular soil layer, e_{tot} the total evaporation (= -d), z de depth of the soil layer, z_e the maximum evaporation-depth and ak is defined by:

$$ak = (moist - wp) / (fc - wp) \qquad (5)$$

In this way, the amount of water and NO_3^- is calculated for each day.

The model was adjusted by adding the α-parameter which denotes the proportion of water above field capacity that drains to the underlying soil layer and has to be specified for each soil layer (Moreels et al., 2003)[2]. This adjustment allows simulation of moisture contents between field capacity and saturation. The α-parameter was calibrated using the PEST model.

N mineralization

The mineralization of soil organic matter is described by a zero-order kinetics model (De Neve et al., 1996)[3], while the mineralization from crop residues follows a first-order kinetics model (De Neve and Hofman, 1998)[4].

[1] Mary B, Beaudoin N, Justes E, Machet JM (1999) Calculation of nitrogen mineralization and leaching in fallow soil using a simple dynamic model. Eur J Soil Sci 50: 549-566.

[2] Moreels E, De Neve S, Hofman G, Van Meirvenne M (2003) Simulating nitrate leaching in bare fallow soils: a model comparison. Nutr Cycl Agroecosyst 67: 137-144.

[3] De Neve S, Pannier J, Hofman G (1996) Temperature effects on C- and N mineralization from vegetable crop residues. Plant Soil 181: 25-30.

[4] De Neve S, Hofman G (1998) N mineralization and nitrate leaching from vegetable crop residues under field conditions: a model evaluation. Soil Biol Biochem 30: 2067-2075.

Soil organic matter: $N(t) = k.t$ (6)

with N the amount of nitrogen released after time t and k the mineralization rate constant.

Crop residues: $N(t) = N_A (1 - \exp(-k\, t))$ (7)

where N_A is the amount of mineralized N and *k* is the rate constant for N mineralization.

Denitrification

In order to estimate the denitrification in the field, the NEMIS model (Hénault and Germon, 2000)[5] is used. De general form of the model is given by:

$$D_A = D_P\, F_N\, F_W\, F_T \quad (8)$$

in which D_A is actual denitrification in the field (kg ha^{-1} day^{-1}), D_P is the potential denitrification (kg ha^{-1} day^{-1}) and F_N, F_W and F_T are the effects of the soil nitrate content, the soil water-filled pore space and the soil temperature on denitrification, respectively. These functions are dimensionless and are given by:

$$F_N = \frac{\left[NO_3^-\right]}{22 + \left[NO_3^-\right]} \quad \text{with } [NO_3^-] \text{ in mg } kg^{-1} \text{ soil} \quad (9)$$

$$F_W = \left[\frac{\delta_{WF} - 0.62}{0.38}\right]^{1.74} \quad \text{with } \delta_{WF} = \text{moist / sat} \quad (10)$$

(denitrification starts at a WFPS of 0.62)

[5] Hénault C, Germon JC (2000) NEMIS, a predictive model of denitrification on the field scale. Eur J Soil Sci 51: 257-270.

$$F_T = e^{\frac{(t-11)\ln(89)-9\ln(2.1)}{10}} \quad \text{at } t < 11°C \tag{11}$$

$$F_T = e^{\frac{(t-10)\ln(2.1)}{10}} \quad \text{at } t \geq 11°C \tag{12}$$

Curriculum vitae

Personalia

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Studies

1985-1991:	Lagere school, Visitatie Mariakerke
1991-1996:	Middelbaar onderwijs Visitatiehumaniora, Onderstraat 8, 9000 Gent, richting: Wetenschappen - wiskunde (8 uur wiskunde)
1996-2001:	Universitaire studies bio-ingenieur Ugent, opleiding landbouwkunde, optie plantaardige productie
2003-2005:	Aggregaatsopleiding aan Ugent

Wetenschappelijke loopbaan

2001-2006:	Wetenschappelijk medewerker aan de vakgroep Bodembeheer en Bodemhygiëne, Prof. Dr. ir. G. Hofman Prof. dr. ir. S. De Neve, Faculteit Bio-ingenieurswetenschappen, Universiteit Gent

Wetenschappelijke publicaties

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A2 publicaties

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Internationale congressen

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ISHS Symposium Towards ecologically sound fertilisation strategies for field vegetable production, June 2004**,** Perugia, Italië, Mondelinge presentatie: Conserving N from high N crop residues under field conditions by using on- and off-farm organic waste products

VIII ESA Congress, July 2004, Kopenhagen, Denemarken, Mondelinge presentatie: Influence of incorporating crop residues and organic waste products on N_2O emissions from soil

14th N Workshop, October 2005, Maastricht, Nederland, poster presentatie en mondelinge presentatie (in working group): Manipulating the N release from crop residues by using organic wastes: a field study

Nationale congressen

8th Symposium on Applied Biological Sciences, October 2002, Gent, België, Poster presentatie: Modelling the N mineralization of vegetable root residues and green manures using the (bio)chemical composition

9th Symposium on Applied Biological Sciences, October 2003, Leuven, België, Poster presentatie: Screening of organic biological waste products for their potential to manipulate the N release from crop residues

Day of the Young Soil Scientists, February 2004, Brussel, België, Poster presentatie: Screening of organic biological wastes for their potential to manipulate the N release from crop residues

Studienamiddag: Oogstresten op groentenbedrijven, February 2005, Provinciaal Proefcentrum voor de Groenteteelt, Kruishoutem, België, Mondelinge presentatie: Stikstofdynamiek in de bodem na het inwerken van oogstresten van groenten en organisch biologische reststoffen

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