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ESTIMATING NITROGEN MINERALIZATION POTENTIAL OF SOILS AND THE EFFECT OF WATER AND TEMPERATURE AND CROP RESIDUES ON NITROGEN NET MINERALIZATION

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ESTIMATING NITROGEN MINERALIZATION POTENTIAL OF SOILS AND THE EFFECT OF WATER AND TEMPERATURE AND CROP RESIDUES ON NITROGEN NET MINERALIZATION

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ABSTRACT

Chemical and physical procedures were tested to forecast soil net N mineralization in a group of eight soils of Lower Saxony, varying in pedological characteristics as well as management. Most of the indexes tested were well related to N mineralization obtained through incubation under controlled conditions, being less related to the N uptake by crops, probably because this parameter was limited by differences among crops. Factors affecting N mineralization were studied in soils of Uruguay through incubation, assessing simultaneously soil microbial activity through CO_2 evolution. To evaluate the influence of the amount and quality of plant residues the effect of wheat straw (WS) and N addition was tested. The WS rate determined the extent of N immobilization, which was very fast. Mineral N availability influenced the remineralization of immobilized N, being higher in N depleted soils. There was a negative effect of fertilizer N addition on soil biomass, partially counteracted by WS amendment. This effect could be caused by the pH decrease and increased salt concentration of the soil solution. The next study compared decomposition patterns of different plant materials, crop residues and green manures. Chemical composition of plant materials influenced the pace of the decomposition process. No single chemical component could explain differences in decomposition patterns. The soluble C content was responsible for the initial decomposition flush, later cellulose and hemicellulose determined in a greater extent the decomposition pace. The phenolic compounds were negative along the whole incubation. Plant materials with low N concentrations at low N levels did not show differences in decomposition patterns, despite differences in composition. Although N concentration of residues did not affect decomposition pace, was the most important characteristic explaining net N mineralization. The influence of temperature on soil organic matter (SOM) mineralization followed an exponential model in the range from 5 to 40ºC. The calculated Q_{10} values, for both N and C mineralization, indicate a slightly more than two fold mineralization rate increase per each 10ºC increase in the two studied soils, despite differences in texture and SOM. There was a direct relationship between C and N mineralization and gravimetric soil water content, although the two studied soils showed differences in the response to changes in water content. Substantial microbial activity was observed at high water tensions, indicating that in dry periods mineral N is likely to accumulate in the soil.

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List of abbreviations

Chapter 2

Abbreviations of sites:

Be	Berwartshausen
GM	Gross Malchau
Ce	Celle
Jue	Jühnde
KL	Königslutter
Ot	Otterndorf
HH	Höckelheim
R	Reinshof

Abbreviations of treatments:

GV	Without exports
SBV	Grain exported
GA	Grain, leaves and straw exported

Abbreviations of soil N availability indexes:

PB	Nitrogen extraction with phosphate-borate
HW	Nitrogen extraction with hot water
HCl	Acid hydrolysable N
$CaCl_2$-t	Nitrogen extraction with $CaCl_2$
$CaCl_2$-o	Organic N extraction with $CaCl_2$
UV260	Absorbance at UV 260 nm
UV205	Absorbance at UV 205 nm
>0.200 mm	Nitrogen in fraction greater than 0.200 mm
0.200-0.063 mm	Nitrogen in fraction between 0.200 and 0.063 mm

Chapter 3

Abbreviations of treatments:

Experiment 1

N0-WS0	Without fertilizer, without wheat straw
N0-WS0.8	Without fertilizer, with 0.8 g kg^{-1} of wheat straw
N0-WS2.4	Without fertilizer, with 2.4 g kg^{-1} of wheat straw
N0-WS4.8	Without fertilizer, with 4.8 g kg^{-1} of wheat straw
N80-WS0	With N fertilizer (80 mg kg^{-1}), without wheat straw
N80-WS0.8	With N fertilizer (80 mg kg^{-1}), with 0.8 g kg^{-1} of wheat straw
N80-WS2.4	With N fertilizer (80 mg kg^{-1}), with 2.4 g kg^{-1} of wheat straw
N80-WS4.8	With N fertilizer (80 mg kg^{-1}), with 4.8 g kg^{-1} of wheat straw

Experiment 2

WS-N0	With wheat straw, without N fertilizer
WS-U	With wheat straw, fertilized with urea (N 80 mg kg^{-1})
WS-AS	With wheat straw, fertilized with ammonium sulphate (80 mg kg^{-1})
WS-PN	With wheat straw, fertilized with potassium nitrate (80 mg kg^{-1})

N0 Without wheat straw, without N fertilizer
U Without wheat straw, fertilized with urea (N 80 mg kg^{-1})
AS Without wheat straw, fertilized with ammonium sulphate (80 mg kg^{-1})
PN Without wheat straw, fertilized with potassium nitrate (80 mg kg^{-1})

Chapter 4

Abbreviations of treatments:
Experiment 1
N0-R0 Without N fertilizer, without crop residue
N0-WS Without N fertilizer, with 2.4 g kg^{-1} of wheat straw
N0-Sun Without N fertilizer, with 2.4 g kg^{-1} of sunflower residue
N0-M Without N fertilizer, with 2.4 g kg^{-1} of maize residue
N80-R0 With N fertilizer (80 mg kg^{-1}), without crop residue
N80-WS With N fertilizer (80 mg kg^{-1}), with 2.4 g kg^{-1} of wheat straw
N80-Sun With N fertilizer (80 mg kg^{-1}), with 2.4 g kg^{-1} of sunflower residue
N80-M With N fertilizer (80 mg kg^{-1}), with 2.4 g kg^{-1} of maize residue

Experiment 2
C-y Egyptian clover, young shoots
C-m Egyptian clover, mature shoots
C-r Egyptian clover, roots
Sun-L Sunflower leaves
Sun-S Sunflower stems
Soy-L Soybean leaves
Soy-S Soybean stems
M Maize shoots

1 INTRODUCTION

1.1 Soil N mineralization

Mineralization of organic materials in soils is one of the key processes that enables plant growth and therefore crop production because, as consequence of the mineralization process, readily available nutrients are released. Organic N is the main form of N in soils, hence mineralization, which is performed by the soil microbial population, acquires special importance in the N dynamics of the soil.

Nitrogen mineralization process consists mainly in three steps: The first step is ammonification, implying degradation of the organic materials by a wide group of heterotrophic microorganisms. These organisms use the organic matter C for their own growth and as energy source, while part of the organic N present in decomposed organic materials is incorporated to the biomass and part is released. Although most of N in soil organic matter (SOM) has not been identified yet, and it is believed to be integrated to humic substances, it is known that a significant portion of organic N is in amino-N form (Castrou and Schnitzer, 1987). Mineralization involves a sequence of enzymatic processes, the most important enzyme types being proteases and deaminases for the subtract peptide, and O-glycosidases, deaminases and acetyl hydrolases for the polymers of various amino sugars (Mengel, 1996). The ammonification process leads to NH_4^+ release, however not all the N present in decomposed organic matter is mineralized because the soil microorganisms assimilate a fraction of this N (Janssen, 1996). The N assimilation depends on the C flow and the C:N ratio of the microorganisms (Recous et al., 1996). Under field conditions decomposition of soil organic matter is limited by readily available C, while decomposition of plant residues is frequently limited by N availability (Paustian and Schnürer, 1987).

The following steps of N transformation consist in oxidation of the N compounds, producing nitrite (NO_2^-) and, through a further incorporation of oxygen, nitrification with the final product NO_3^-. These processes are performed by a small group of strictly aerobic autotrophic microorganisms, which derive their energy from the oxidation of either NH_4^+ or NO_2^-. It has been reported that nitrification is also performed by heterotrophic microorganisms, but the amounts of N involved are irrelevant compared to

the former (Haynes, 1986). In most soils and environmental conditions nitrification pace is faster than ammonification, in consequence low amounts of NH_4^+ are generally present, being NO_3^- the dominant form of soil mineral N (Rosswall, 1982).

1.2 Gross and net N mineralization

Net mineralization is the result of two opposite processes: gross mineralization (N release) and immobilization (N assimilation) by the microbial population. Gross mineralization and immobilization can be measured using ^{15}N tracers that enable to separate the portion of N, either in biomass or in the mineral pool, coming from the organic material (Bjarnason, 1988; Jensen, 1993; Shindo and Nishio, 2005). When gross mineralization exceeds assimilation a net gain of soil mineral N occurs, on the contrary when the biomass growth requires more N than the released amount, a net immobilization of soil mineral N is observed (Rosswall, 1982). In most soils C availability determines microbial growth; in consequence since most soils are C limited, the mineralization of native SOM produces net N mineralization (Agren and Bosatta, 1998; Shindo and Nishio, 2005). In contrast the net result of the N mineralization of plant materials in soil depends on their composition (Reinertsen, et al. 1983; Frankenberger and Abdelmagid, 1985; Kirchmann and Bergqvist, 1988; Vigil and Kissel, 1991a).

1.3 Soil N mineralization potential

In hypothetical terms the entire pool of soil organic N is potentially mineralizable, however this extreme case is irrelevant from the agronomic point of view. On the contrary, only 1 to 3% of the organic soil N is mineralized each year (Bremner, 1965). Moreover the soils differ in their ability to provide mineral N to crops, and this ability is not always directly related to the total amount of N (Warren and Whitehead, 1988; Campbell et al., 1991). The capacity of the soils to release N has been studied in order to assess the N mineralization potential which is meaningful in terms of N supply to plants.

Studies from Stanford and Smith (1972) were aimed to establish the N mineralization capacity through long-term incubation procedures. From their studies they proposed an asymptotic model of time course of N mineralization, making it possible to calculate the N mineralization potential of the soils. Nitrogen mineralization potential has been used for assessing the effect of management practices on the soil mineralizable N pool

(Doran, 1980; Campbell and Souster, 1982). Nevertheless the Standford model is not always applicable to mineralization data sets, in consequence models considering more than one pool of mineralizable organic N, with different mineralization rates have been proposed (Nuske and Richter, 1981; Ellert and Bettany, 1988).

1.4 Factors affecting soil N mineralization

Nitrogen mineralization, like all biological processes, is affected by environmental factors especially temperature and soil water content, which affect the growth and activity of the microbial population (Harris 1981; Haynes, 1986).

A positive effect of temperature increase on N mineralization is generally acknowledged (Alexander, 1977; Ellert and Bettany, 1992; Zak et al, 1999), however the optimum temperature as well as the effect of temperature on mineralization kinetics seems to be different in different soils and environments (MacDonald et al., 1995; Zogg et al., 1997; Kirschbaum, 1994). On the other hand it has been observed that temperature changes promote changes in microbial population, with predominance of the microorganisms better adapted to the new conditions (Zogg et al., 1997). The effect of temperature fluctuations on N mineralization is a controversial aspect. Some experiments have showed an enhancing effect of these fluctuations (Carlyle, 1988), while Lochmann et al., (1989) concluded that normal temperature fluctuations in the field have a very low impact on soil N mineralization.

There effect of soil moisture on N mineralization indicates that water availability not only has a positive effect on microbial growth and nutrition, but also increases the ability of the microorganisms to reach the substrate (Killham et al. 1993). The water shortage on the other hand produces negative impacts due to increase in osmotic pressure, which in turn increases the energy requirements of the microorganisms for osmoregulation (Harris, 1981). Drying soils also affect soil microbial biomass, through restriction of bacterial movement (Wong and Griffin, 1976). On the other hand differences in microbial tolerance to low moisture potentials have been reported (Howard and Howard, 1983). It has been observed that repeated drying and rewetting of the soil promote strong increases in SOM mineralization after rewetting (Jager and Bruins, 1975; Orchard and Cook, 1983; Cabrera, 1993). According to Magid et al, (1999), the principal reasons for this effect are increasing solubilization of humic substances, the weakening

of aggregates, exposing physically protected SOM and microbial death during the drying process, with consequent remineralization. The importance of this enhanced mineralization is however matter of discussion, since some studies show that the mineralization flush after rewetting is counteracted by the decreasing mineralization during the drying period (Mikka et al., 2005).

Soil acidity and high salt content of the soil solution are factors that affect organic matter mineralization. The optimal pH for soil biomass growth has been established near neutrality, being mineralization restricted at low pH levels (Haynes, 1986; Appel and Mengel, 1990). Nevertheless a significant N mineralization has been detected in soils with pH values between 4 and 5, indicating that the microorganisms can be adapted to acid conditions (Dancer et al. 1973; Shah et al., 1990). According to Beck (1983) while soil pH has a strong influence on nitrification its effect on ammonification is rather small. The high salt content of the soil solution has been also reported as negatively affecting biomass growth, and in consequence N mineralization (Laura, 1977; Haynes, 1996). The cause of this negative effect is related to the required osmorregulation of the microbial tissue as a response against external high salt concentration.

From the point of view of the organic matter as substrate for microorganisms, the amount and characteristics of native SOM and the plant materials incorporated to the soil strongly influence the mineralization process. When residues of harvested crops, which are frequently rich in C, are incorporated to the soil, their decomposition process imply the incorporation of soil mineral N to the microbial biomass, hence net N immobilization. Conversely, residues from N rich crops, like legumes (low C:N ratio), lead to net N mineralization. In consequence one of the parameters used for the assessment of net mineralization of plant materials is the C:N ratio (Janzen and Kucey, 1988; Jama and Nair, 1995). The quality of the carbonaceous compounds influences the kinetics of the decomposition process (Janssen, 1996), being decomposition of lignin rich organic materials slower than decomposition of young tissues (Jawson and Elliot, 1986). Also polyphenol content has been found to produce a retarding effect of residue decomposition (Constantinides and Fownes, 1993).

The study of the effect of inorganic N availability on mineralization rate has produced contradictory results. It has been found that soil mineral N shortage can slow the pace

of decomposition of crop residues with high C:N ratio (Recous et al., 1996). On the other hand negative effects of high soil mineral N levels on microbial activity, and consequently SOM and residues mineralization have also been reported (Fog, 1988). Possible explanations for this behaviour are decrease in soil pH and increase in salt concentration of the soil solution caused by N fertilization (Kowalenko et al., 1978, Huntjens et al., 1981) and inhibition of lignin decomposition by high N concentrations (Fog, 1988).

It is difficult to determine the relative importance of the previously mentioned factors that affect mineralization. Laboratory studies indicate that soil moisture content and temperature have a greater effect during the first steps of plant material decomposition, when organic labile compounds are available, while in the final stages of decomposition the limiting factors are C and N availability (Knapp et al., 1983).

1.5 Indexes of N mineralization

Considering that N mineralization occurs during the crop development it is important to be able to forecast the net effect of mineralization in order to evaluate the capacity of the soil to provide the N required by the crop, and the need of fertilizer application when this amount is insufficient. With this aim a number of chemical indexes have been developed (Bremner, 1965; Fox and Piekelek, 1978a). These indexes are aimed to extract the portion of N which will be more easily decomposed, being therefore directly related to the N mineralized during the growth of the crop (Wang et al., 2001). The chemical indexes are in general empirical, and in consequence do not ensure a selective extraction of the labile SOM (Khan et al., 2001). Although no one of these indexes has been extensively used, they have proved to be adequate for specific areas and production systems. As an example the electroultrafiltration method has been extensively used in Germany for N fertilizer assessment. Other researchers suggested that the pool of N likely to be mineralized can be assessed by the organic matter physical fractionation, being the particulate organic matter directly related to the available portion of soil organic matter (Janzen et al., 1992).

On the other hand it is possible that the factors that affect SOM decomposition influence the success of soil N assessment indexes. Jenkinson, (1968) suggests that probably these methods show a good behavior only when the immobilization process is not very

important. According to this author the behavior of the chemical methods will improve when the sampling is made long after the crop residues are incorporated to the soils, rather than when residues with a high C:N ratio were recently incorporated. From the practical point of view, however, this fact represents a disadvantage, because the use of the chemical indexes, instead of the measurements of the mineral N content of the soil, is intended to provide time for planning of the fertilizer recommendation and application. Hence the adoption of the indexes, which usually imply more complex analysis than mineral N, is only justified in advance of sowing.

1.6 Objectives

In this study the evaluation of different soil N availability indexes in soils of Lower Saxony was aimed to find parameters that characterize the mineralization potential of soils. The objective of the study in Uruguay was the evaluation of different factors (crop residue incorporation, N availability, temperature and soil moisture) that influence soil N mineralization in agricultural soils.

For the first objective a number of soil N availability indexes were compared to net N mineralized, and N absorbed by crops in 8 soils of Lower Saxony, varying in soil type and management (Chapter 2). For the second objective the effect of quality and amount of crop residues, the effect of fertilizer addition and the effect of soil temperature and moisture on net N mineralization were studied. The effect of amounts of wheat straw on the mineralization-immobilization process, as affected by soil mineral N and fertilizer N addition are presented in chapter 3. Patterns of residue decomposition, as depending on chemical composition and N availability are presented in chapter 4. The effects of temperature and moisture on soil organic matter (C and N) mineralization patterns of Uruguayan soils were also examined (chapter 5).

2 NITROGEN MINERALIZATION CAPACITY OF AGRICULTURAL SOILS OF LOWER SAXONY - RELATIONSHIP WITH SOIL N AVAILABILITY INDEXES

2.1 INTRODUCTION

The application of N fertilisers is necessary for agricultural crops in the vast majority of the production systems, however there are still uncertainties about the amount of N required to reach high yields. Moreover the potential environmental damage that the excess of fertilizer can cause should be considered. These two points highlight the need of improvement of the tools for the decision regarding the N fertilizer recommendation.

The principal cause of these problems rely on the difficulties for the assessment of the amount of mineral N that the soil is capable to release during the growing season, since most of the soil N is in stable forms and thus do not contribute to the N mineral pool (Standford and Smith, 1972). There are two main approaches to this problem: The first is the development of N availability indexes. Different chemical procedures that extract part of the organic N from the soil have been developed with the objective to estimate the pool of N susceptible to mineralization of the soil. These methods in general represent an empirical approach, without causal relationship with the mineralization process (Gallagher and Bartolomew, 1964). The hypothesis behind these procedures is that the extracted pool will be more easily attacked by microorganisms (Serna and Pomares, 1992), being therefore directly related to the mineralized N during the growth of the crop. Other researchers suggested that this pool can be assessed by the organic matter physical fractionation, being the particulate organic matter directly related to the available portion of soil organic matter (Warren and Whitehead, 1988).

The different indexes of soil N availability should be tested in order to evaluate their adequacy for N mineralization forecast. The references for N assessment can be the N accumulated after a given incubation period (aerobic or anaerobic incubation) or N uptake by plants grown in greenhouse or field experiments (Keeney, 1982). The use of field experiments for this purpose has been however criticised, because the index can not take into account the mineral N already present in the soil at the beginning of the evaluation (Wang et al., 2001), while other authors warned about the possibility that the crops extract N accumulated in deep soil layers (Bundy and Meisenger, 1994). The

other problem related to the use of crop N uptake as reference is the fact that other factors, for example water availability and temperature, which cannot be standardized, affect crop growth and N mineralization (Wang et al., 2001).

Mineralized N through aerobic and anaerobic incubation has been used as index (classified as biological index) and as reference of N mineralization Examples of the mineralized N as index can be found in Gallagher and Bartolomew, (1964) as well as in Serna and Pomares (1992) work. On the other hand Smith and Standford, (1971); Gianello and Bremner, (1986); Groot and Houba, (1995); Jalil et al., (1996) used results from incubations for chemical tests evaluation. Surprisingly Gallagher and Bartolomew, (1964) reported that chemical indexes correlated better than biological indexes respect to N absorbed by crops in a greenhouse experiment. In general the incubation methods, as indexes of soil N mineralization, are considered superior to other methods, however difficulties arise for their use as routine laboratory methods, especially due to the necessary long term of the incubation (Groot and Houba, 1995; Mulvaney and Hoef, 2001). In addition Keeney and Bremner, (1966) warned about the effect of treatment of the soil samples previous to the incubation, which can affect the results of the biological indexes. Chemical methods on the other hand are seen as quick and simple methods, hence better adapted as routine tests for recommendation of N fertilization (Fox and Piekielek, 1978b).

Even though many indexes have shown good relationships with the potential of N mineralization, they are still unable to forecast the amount of mineral N effectively released, because it depends not only on soil organic matter proportion and composition, but also on weather conditions, specially temperature and water content of the soil. The second approach thus, proposes the use of simulation models, which include parameters of temperature and water content as well as estimation of N losses, in order to overcome this problem (De Willigen and Neetson, 1985; Duivenbooden, 1996; Richter and Benbi, 1996).

In order to evaluate different chemical and physical indexes of soil N availability soil samples were taken from eight field trials varying in soil type as well as long term N fertilizer additions and soil management. Each soil sample was analyzed for the different soil tests. The methodology of evaluation implies the comparison of the results

of the different indexes with mineralization parameters. In this research N mineralized through aerobic incubation (laboratory) and N absorbed by crops (field) were used as mineralization parameters.

The objective of this research was to evaluate chemical and physical N availability indexes for prediction of the N supply capacity of agricultural soils in Lower Saxony, Germany.

2.2 MATERIALS AND METHODS

2.2.1 Soils

The soils considered (table 2.1) were in all instances either from N fertilization trials or from experiments that have received contrasting soil management treatments.

Table 2.1 - Experimental sites

Experiment – Soil type	Crop	Treatment	N source	Start year
1- Berwartshausen (Be) Luvisol	W Barley	N 0		1990
		Required N rate	Mineral	
		Required N rate + 40%	Mineral	
2- Gross Malchau (GM) Cambisol	W. Rye	N 0		1990
		Required N rate	Mineral	
		Required N rate + 1/3	Mineral	
3- Celle (Ce) Stagno-gleyic Cambisol	Maize	N 0		1991
		Required N rate	Mineral	
		Required N rate + 40%	Mineral	
4- Jühnde (Jue) Rendzina-	W. Barley	N 0		1995
		Required N rate	Mineral	
		Required N rate + 30%	Mineral	
		Required N rate	Organic	
5- Königslutter (KL) Luvisol	W. Barley	N 0		1990
		Required N rate	Mineral	
		Required N rate + 40%	Mineral	
6- Otterndorf (Ot) Fluvisol	W. Wheat	N 0		1990
		Required N rate	Mineral	
		Required N rate + 40%	Mineral	
7- Höckelheim (HH) Luvisol	W. Wheat	N 0		2001
		180 kg N ha^{-1}	Mineral	
8- Reinshof (R) Fluvisol	W. Wheat	P 0 GV	Mineral	1983
		P 0 SBV	Mineral	
		P 0 GA	Mineral	

Soils 1 to 6 were from experiments of the Landwirschaftskammer - Hannover. These are long-term experiments that include crop rotations with different N fertilizer rates; each plot was fertilized at the same N level every year. For our purpose the selected treatments were control without N, Required N rate: “Sollwert“ (amount estimated for reaching maximum yield, according to the local calibration based on soil mineral N) and 130 or 140 % of the required N rate. In soil 4 samples were taken also from a treatment that includes organic matter application (liquid manure). Soils 7 and 8 were from experiments of the Institut für Agriculturchemie, being soil 7 from an experiment of N application strategies, and soil 8 from a long term experiment that evaluates crop responses to soil organic matter management and P application rates. In soil 7 the treatments sampled were control and the treatment fertilized with a N rate of 180 kg ha^{-1}. In soil 8 the treatments selected were GV without exports (besides leaves and straw also grain and beets of sugar beet were incorporated into the soil), SBV (only product was exported while the straw and leaves remained in the plots) and GA (leaves or straw were also removed with products). In this experiment mineral N fertilization was the same on all treatments (230 kg ha^{-1}). Texture of the superficial soil layer of soils 2 and 3 was sandy, while the other soils presented loamy to clay texture.

The evaluated crops were winter barley in Be, Jue and KL, winter rye in GM, maize in Ce and winter wheat in Ot, HH and R sites. In all experiments the treatments were in randomized block designs, with 4 replications, and composite soil samples were taken from each of the 4 replications.

2.2.2 Chemical Methods for Assessment of Soil N Supply

All methods were performed on air dried soils ground to pass a 2 mm mesh.

Soil mineral N: The soil (25 g) was extracted with 0,0125 M $CaCl_2$ (soil:solution 1:10), after shaking for 1 hour the extracts were filtered and kept in the fridge. The extracted mineral N (NO_3^- and NH_4^+) was determined with Autoanalyzer (Skalar SANplus) and the mineral N present at the beginning of the experiment was subtracted.

Total soil N: The soil (0.5 g) was mineralized with concentrated H_2SO_4 at 380 °C using a catalyst mixture of Se, K_2SO_4 and $CuSO_4$, followed by distillation with 10N NaOH, recovery of NH_4^+ in 2% boric acid-indicator solution and titration with 0.005 N H_2SO_4).

Phosphate - Borate buffered pH 11,2 (PB): Gianello and Bremmer (1986).
Steam distillation of 4 g of soil with 40 mL Phosphate-Borate solution (10% $Na_3PO_4.12 H_2O$ and 2.5 % $Na_2B_4O_7$ buffered at pH 11.2) for 8 minutes (soil:solution 1:10). NH_4^+ was recovered in 2% boric acid-indicator solution and titrated with 0.005 N H_2SO_4). The exchangeable NH_4^+ extracted with $CaCl_2$ 0.0125 M was subtracted.

Hot water (HW): Keeney and Bremmer (1966) after Livens (1959).
The soil (10 g) was boiled with distilled water (soil:water 1:6) for 1 hour, 10% K_2SO_4 was added for improve filtration, diluted to 100 mL with water and the N in the extract was analyzed by Kjeldahl (digestion of 20 mL of the extract with concentrated H_2SO_4, followed by distillation with 10N NaOH, recovery of NH_4^+ in 2% boric acid-indicator solution and titration with 0.005 N H_2SO_4).

Hot 6N HCl (acid hydrolysable N): Bremmer (1949).
The soil (3 g) was boiled with 6N HCl (soil:solution 1:3) for 12 hours with glass beads and 5 drops of octanol, diluted to 100 mL with water, filtered and N in the extracts analyzed as described for HW.

UV absorbance of 0.01 M $NaHCO_3$ (UV 205 and UV 260): Fox and Piekielek (1978) after extraction from Mc Lean (1961).
The soil (10 g) was shaken with 100 mL 0.01 M $NaHCO_3$ for 15 minutes (soil:solution 1:20), centrifuged at 3000 rpm during 10 minutes, filtered and 3 drops of concentrated HCl were added in order to eliminate HCO_3^-. Extracts were measured at UV 205nm and 260 nm wavelengths.

$CaCl_2$ (0.01 M) extraction (total $CaCl_2$-t N and organic $CaCl_2$-o N): Houba et al., (1986). The soil (10 g) dried at 40°C for 48 hours was shaken for 2 hours with 0.01M $CaCl_2$ (soil:solution 1:10) and filtered. Total N and mineral N in the extracts were measured in Autoanalyzer.

Physical fractionation:
Wet sieving (Cambardella and Elliot, 1992). The soil (50 g) was shaken with 100 mL of 5 g L^{-1} Sodium Polyphosphate for 16 hours in order to disperse aggregates. The fractions > 0.200 mm, > 0.063 mm and > 0.063<0.200 mm were recovered by wet

sieving. The recovered material was dried at 40 °C, weighed, ground and total N in the fraction analyzed as previously described. No separation between organic and mineral material of the fractions was made.

All analysis were performed in duplicate, the results presented are the average of both measurements.

2.2.3 Parameters of soil N mineralization

Soil N mineralization capacity measured through aerobic incubation: Briefly 25 g of air dried soil, with sufficient water to reach 60 % of water holding capacity, was incubated at 25 °C in 250 mL flasks covered with parafilm. After 14; 28; 42; 56; 70 and 84 days 4 flasks from each plot were removed for mineral N measurement.

Nitrogen absorbed by crops: Plant and soil samples were taken at each site at harvest. Ears were separated from straw and both plant parts dried and grinded. N in grain and straw was analyzed by Leco furnace. The N mineralized during the crop growth was calculated adding the final mineral N amount and subtracting the initial mineral N amount from the total N present in grain and straw at harvest. This parameter will be called N mineralized in the field.

2.2.4 Statistical analysis

Regression analysis was performed between the results from N availability indexes and mineralization parameters. Correlation analyses were performed comparing the N availability indexes. Analysis of variance of the results of N availability indexes and mineralization parameters of the different treatments were performed for each site.
The statistical analyses were carried out using the GLM procedure (SAS Institute, Inc. 1985). The results presented in graphics and tables are the average of the four replications.

2.3 RESULTS

2.3.1 Mineralization Parameters

Time courses of the mineral N content of the incubated soil samples are presented in Fig. 2.1. The shape of the curves show a steep increase in mineral N in the first 14 days

followed by a slow linear increase thereafter. There was not a clear maximum in the net mineralization curves of any soil, due to the increasing trend even at the end of the incubation period.

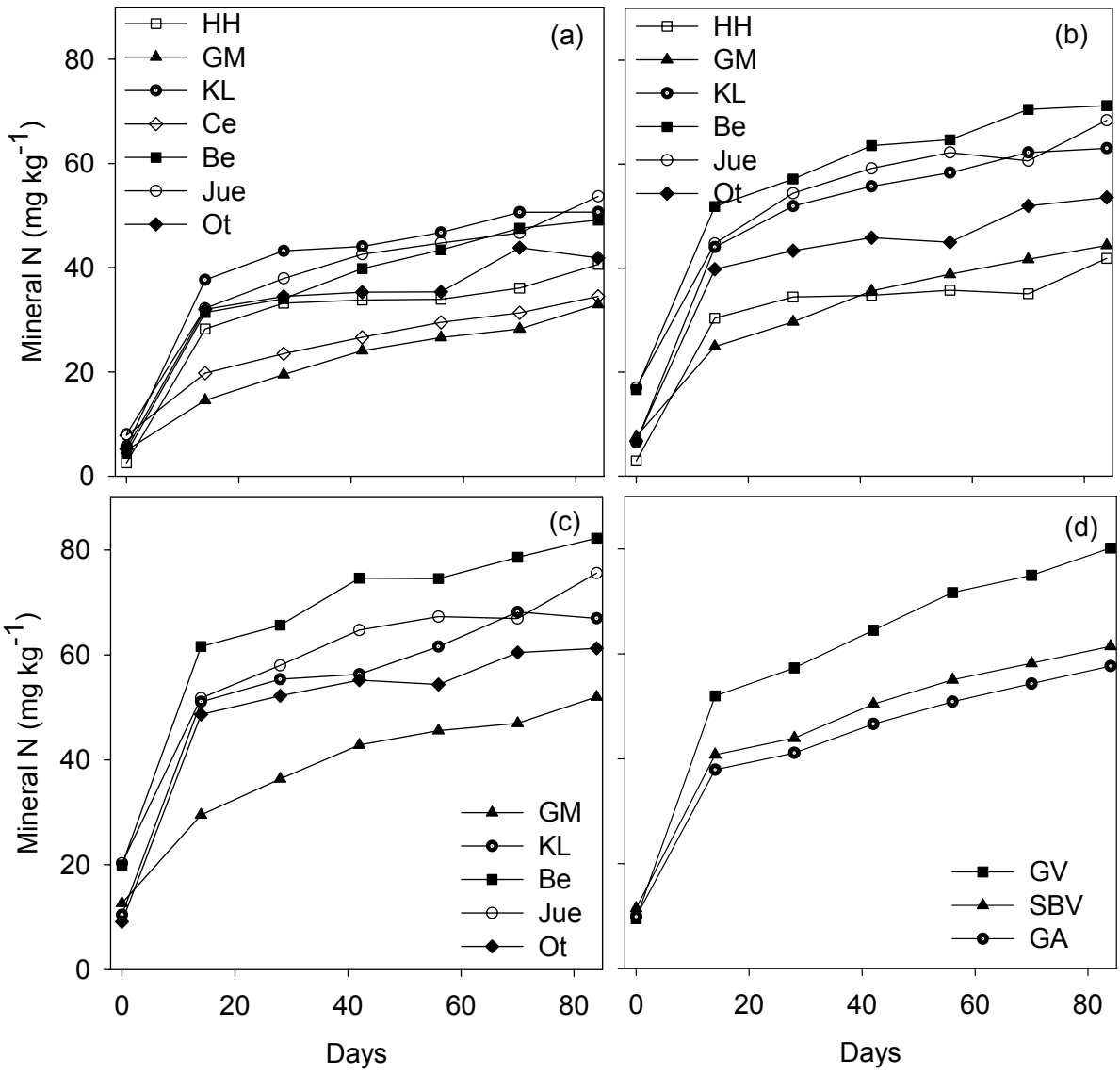

Figure 2.1. Time course of mineral N in seven soils (a) without N fertilizer (b) with the required N rate (c) with 40% over the required N rate and (d) Reinshof soil with different soil management systems. The soils are Höckelheim (HH),Gross Malchau (GM), Königslutter (KL), Celle (Ce), Bewartshausen (Be), Jühnde (Jue), Otterndorf (Ot), Soil management systems in Reinshof are without exports (GV), only products exported (SBV) and products leaves and straw removed (GA).

Taking these results into consideration, both the mineral N released in the first 14 days (2 weeks) and after 84 days (12 weeks) of incubation are used as mineralization

parameters, the first especially related to short term net mineralization and the second to long term net mineralization.

It was also possible to fit the first order kinetics equation:

$N_t=N_0$ [1-exp(-kt)]

Where:
N_t is the amount of N mineralized at time t,
N_0 is the potentially mineralizable N at t_0
k is a rate constant

The rate constants calculated from these equations were used as mineralization parameter following Campbell et al., (1984) and Curtin and Wen, (1999).

The net mineralization patterns presented in Fig. 2.1 enabled to fit a linear regression of mineral N on days of incubation between 14 and 84 days for each soil. The slope of the curve from the third week onwards represents the daily net mineralization rate after the new equilibrium was reached and was also compared with the results of the N availability indexes evaluated.

The amounts of mineral N released in the first 14 days of incubation ranged from 8 mg kg^{-1} in GM-N0 to 47 mg kg^{-1} in KL-N2, while after 84 days of incubation the minimum mineral N released was found in the same plot in the sandy soil of Gross Malchau (24 mg kg^{-1}) and the maximum in one of the plots of the treatment without exports (GV) in Reinshof (77 mg kg^{-1}). Most of the mineral N in the incubated samples was in the NO_3^- form, being the amounts of NH_4^+ practically insignificant in all soils and treatments. The proportion of total N mineralized in 14 and 84 days of incubation was in average 2.3 % and 4.0 % respectively. There was a close correlation (r=0.89) between the amounts of N released in the first 14 days and in 84 days of incubation (Fig. 2.2, Table 2.6).

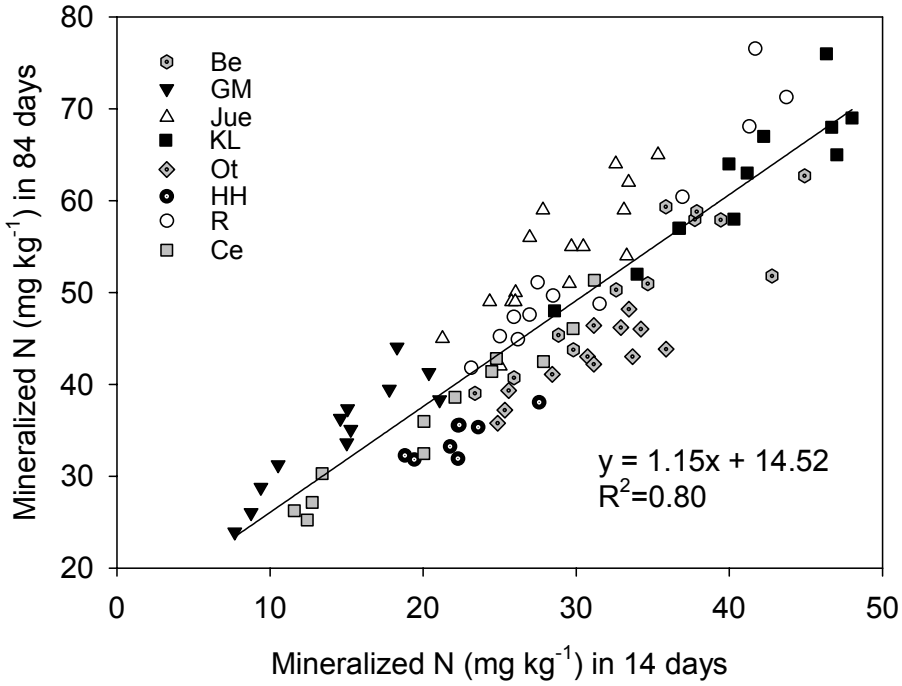

Figure 2.2. Regression of net mineralized N in 84 days of incubation on net mineralized N in 14 days of incubation in eight soils: Höckelheim (HH),Gross Malchau (GM), Königslutter (KL), Celle (Ce), Bewartshausen (Be), Jühnde (Jue), Otterndorf (Ot) and Reinshof (R).

In the first 14 days of incubation, in all but Höckelheim experiment, the amounts of N released in the fertilized treatments were significantly higher than those of the unfertilized treatments (Fig. 2.3). In Jühnde the control treatment without fertilizer resulted in significantly lower amounts of released N (24 mg kg^{-1}) than the fertilized treatments, but there were no significant differences between the fertilized treatments. However the treatment fertilized with farmyard manure was in average higher (30.5 mg kg^{-1}) than the same rate of mineral fertilizer, which released in average 28.5 mg kg^{-1}. In Reinshof experiment, where all treatments received the same amount of mineral fertilizer N, there were differences between soil organic matter (SOM) management systems, being the treatment without exports (GV) significantly higher (41 mg kg^{-1}) than the others (SBV=28.5 and GA 25 mg kg^{-1}). After 12 weeks incubation the sites that did not show responses to N treatments, in terms of mineralized N, were Höckelheim and Jühnde. In Reinshof the results were similar to the 2 weeks incubation period, being GV significantly higher than the others.

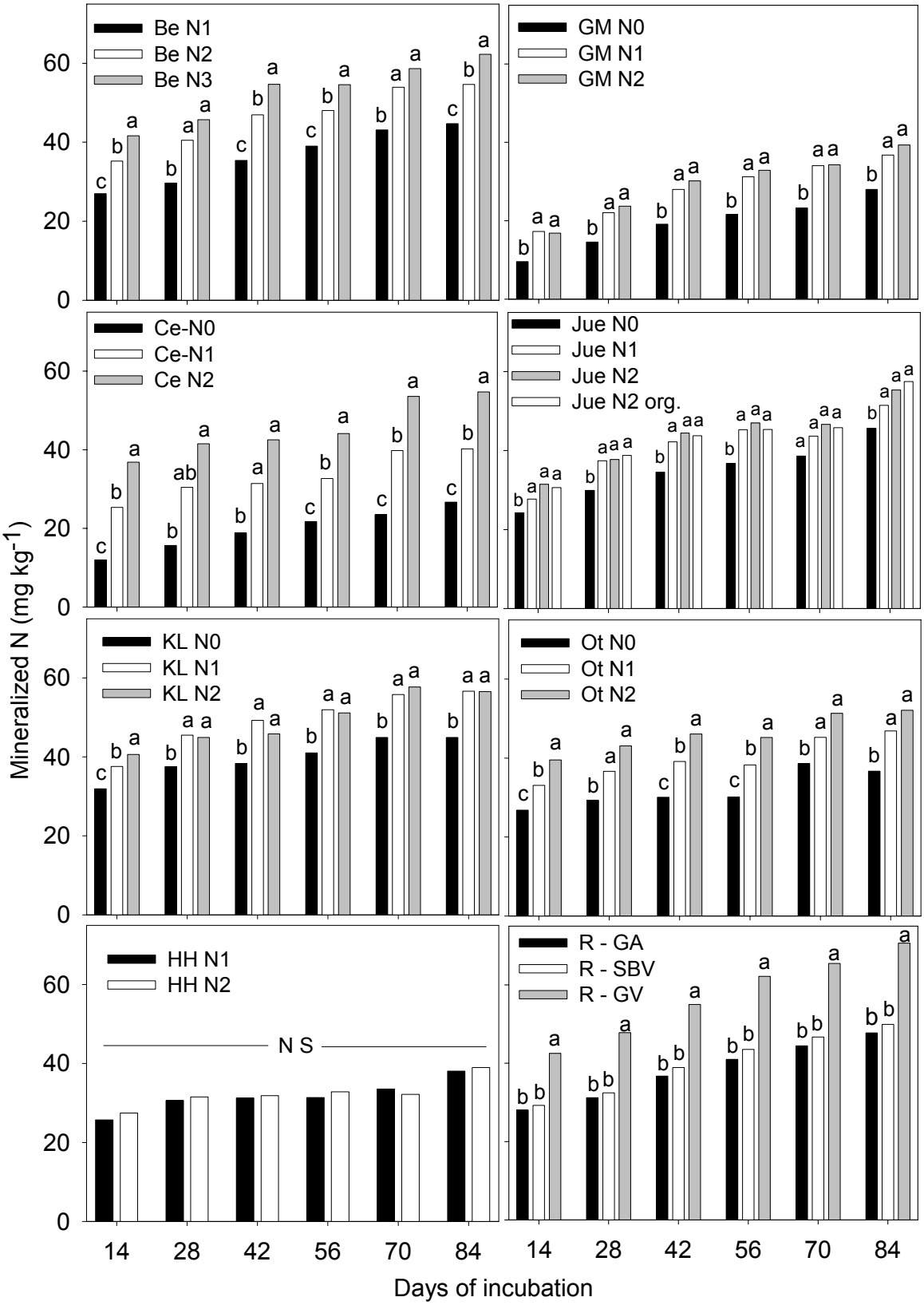

Figure 2.3. Time course of mineralized N in eight soils: Höckelheim (HH),Gross Malchau (GM), Königslutter (KL), Celle (Ce), Berwartshausen (Be), Jühnde (Jue), Otterndorf (Ot), Reinshof systems are: without exports (GV), products exported (SBV) and products leaves and straw removed (GA). Different letters indicate significant differences at each date (P<0.05)

It is not possible to separate the effect of the long-term fertilizer treatment from the effect of the recently applied N. The recent fertilizer application seemed to be important in Celle, where a high N rate (180 $kgha^{-1}$) had been applied and the crop was emerging, hence unable to absorb large amounts of N. Moreover the high dispersion of the results in this site seems to indicate that in this case the sampling and homogenization of the sample were not adequate. In contrast at the other experimental sites the rates of N applied were sensibly lower and the crop was already growing, therefore the amounts of mineral N present in the soil were lower and it is possible to expect that the influence of the recently applied fertilizer on the results was also smaller. Taking these issues into consideration the results from the incubation of the fertilized treatments in Celle were not included in the comparison with the N availability indexes.

Table 2.2 shows N mineralization rates and rate constants for the different soils and treatments. These parameters were not significantly related. There was a weak (R^2=0.20) relationship between the net N mineralization rate and N released in 14 days of incubation (Fig. 2.4), while the relationship with the amount of N released after 84 days of incubation was higher (R^2=0.55). The analysis of variance did not show significant differences among mineralization rates of the different treatments in soils 1 to 7 (Appendix 2.1), being in Reinshof the treatment without exports (GV) significantly higher than the others (GA and SBV). Rate constants showed a week, although significant, relationship with the net mineralized N in the first 14 days (R^2=0.24), while the relationship between k and the amount of N released in 84 days was not significant.

Table 2.2. Nitrogen mineralization rates between 14 and 84 days of incubation, from linear regression of mineral N on days of incubation and rate constants from first order kinetics equation.

Site	Treatment	N mineralization rate	Rate constant (k)
		(mg $kg^{-1}d^{-1}$)	(d^{-1})
Be	N0	0.271	0.052
Be	N1	0.282	0.066
Be	N2	0.290	0.074
GM	N0	0.246	0.022
GM	N1	0.279	0.035
GM	N2	0.300	0.035
Ce	N0	0.203	0.032
Ce	N1	0.212	0.066
Ce	N2	0.259	0.079
Jue	N0	0.277	0.049
Jue	N1	0.287	0.057
Jue	N2	0.303	0.058
Jue	N1 org	0.320	0.055
KL	N0	0.184	0.088
KL	N1	0.263	0.074
KL	N2	0.252	0.084
Ot	N0	0.158	0.093
Ot	N1	0.191	0.093
Ot	N2	0.177	0.108
HH	N0	0.144	0.094
HH	N1	0.123	0.110
R	GV	0.410	0.055
R	SBV	0.307	0.050
R	GA	0.291	0.050

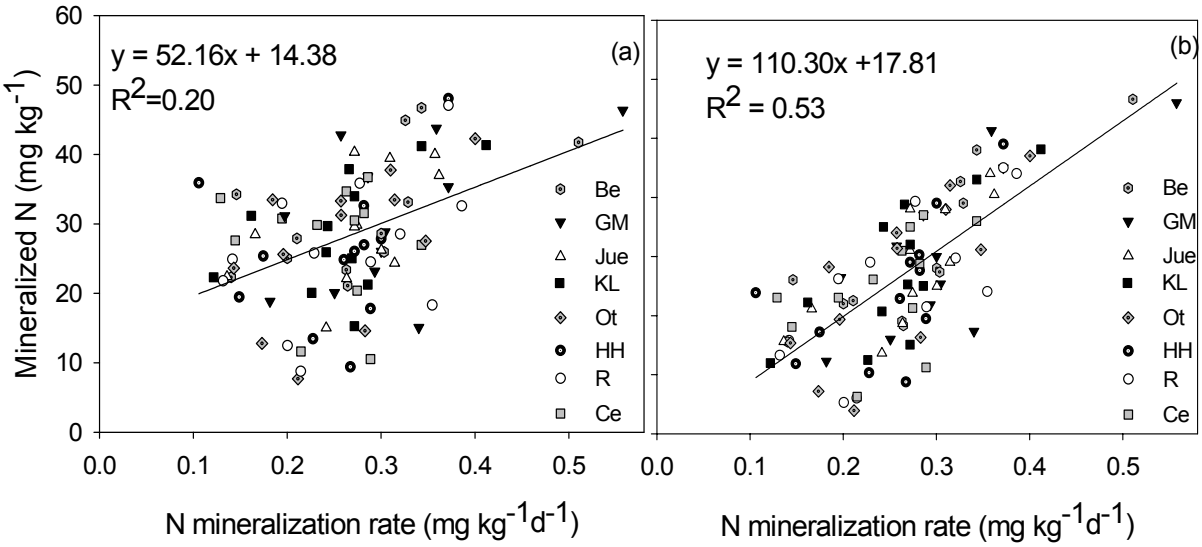

Figure 2.4. Regression of N mineralization rates on mineralized N in (a) 14 and (b) 84 days of incubation

The total amount of N taken up by crops in the field in the different sites as well as the calculated amounts of N mineralized during the growth period in the control treatments (without N fertilization) are presented in table 2.3.

Table 2.3. Amounts of N taken up by crops and mineralized in control treatments.

Site	Crop	Block	Taken up N	Mineralized
			(kg ha^{-1})	N (kg ha^{-1})
Be	Barley	1	50	53
Be	Barley	2	57	77
Be	Barley	3	50	49
Be	Barley	4	45	38
Jue	Barley	1	53	46
Jue	Barley	2	49	47
Jue	Barley	3	61	50
Jue	Barley	4	45	36
KL	Barley	1	59	69
KL	Barley	2	77	79
KL	Barley	3	80	87
KL	Barley	4	78	78
GM	W. Rye	1	58	57
GM	W. Rye	2	59	63
GM	W. Rye	3	57	57
GM	W. Rye	4	58	55
HH	W. Wheat	1	74	88
HH	W. Wheat	2	66	75
HH	W. Wheat	3	58	66
HH	W. Wheat	4	62	72
Ot	W. Wheat	1	38	39
Ot	W. Wheat	2	45	55
Ot	W. Wheat	3	54	53
Ot	W. Wheat	4	57	55

The total amount of N taken up by the crops was not significantly related to the N released after 14 or 84 days of incubation when the whole group of soils were included (Fig. 2.5). However when only the barley experiments were analyzed there was a significant linear relationship between the results of 14 and 84 days of incubation and the amount of N taken up by the crop (R^2 = 0.64 and 0.62 respectively). Similar results were obtained for the calculated N mineralized in the field. The N uptake was similarly poorly correlated to the mineralization rate. With regard to the k constant from first order kinetics equation when all crops were considered no significant relationship was found, however a highly significantly relation was obtained when only the barley soils were included (R^2 = 0.63). Unfortunately the different behaviour of the crops, even though all

of them are winter cereals, did not allow the comparison between different soils, specially regarding to sandy soils. Hence these mineralization parameters (N absorbed by the crops and calculated mineralized N in the field) have in this study a relatively lower value in terms of evaluation of the N availability indexes.

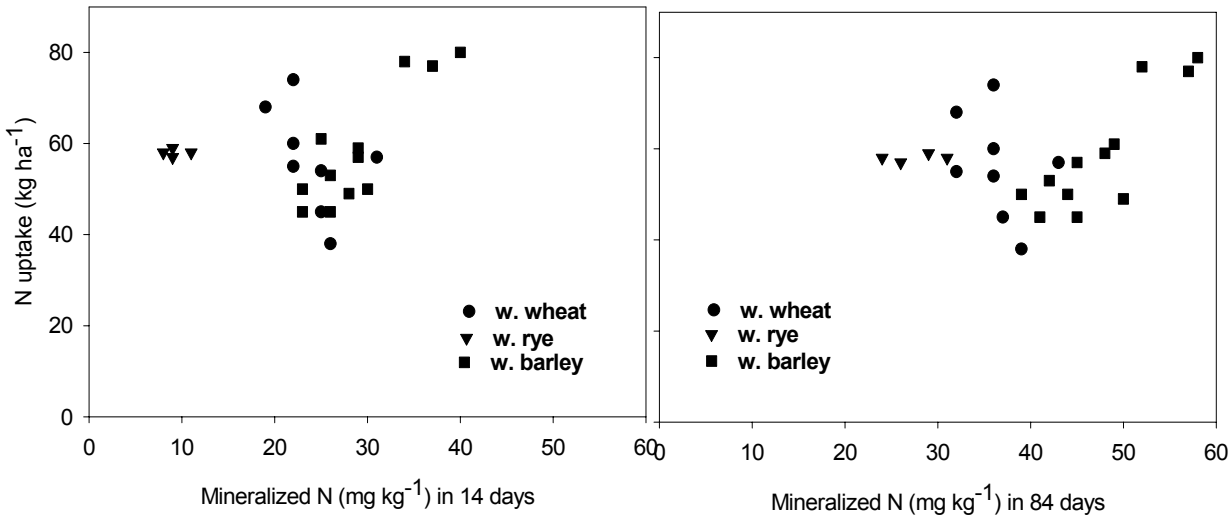

Figure 2.5. Relationship between N uptake by crops and mineralized N in (a) 14 and (b) 84 days of incubation.

The amounts of N taken up by the crops in the fertilized treatments were in general lower than those applied (Table 2.4). Only in site 5 (KL) and in site 7 (HH) the amounts taken up were higher than the N rates. In Reinshof the amounts of N taken up by the GV treatment were appreciably higher (plus 40 kg N ha^{-1}) than the fertilizer rate, while in the other treatments the amounts taken up were very close to the applied N (10 kg N ha^{-1} and -6 kg N ha^{-1} for treatments SBV and GA respectively):

Table 2.4. Amounts of N taken up by crops (U) compared to N fertilization rates (F).

	Be		GM		Jue		KL		Ot		HH		R	
	U	F	U	F	U	F	U	F	U	F	U	F		U[1]
							N (kg ha^{-1})							
N0	50	0	58	0	52	0	78	0	48	0	65	0	GV	270
N1	164	162	127	180	167	187	216	170	179	210	196	180	GA	240
N2	206	227	146	240	219	247	276	246	241	322			SBV	224
N1-o					185	189								

[1]- In Reinshof the N fertilizer rate was 230 kg ha^{-1} for all treatments.

When the N present in the grain was subtracted from the amount of N in the applied fertilizer, in order to evaluate the overall effect of the treatments on the N status of the system, only the control treatments (N0) and KL-N1 showed a negative result, while the inputs exceeded largely the outputs in most cases (Table 2.5).

Table 2.5.Nitrogen inputs - N outputs in experimental sites under wheat, barley and rye production (negative values indicate net N export).

	Be	GM	Jue	K L	Ot	HH	R	
			N (kg ha^{-1})					
N0	-45	-48	-46	-65	-43	-52	GV	230
N1	15	79	46	-2	50	19	SBV	23
N2+	49	130	61	53	113		GA	6
N1org			30					

The different mineralization parameters above discussed are intended to estimate the net N mineralization in the soils tested. In order to compare those parameters Table 2.6 presents the correlation between the parameters for the control treatments.

Table 2.6. Coefficients (r) of the correlation between N mineralization parameters. For N absorbed by crops the results for barley alone are in parenthesis (*) indicates P<0,05.

Parameter	84 days mineralized N	Mineralization rate (14 - 84 days)	Rate constant	Absorbed N
14 days mineralized N	0.89*	0.20	0.66*	0.31 (0.80*)
84 days mineralized N		0.54*	0.52*	0.35 (0.79*)
Mineralization rate (14-84 days)			-0.38	0.12 (0.24)
Rate constant				0.32 (0.84*)

2.3.2 Nitrogen availability indexes

The matrix correlation of the N availability indexes is presented in table 2.7, while tables 2.8 and 2.9 show the results of the regression analysis of the N availability indexes and mineral N released after 14 and 84 days of incubation. Tables 2.10 and 2.11 show the results of the regression analysis of the N availability indexes and the calculated weekly mineralization rate and the rate constant (k) respectively. Finally Table 2.12 shows the results of the regression analysis of the N availability indexes and the calculated amounts of N taken up by the crops in the soils without fertilizer N addition.

Table 2.7. Coefficients (r) of the correlation between different N availability indexes. (*) indicates P<0,05

Method	PB	HW	HCl	UV260	UV205	N min	$CaCl_2$-t	$CaCl_2$-o	N>0.200 mm	N 0.200-0.063mm
Tot N	0.89*	0.87*	0.96*	0.58*	0.62*	0.30*	0.33*	0.24*	-0.40*	0.57*
PB		0.77*	0.87*	0.58*	0.74*	0.52*	0.57*	0.21	-0.34*	0.52*
HW			0.87*	0.45*	0.48*	0.22*	0.36*	0.45*	-0.18	0.70*
HCl				0.44*	0.52*	0.32*	0.33*	0.24*	-0.31*	0.60*
UV260					0.80*	0.08	0.31*	0.37*	-0.60*	0.15
UV205						0.59	0.73*	0.12	-0.44*	0.36*
N min							0.84*	-0.24	0.08	0.36*
$CaCl_2$-t								0.13	-0.01	0.40*
$CaCl_2$-o									-0.14	0.17
N<0.200 mm										0.22*

Table 2.8. Coefficients (R^2) of the linear regression between different N indexes and net mineralized N in the first 14 days of incubation for the whole group of soils (all), the N0 treatment of site 1 to 7 and for each site. (*) indicates P<0,05

Site	Total N	PB	HW	HCl	UV260	UV205	Nmin	$CaCl_2$-t	$CaCl_2$-o	N>0.200 mm	N 0.200 - 0.063 mm
all	0.40*	0.52*	0.42*	0.32*	0.62*	0.68*	0.10*	0.38*	0.22*	0.17*	0.18*
N0	0.54*	0.52*	0.53*	0.31*	0.67*	0.71*	0.01	0.24*	0.43*	0.67*	0.12
Be	0.49*	0.80*	0.42*	0.77*	0.64*	0.82*	0.48*	0.63*	0.40*	0.39*	0.55*
GM	0.00	0.63*	0.50*	0.27	0.15	0.78*	0.50*	0.73*	0.06	0.56*	0.41
Jue	0.04	0.02	0.20	0.12	0.46	0.34*	0.12	0.28*	0.03	0.44*	0.01
KL	0.47*	0.53*	0.51*	0.59*	0.61*	0.61*	0.36*	0.56*	0.43*	0.50*	0.43*
Ot	0.61*	0.83*	0.63*	0.37*	0.11	0.76*	0.58*	0.66*	0.21	0.38*	0.47*
HH	0.62*	0.67*	0.68*	0.29	0.01	0.01	0.46*	0.52*	0.44	0.77*	0.66*
R	0.85*	0.38*	0.76*	0.82*	0.66*	0.45*	0.22	0.42*	0.25	0.87*	0.91*

Table 2.9. Coefficients (R^2) of the linear regression between different N indexes and net mineralized N in 84 days of incubation for the whole group of soils (all), the N0 treatment of sites 1 to 7 and for each site. (*) indicates P<0,05

Site	Total N	PB	HW	HCl	UV260	UV205	Nmin	$CaCl_2$-t	$CaCl_2$-o	N>0.200 mm	N 0.200-0.063 mm
all	0.49*	0.60*	0.59*	0.46*	0.36*	0.52*	0.17*	0.40*	0.16*	0.04	0.29*
N0	0.71*	0.60*	0.73*	0.52*	0.44*	0.51*	0.00	0.23*	0.42*	0.45*	0.27*
Be	0.65*	0.79*	0.44*	0.75*	0.54*	0.80*	0.53*	0.67*	0.32*	0.16	0.82*
GM	0.03	0.83*	0.68*	0.21	0.35*	0.90*	0.69*	0.76*	0.06	0.49*	0.64*
Jue	0.14	0.00	0.22	0.29*	0.04	0.30*	0.01	0.18	0.01	0.43*	0.01
KL	0.50*	0.51*	0.64*	0.66*	0.82*	0.82*	0.52*	0.76*	0.54*	0.75*	0.47*
Ot	0.64*	0.63*	0.49*	0.37*	0.01	0.36*	0.24	0.43*	0.14	0.44*	0.45*
HH	0.66*	0.39	0.74*	0.31	0.04	0.02	0.25	0.26	0.31	0.59*	0.44
R	0.86*	0.35*	0.87*	0.89*	0.66*	0.48*	0.16	0.51*	0.31	0.92*	0.94*

Table 2.10. Coefficients (R^2) of the linear regression between different N indexes and the net N mineralization rate from 14 to 84 days of incubation for the whole group of soils (all), the N0 treatment of sites 1 to 7 and for each site (*) indicates P<0,05

Site	Total N	PB	HW	HCl	UV260	UV205	Nmin	$CaCl_2$-t	$CaCl_2$-o	N>0.200 mm	N 0.200-0.063 mm
all	0.16*	0.23*	0.30*	0.22*	0.22*	0.07*	0.12*	0.22*	0.13*	0.04	0.30*
N0	0.12	0.07	0.16*	0.13	0.00	0.00	0.06	0.10	0.18*	0.00	0.10
Be	0.43*	0.15	0.15	0.23	0.47*	0.20	0.09	0.12	0.16	0.11	0.28
GM	0.13	0.55*	0.48*	0.17	0.29	0.41*	0.38*	0.23	0.26	0.03	0.50*
Jue	0.22	0.10	0.04	0.36*	0.00	0.05	0.02	0.05	0.00	0.13	0.12
KL	0.46*	0.46*	0.52*	0.67*	0.73*	0.78*	0.56*	0.68*	0.41*	0.80*	0.44*
Ot	0.09	0.12	0.18	0.10	0.23	0.36*	0.33	0.25	0.02	0.03	0.14
HH	0.01	0.09	0.17	0.01	0.12	0.07	0.00	0.06	0.00	0.14	0.29
R	0.70*	0.22	0.77*	0.84*	0.54*	0.46*	0.05	0.59*	0.30	0.71*	0.70*

Table 2.11. Coefficients (R^2) of the linear regression between different N indexes and the rate constant k. (*) indicates P<0,05

Site	Total N	PB	HW	HCl	UV260	UV205	Nmin	$CaCl_2$-t	$CaCl_2$-o	>0.200 mm	0.200-0.063 mm
all	0.28*	0.22*	0.35*	0.23*	0.51*	0.66*	0.09*	0.18*	0.08*	0.20*	0.18*
N0	0.58*	0.46*	0.60*	0.45*	0.63*	0.64*	0.14	0.05	0.32*	0.44*	0.24*
Be	0.57*	0.87*	0.42	0.81*	0.80*	0.88*	0.57*	0.71*	0.30	0.36	0.60*
GM	0.29	0.66*	0.58*	0.20	0.33	0.78*	0.41	0.70*	0.04	0.56	0.68*
Jue	0.22	0.22	0.24	0.36*	0.02	0.31	0.14	0.04	0.01	0.37*	0.17
KL	0.35	0.51*	0.51*	0.51*	0.57*	0.65*	0.35*	0.74*	0.52*	0.70*	0.30
Ot	0.93*	0.81*	0.78*	0.21	0.51*	0.91*	0.75*	0.79*	0.35	0.20	0.78*
HH	0.71*	0.72*	0.73*	0.52	0.60	0.64	0.72*	0.64	0.53	0.73*	0.83*
R	0.85*	0.36	0.91*	0.92*	0.66*	0.54*	0.40	0.57*	0.33	0.92*	0.92*

Table 2.12. Coefficients (R^2) of the linear regression between different N indexes and the amount of N taken up by the crops in sites Be, GM, Jue, KL, Ot, HH and R without fertilizer applications (N0) and sites Be, Jue and KL (Barley). (*) indicates P<0,05.

Site	Total N	PB	HW	HCl	UV260	UV205	Nmin	$CaCl_2$-t	$CaCl_2$-o	N >0.200 mm	N 0.200-0.063 mm
N0	0.04	0.05	0.29*	0.04	0.00	0.01	0.00	0.09	0.19*	0.00	0.22*
Barley	0.06	0.12	0.36*	0.00	0.71*	0.65*	0.00	0.22	0.30*	0.00	0.16

In the next section the results from the analysis by each N availability index are described. The comparisons are based on the data presented in tables 2.7 to 2.12. The complete set of results is presented in Appendix 2.2.

Mineral N: The amount of mineral N present in the first 30 cm layer of the soil ranged from 2 mg kg^{-1} in HH-N0 to 27 mg kg^{-1} in Be-N2. These amounts were not closely correlated to the total content of N in the soil (table 2.7), neither to the N mineralization indexes evaluated (tables 2.8 to 2.12). The contents of mineral N in the fertilized treatments of sites Be, GM and Ot were significantly higher than the control treatment; while in Reinshof experiment there were no significant differences.

There was a significant, although weak relationship of the initial mineral N with the N released in the first 14 days and in 84 days of incubation (R^2=0.10 and R^2=0.17 respectively). Similarly the relationships between the initial amount of mineral N and the mineralization rate and the rate constant k were rather poor (R^2=0.12 and R^2=0.09 respectively). No significant relationship between initial mineral N and the amount of N taken up by barley or mineralized in the field was observed.

Total N: The total N content of the soils ranged from 619 mg kg^{-1} in GM-N2 to 2387 mg kg^{-1} in KL-N2, being in average 1239 mg kg^{-1}. In two cases, Jue and KL, there was a marked variability among plots. Even though there was a trend towards higher total N content in the fertilized treatments of sites Be, GM, Ce, KL, and Ot the differences were not significant. In the experiment of Jühnde, the treatment N1 org (farmyard manure) showed similar values as the other treatments. In Reinshof the average total N content in GV (without exports) was significantly higher than the others (GA and SBV).

There was a significant linear relationship between total soil N and N mineralized in the incubation experiments (Fig. 2.6). This relationship was higher for the long-term incubation compared to the first 14 days (R^2=0.40 and R^2=0.49 for 14 and 84 days respectively, Tables 2.8 and 2.9). In contrast the relationships between total N and the mineralization rate from 14 to 84 days and rate constant k (Tables 2.10 and 2.11) were rather poor (R^2=0.16 and 0.28 respectively), as well as the regression between total N and N absorbed by barley or N mineralized in the field (not significant, Table 2.12). When only the control treatments were analyzed the relationship between total N and the N released during incubation showed a substantial improvement (R^2=0.52 and R^2=0.71 for 14 and 84 days respectively).

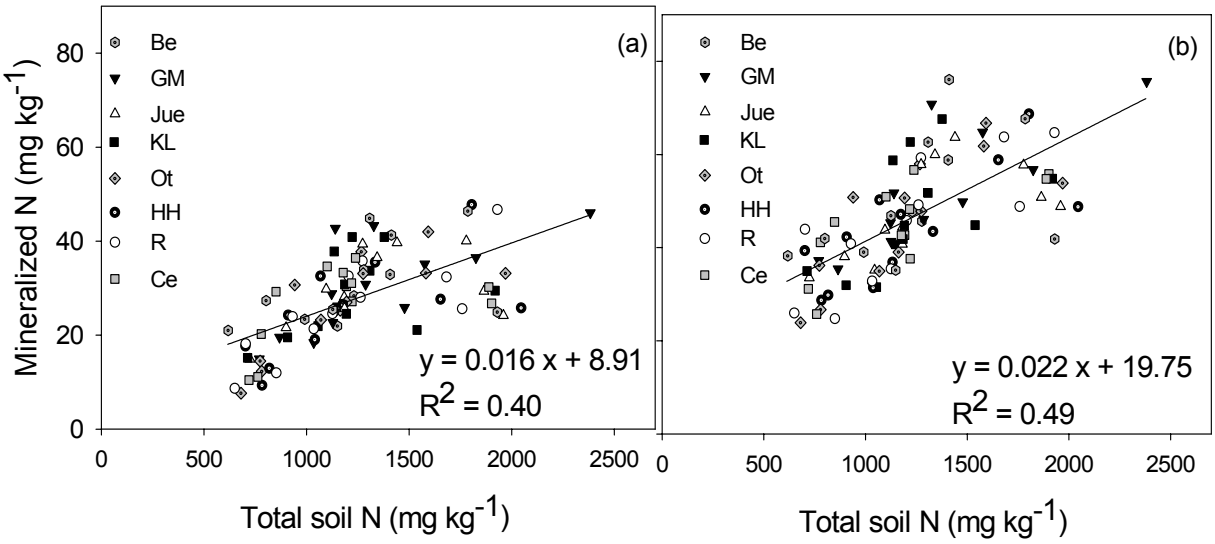

Figure 2.6. Regression of net mineralized N in (a) 14 and (b) 84 days of incubation on total soil N.

Hot 6N HCl: The amount of N extracted by the acid hydrolysis ranged from 476 mg kg^{-1} in GM-N0 to 1678 mg kg^{-1} in KL-N2. There was a high correlation between this index and total N, (r=0.96, table 2.7). The proportion of total soil N extracted with 6N HCl was in average 67.2 %, indicating that this extraction procedure is very strong, hence only the most recalcitrant pool of N remains.

The regression analysis between this method and N mineralized in 14 and 84 days of incubation showed similar trends as total N (tables 2.8 and 2.9), with a closer linear relationship with N released in long term than short term incubation (R^2=0,32 and R^2=0,46 for 14 and 84 days respectively). The relationship of this index with the N mineralization rate between 14 and 84 days was very weak (R^2=0.22), similar to the relationship with the constant rate k (R^2=0.23, Tables 2.10 and 2.11 respectively).

Phosphate - Borate buffered pH 11,2: The amounts of N measured through this index ranged from 9 mg kg^{-1} in GM-N0 to 34 mg kg^{-1} in KL-N2, representing in average 1,7 % of total soil N. This index correlated well with total N and acid hydrolysable N (r=0.89 and 0.87 respectively, table 2.7). The results from this method in the fertilized treatments of sites Be; GM and Ot were significantly higher than the control treatment, while in Reinshof experiment GV (without exports) was significantly higher than the others (GA and SBV).

The relationships between N extracted by PB and the N released in incubation experiments (Fig 2.7) were also very close (R^2=0.52 and R^2=0.60 for 14 and 84 days respectively). On the contrary the relationship of this index with the N mineralization rate between 14 and 84 days and the rate constant k were very weak (R^2=0.23 and 0.22 respectively, Tables 2.9 and 2.10). Unlike the two indexes previously analyzed, in this case the regression against mineralized N in incubation experiments remained almost identical when only the control treatments were evaluated (R^2=0.50 and R^2=0.60 for 14 and 84 days respectively). The regression between this index and N absorbed by barley or N mineralized in the field was not significant (table 2.12).

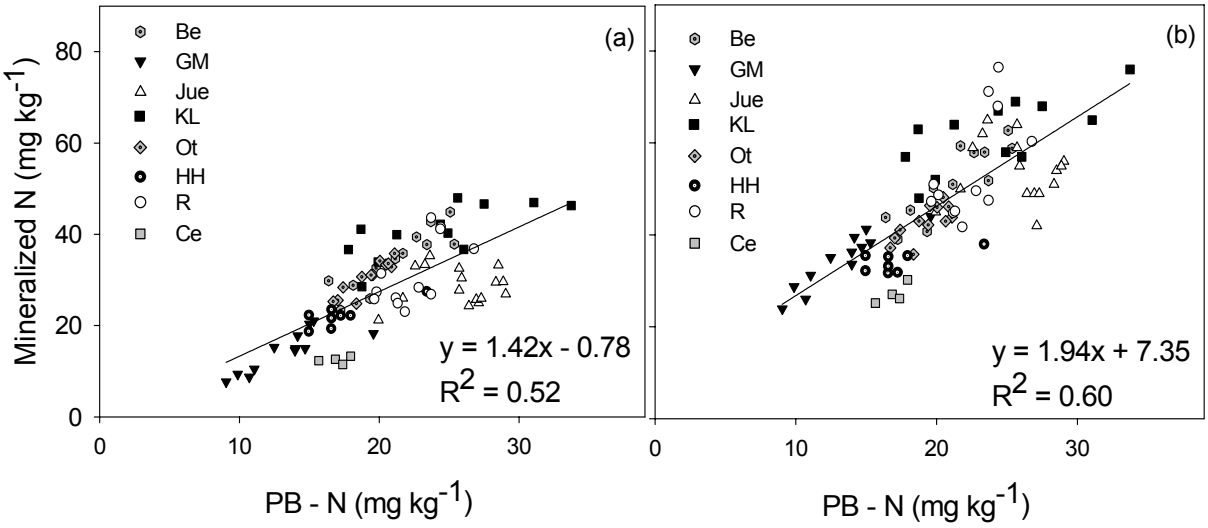

Figure 2.7. Regression of net mineralized N in (a) 14 and (b) 84 days of incubation on N extracted by PB.

Hot water: The amounts of N extracted with hot water ranged from 39 mg kg^{-1} in Ce-N0 to 133 mg kg^{-1} in KL-N2, being in average 5,5 % of total soil N. It was well correlated to total N, acid hydrolysable N and Phosphate Borate (r=0.87; 0.87 and 0.77 respectively, Table 2.7). The results from this method in the fertilized treatments of sites GM and Jue were significantly higher than the control treatment, and GV (without exports) was significantly higher than the others (GA and SBV) in Reinshof experiment.

The coefficients of the regressions of HW extractable N with N mineralized in 14 and 84 days were R^2=0.43 and 0.59 respectively (Fig 2.8). The relationship of this index with the N mineralization rate between 14 and 84 days was poor (R^2=0.30, Table 2.10),

being the relationship of HW and the rate constant k higher (R^2=0.35, Table 2.11). Like the total N this index was more closely related to the results of incubation when only the control treatments were compared (R2=0.52 and 0.73 for 14 and 84 days respectively and R^2=0.60 for rate constant k). There was a significant relationship between this index and N absorbed by barley in sites 1; 4 and 5 (R^2=0.36, table 2.12).

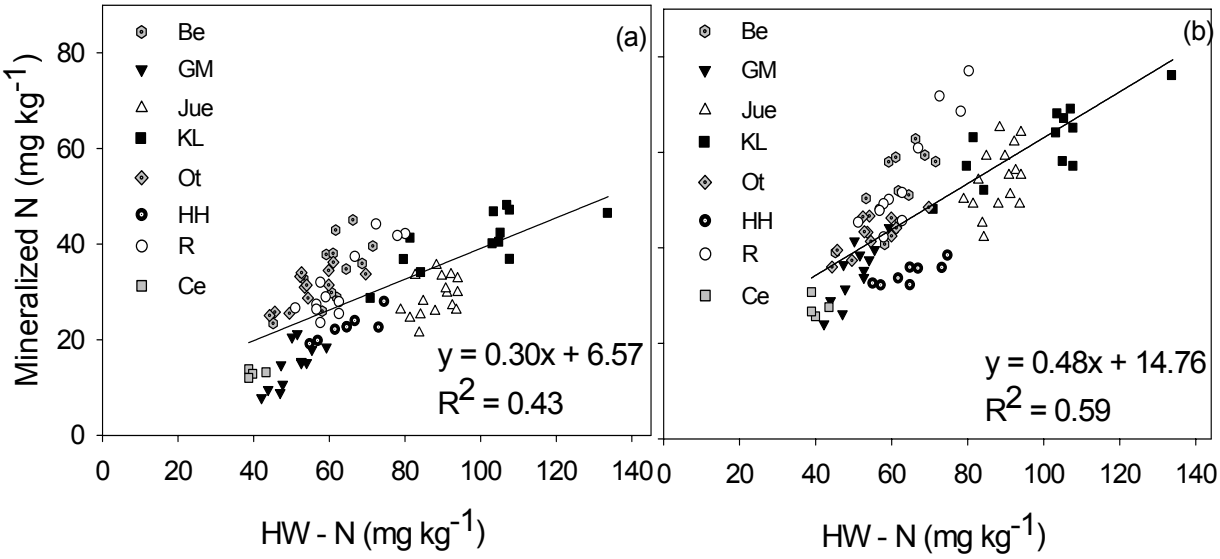

Figure 2.8. Regression of net mineralized N in (a) 14 and (b) 84 days of incubation on N extracted by HW.

$CaCl_2$ (0.01 M) extraction (total $CaCl_2$-t N and organic $CaCl_2$-o N): The total amounts of N measured in the extracts of $CaCl_2$ 0.01 M ranged from 7 mg kg^{-1} in GM-N0 to 34 mg kg^{-1} in Be-N2, representing in average 1,4 % of total soil N. The results from this method were poorly correlated to total soil N (r=0.33, table 2.7) and all the other methods tested. The coefficients of the linear regression of this method with the mineralization parameters were R^2=0.38 and 0.40 for 14 and 84 days of incubation respectively (tables 2.8 and 2.9). The relationships of this index with the N mineralization rate between 14 and 84 days and the rate constant k were very weak (R^2=0.22 and 0.18 respectively, Tables 2.10 and 2.11). When only the control treatments were analyzed the relationship with the incubation results did not improve (R^2=0.24 and 0.23 for 14 and 84 days of incubation respectively). The results from this method in the fertilized treatments of sites Be, GM, Jue, KL and Ot were significantly higher than the control treatment, while it did not detect differences between the soil management treatments in Reinshof.

The organic N in the $CaCl_2$ extracts ($CaCl_2$-o) ranged from 1 mg kg^{-1} in Jue-N0 and R-GA to 14 mg kg^{-1} in KL-N2, representing in average 0.5 % of total N. Like the total amount extracted the organic N in the extract was poorly correlated to total N (r=0.24, table 2.7) and the other indexes. The relationships of this method with the mineralization parameters were also rather poor (tables 2.8 and 2.9); the determination coefficients of the linear regression were R^2=0.23 and 0.16 for 14 and 84 days of incubation respectively. The relationship of this index with the N mineralization rate between 14 and 84 days (Table 2.10), although significant was very weak (R^2=0.13), as well as the relationship with the rate constant k (R^2=0.08). The determination coefficients for the control treatments were R^2=0.43 and 0.42 for 14 and 84 days of incubation respectively, representing an important improvement respect of the whole group of soils. There was a significant, although weak, relationship between this index and N absorbed by crops (R^2=0.19, Table 2.12).

Measurement of UV absorbance of 0.01 M $NaHCO_3$ extracts:

These two indexes (UV absorbance at 205 nm and 260 nm wavelength) represent a different approach to the assessment of available N in soil. After the extraction, instead of analysing the N content of the extract, the UV absorbance is measured, but in this case not only N but also dissolved organic matter forms are detected. Therefore the results cannot be expressed, like the other indexes, in terms of N content; they are expressed in absorbance. The two wavelengths differ in that at 205 nm soluble organic matter as well as NO_3^- is detected, while at 260 nm it is expected that only organic matter in the extract is detected. In order to assess the sensibility of the methods respect of the NO_3^- content of the sample NO_3^- standards ranging from the equivalent to 4 to 40 mg N kg^{-1} of soil were measured at 260 and 205 nm wavelength. At 260 nm all the standards yielded nearly zero, while a linear relationship was found between absorbance at 205 nm and NO_3^- concentration. However the absorbance values found were rather low, ranging from 0.057 to 0.221, indicating that the influence of the NO_3^- content in soil can only seldom be responsible for the majority of the UV absorbance.

At 205 nm wavelength the absorbance ranged from 0.358 in GM-N0 to 1.303 in KL-N2, being in average 0.825. This method was not highly correlated to total N (r=0.62), and showed a poor correlation with the other indexes except Phosphate Borate (r=0.74, table 2.7) and absorbance at 260 nm (r=0.80). Even the correlation with N-NO_3 was not

very close (r=0.62). In contrast, the relationship of UV 205 with the N mineralized in 14 days of incubation was the highest of all methods (R^2=0.68) being lower for the long-term incubation (R^2=0.52). These different relationships are presented in Fig. 2.9. The relationship of this index with the rate constant k was also high (R^2=0.66, Table 2.11); in contrast there was a very poor, although significant relationship of PB with the N mineralization rate between 14 and 84 days (R^2=0.07, Table 2.10). There was a close linear relationship between this index and N taken up by barley (R^2=0.65 Table 2.12).

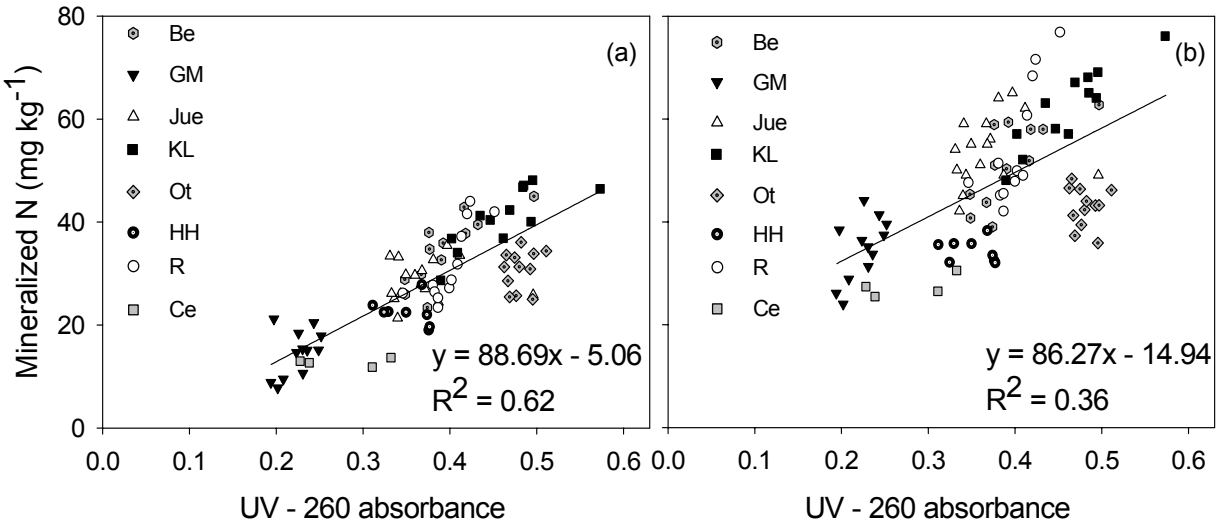

Figure 2.9. Regression of net mineralized N in (a) 14 and (b) 84 days of incubation on absorbance of the Na_2CO_3 extracts at 260 nm.

The results from UV 205 in the fertilized treatments of sites Be, GM, Jue, KL and Ot were significantly higher than the control treatment, while it did not detect differences between the soil management treatments in Reinshof.

At 260 nm wavelength the absorbance ranged from 0.194 in GM-N0 to 0.573 in KL-N2, being in average 0.379. The behaviour of this method is similar to UV 205 respect of the correlation with total N (r=0.58) and was poorly correlated with the other indexes, except for UV 205 (r=0.80). The relationship of absorbance at 260 nm with N released by incubation was R^2=0.62 for 14 days and R^2=0.36 for 84 days incubation (Fig 2.10). While the relationship between this index and the rate constant k was relatively high (R^2=0.51), the regression of this index with the N mineralization rate between 14 and 84 days was not significant (Tables 2.10 and 2.11 respectively). There was a significant linear relationship between UV 260 and N absorbed by barley (R^2=0.71, Table 2.12).

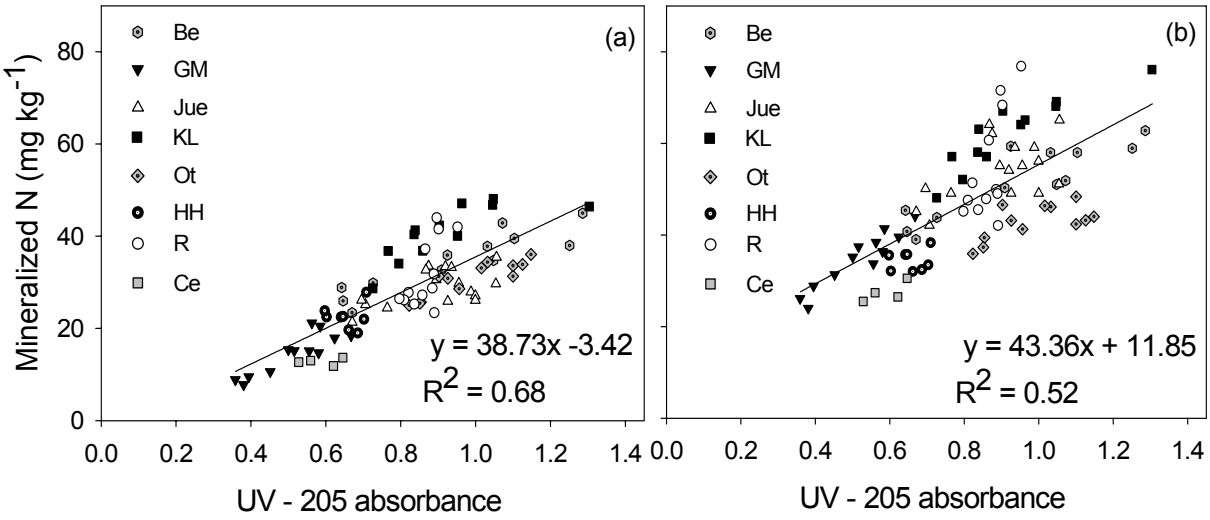

Figure 2.10. Regression of net mineralized N in (a) 14 and (b) 84 days of incubation on absorbance of the Na_2CO_3 extracts at 205 nm.

The results from the 260 nm measurements in the fertilized treatments of sites Be; and KL were significantly higher than the control treatment, and in Reinshof experiment GV (without exports) was significantly higher than treatment GA.

Physical fractionation:

The total N in the fraction of particles greater than 0.200 mm ranged from 22 mg kg^{-1} in R-GA to 177 mg kg^{-1} in GM-N2. This range shows an important scattering in the results from the analysis, furthermore it is possible to distinguish two groups of soils: the sandy soils (GM and Ce) where the fraction constitutes the majority of the soil mass (63,1% in average), and the rest of the soils with a small proportion (average 5,8 % of particles greater than 0.200 mm). The different characteristics of the two groups of soils affected as well the proportion of total N in the fraction. While for sandy soils this proportion is in average 12,8%, it accounts for only 2,9 % of the total N in the other group of soils. Taking these characteristics into account it can be expected that the behaviour of this fraction in terms of assessment of mineralization potential of the soils differ substantially in the two groups of soils, which was confirmed (Fig 2.11). When both groups were analysed separately the relationship between N in this fraction and N released by 14 days of incubation presented similar coefficients (R^2 =0,32 and 0,29 for sandy soils and the other group respectively), however the parameters of the regression lines were

significantly different. For 84 days of incubation the regression coefficients were R^2=0.58 and R^2=0.40 for sandy soils and the other group respectively.

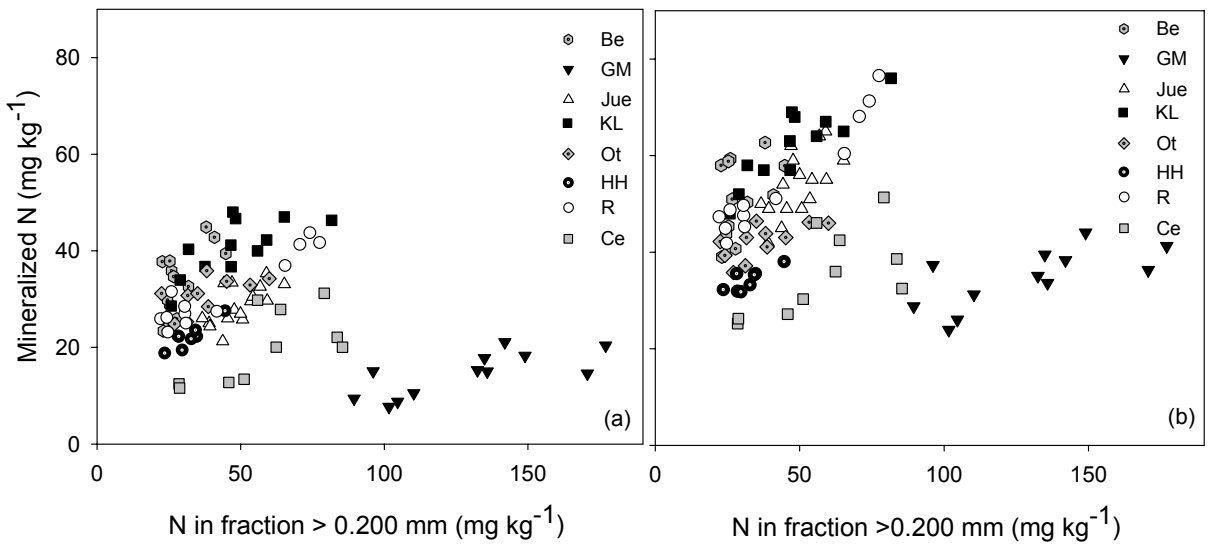

Figure 2.11. Relationship between net mineralized N in (a) 14 and (b) 84 days of incubation and N in the > 0.200 mm fraction.

The results from the measurements in the fertilized treatments of sites Be, Jue and KL were significantly higher than the control treatment, while in Reinshof experiment GV (without exports) was significantly higher than the others.

The total N in the fraction of particles greater than 0.063 mm and smaller than 0,200 mm ranged from 32 mg kg^{-1} in Ce-N0 to 224 mg kg^{-1} in KL-N2, representing in average 9% of total N. In this case the sandy soil of Gross Malchau showed, in accordance to the previous case, a different behaviour than the other soils (average N in the fraction 16.2 % of total N in Gross Malchau compared to 7.4 % in the other group of soils), while the sandy soil of Celle the N content of the 0.200-0.063 mm fraction was rather low (average 9.1 % of total N).

There was a significant, although weak, linear relationship between N in this fraction and N released in 14 and 84 days and the mineralization rate from the incubation of the whole group of soils (R^2 = 0.18; 0.29 and 0.30 respectively, Tables 2.8; 2.9 and 2.10). When the soil from Gross Malchau was removed from the regression analysis the determination coefficients improved substantially (R^2=0.37 and 0.34 for 14 and 84 days

respectively), while the coefficients for Gross Malchau were higher (R^2=0.41 and 0.64 for 14 and 84 days respectively).

The results from this method in the fertilized treatments of sites Be, GM and Ot were significantly higher than the control treatment, and GV (without exports) was significantly higher than the others in Reinshof experiment.

2.4 DISCUSSION

2.4.1 Mineralization parameters

The high amount of net mineralized N at the beginning of the incubation (Fig. 2.1) can be explained by the methodology used, which implies the sieving and drying of the soil followed by rewetting and incubation. It is possible that the disturbance produced in the soil organic matter, especially by drying, caused a weakening of the structure of the stable organic matter, as well as leading part of the biomass to death. Once the soil was watered the microorganisms were able to use as substrate these organic materials, and consequently a flush of mineral N occurred (Seneviratne and Wild, 1985, Cabrera, 1993). Considering that the soil remained without disturbance until the end of the incubation, the second portion of the curve probably reflects a new equilibrium in mineralization conditions (Beck, 1983). In consequence the whole amount of net mineralized N at the end of the incubation was very much influenced by the amount released in the first two weeks (Fig. 2.2).

In this study 8 sites, covering a wide range of soil characteristics, were used for the evaluation of soil N availability indexes. But in each site the applied treatments have developed a series of different N availability conditions. The significant differences between the N released by incubation in fertilized and control plots in sites 1 to 6 indicate that the continuous addition of fertilizer N, which in turn produced higher yields (LWK-Hannover), promoted an increase in the organic N pool susceptible to mineralization in the soils under these treatments (Fig. 2.3). In contrast site 7 (HH) was not a long-term experiment and the treatments were located in a homogeneous soil, consequently in this site no effect of the fertilizer treatment on the pool of potentially mineralizable N was observed (no significant differences between N0 and N1 in terms

of N released in 14 or 84 days of incubation). In the Reinshof experiment the differences due to the management of the crop residues were significant only when the whole amount of biomass produced was left in the field (treatment GV), but there was a trend towards higher amounts of net mineralized N when only the product was exported (treatment SBV) respect of the all export GA treatment.

The effects of soil management on build up or depletion of SOM, and in consequence mineralizable N pool, have been extensively documented (Parton et al., 1987; Cambardella and Elliot, 1992; Janzen et al., 1992). In the fertilized treatments of sites Be, GM, Jue, KL and HH and in Reinshof the amount of N exported in all the treatments was lower than that applied (Table 2.4). The fate of the N exceeding exports is however unknown, it is not possible to assume that the whole amount remained in the soil because there were several possibilities to be lost from the system. On the other hand the build up in the pool of readily mineralizable N, observed through the incubation study of the long-term experiments, indicates that part of this surplus of N was incorporated to the SOM.

The different behaviour of the soils and methods respect of the short term (14 days) and long term (84 days) incubation periods raises the question about which of them has a better relationship with the N available for the crops in a given season. According to Jenkinson (1977) these mineralization patterns reflect the readiness for decomposition of the soil organic matter (SOM). In consequence it is possible that the net mineralized N in 14 days of incubation represents the most labile pool, while the N mineralized from 14 to 84 days represent a most resistant pool. On the other hand the relatively low proportion of total N mineralized indicates that the decomposition process did not reach the recalcitrant N pool. Unfortunately the uptake of N by crops in this case, because of differences among crops and the impossibility to include fertilized treatments, cannot be considered a robust parameter in order to evaluate the results of incubation. In consequence both the 14 and the 84 days net mineralized N were considered for evaluation of the N availability indexes.

With regard to the N mineralization rate there was a lack of ability of this parameter to account for changes in the available N pool of the soils (Table 2.10). This poor behaviour is in part caused by the relatively higher N mineralization rates of the sandy

soils compared to the heavier soils, because the sandy soils showed a poor behaviour in terms of amount of N released during incubation and the results of the N availability indexes were also lower than those for heavy soils. Similar results were reported by Groot and Houba, (1995), who attributed the higher mineralization rate of sandy soils to the higher SOM content, but this explanation is not suitable in the present study where the sandy soils presented lower total N contents. Moreover it has been observed that the mineralization process in sandy soils is faster than in heavy soils, (Hassink, 1995; Kösrchens and Schulz, 1999). However, according to these authors the highest mineralization rate does not imply a higher mineralization capacity but is a consequence of the lower stability of the organic matter in the sandy soils respect of the heavy soils related mainly to the higher physical protection of the organic matter in heavy soils.

The rate constant k from the first order kinetics equations fitted was highly related to net mineralized N in the first 14 days of incubation (Table 2.6). However, it showed a lower sensibility to treatment effects than the amount of N released in the first two weeks.

As it was previously mentioned the N uptake by crops was not as successful as mineralization parameter. There were differences between the crops (W. barley, W. wheat and W. rye), which were already growing at the time of the sampling. This fact and the difficulties related to the fertilizer application scheme, that lead to the use of only the control treatments in the comparisons with the N availability indexes, contributed to make this parameter and the calculated mineralization in the field less adequate references. The fact that only the control treatments can be used as references does not allow the evaluation of the effect of the long term build up of the N available pool that occurred through the long term fertilizer application in sites 1 to 6 neither the effect of soil management treatments in Reinshof.

Taking into consideration the above discussed advantages and disadvantages of the different mineralization parameters in the following section the evaluation of the N mineralization indexes will be mainly based on net mineralized N in 14 and 84 days of incubation.

2.4.2 Assessment of soil N availability

The heterogeneity of the soils is reflected in the contents of total N, being the highest content in a medium textured soil (KL) and the lowest in the sandy soils (GM and Ce) (Appendix 2.1). The lack of significant differences between the N fertilizer treatments in each soil, and the increase in the regression coefficient between total N and mineralized N when only the control treatments were tested (Tables 2.8 and 2.9), suggest that total N gives only a coarse indication of the capacity for N mineralization of the soil. This fact allows discriminating types of soil, but the index is not sensitive enough to detect fine differences in a given soil. In other words, the continuous fertilizer application promoted the build up of the pool of mineralizable N but not in such an extent to influence the total N content of the soils. Similarly poor relationships between total N and mineralization parameters (uptake of N by crops and N released by incubation) were reported by Keeney and Bremner, (1966b) ; Smith and Stanford, (1970) and Hong et al., (1990). In the present study it was possible to detect significant differences in total soil N between the soil management in Reinshof experiment, but in this case the treatments were rather extreme in terms of residue management, remaining in the case of GV at least twice as much organic matter in the soil every year respect to the others. The results of the organic fertilizer application from Jühnde were somehow less clear, considering that in other experiments important improvements of the SOM pool were reported when farmyard manure is applied (Körchens, 1997). The reason of the lack of effect in Jühnde can be that the amounts of farmyard manure applied every year were rather moderate and the experiment had been running for only 5 years at the time of sampling.

The higher regression coefficients obtained with total N when long term incubation was analyzed, compared to 14 days incubation, are probably due to the different pools that contribute to the mineral N released in short and long term. While in the first period the pool of N that is decomposed is the most susceptible pool, whose magnitude is influenced by management and environmental factors, in long term the fractions that contribute to the mineralization belong to the more stable pool (Nuske and Richter, 1981).

Regarding to the acid hydrolysis (HCl) most of the previous comments are relevant for this method. The proportion of total N extracted by this method in the present study (67.2%) is higher than that reported by Serna and Pomares, (1992), 53,7% for

calcareous soils; these authors found also high correlation between this index and total N, being the HCl method a good predictor of N released by incubation and absorbed by maize. In contrast Giroux and Sen Tran, (1987) reported a very poor behaviour of this method when compared to N absorbed by sugar beet in Canada. In our study the fact that this index is in such a great extent related to total N and the high proportion of N extracted, can explain its better behaviour in long term than in short term incubation, as well as the incapacity to detect changes produced by fertilizer management in the mineralizable N pool. Since the practical point of view this method implies boiling soil under reflux for 12 hours with a hazardous chemical, hence its adoption should be justified only when the results are very close to the mineralization parameters evaluated and this condition does not follow from our results.

The Phosphate Borate (PB) extraction showed a close relationship with N released by incubation in both 14 and 84 days (Fig. 2.7) and, more important, this relationship seems to be rather constant through types of soil and management (Tables 2.8 and 2.9). The capacity of the index in terms of prediction was not affected by the fact that this method extracted only a very small proportion of soil N (average 1.7%). These amounts are however comparable to that reported by Curtin and Wen, (1999) in Canada (from 0.45 to 2.3 % of total N). In soils from Iowa, USA, Giannello and Bremner, (1986 b) reported similarly good behaviour of this method compared to many others, while Jalil et al, (1996) reported high correlation of this method with N released in the 24 weeks incubation of 42 soils from Canada. On the contrary Hong et al, (1990) found a very poor relationship of this method with the uptake of N by maize in field experiments in Pennsilvania, USA, however in this case the relationship greatly improved when NO_3^- was added to the PB result. In the present study, in contrast to the close relationship with net mineralized N in laboratory incubations, there was not a close relationship of this index and N absorbed by barley.

It has been suggested that distillation with PB extracts not only soluble organic and inorganic N but also N associated to the soil colloids (Curtin and Wen, 1999) due to the high affinity of phosphates for solid particles (Apel and Mengel, 1998), being in this way related to the pool of adsorbed N that is most likely to be attacked by the soil microorganisms. These characteristics suggest that PB is an index specially appropriate for the forecast of short term available N, however in the present study this index

showed also a good behaviour respect of long term released N (total mineral N released in 84 days of incubation, R^2=0.59). Confirming the hypothesis of short-term prediction the relationship of this index with the N released between 14 and 84 days was weaker (R^2=0.31) than that with the N released in the first 2 weeks (R^2=0.52, tables 2.8; 2.9 and 2.10).

The amounts of N extracted by Hot Water (HW) method in this study (from 39 to 133 mg kg^{-1}) were higher than those reported by Keeney and Bremner, (1966) for soils of USA, and lower than those reported by Körschens and Schulz, (1999) from different long term experiments in Europe. The relationship between this method and net mineralized N was less clear, especially for the first 14 days incubation period (Fig. 2.8a). These results were unexpected because it has been reported as a good predictor of available N, compared to other extraction methods in many studies (Keeney and Bremner, 1966 b). The relationship on the other hand was closer for the long-term incubation (Fig. 2.8a). The extraction with hot water was used by Körschens and Schulz, (1999) as measurement of the reactive fraction of soil N. They measured also the extracted C and found that these fractions (soluble N and C) were related to the total amount of organic matter in the soil, but the soil texture had also a strong influence on the organic matter accumulation of the soil. The relationship found for C was :

Total C $=15*C_{HW} + 0.04 *$ % fine fraction (clay + fine silt)

In the present work the relationship of total soil N respect of N extracted by hot water and proportion of sand in the soil was:

Total N (mg kg^{-1})= $14.3*N_{HW}$ (mg kg^{-1}) - 3.4*% sand

Although this index showed higher values for fertilized treatments in many soils, the relationship improved substantially when only the control treatments were tested (Tables 2.8 and 2.9).

On the other hand the HW method showed the highest regression coefficient of all methods when compared to N absorbed by crops (table 2.12). The pool of soil N extracted by this method is expected to be the same as the pool extracted by the PB method (soluble organic matter and N in organic matter weakly adsorbed to soil solids), but in the present study the amounts of N extracted by PB were in average only one third of the amounts extracted by HW. At the same time the HW method was better

related to the N released in 84 than in 14 days of incubation. It seems that in this study the pool of N extracted by the HW method was less influenced by the soluble, readily available pool than that extracted by PB method.

The amounts of N extracted by 0.01 M $CaCl_2$ were in the low edge of the range, both for total and organic N. However the $CaCl_2$-o N values did not differ appreciably from those reported by Mengel et al. (1998) for 20 soils from Germany and Groot and Houba, (1993) for 8 soils of the Netherlands. There is a controversy about this method, because it has been proposed a second extraction at high temperature (Appel and Mengel, 1990), in order to extract a broader pool of organic N, however, according to Appel and Mengel, (1998) the selectivity of the method in terms of available N is greater for the 20 °C extraction. The cited authors report, however a very good behaviour of the method in relation to the assessment of potentially mineralizable N. In the present study probably the low amount of N extracted, which can be very much influenced by the analysis conditions, is one of the causes of the poor behaviour of the method, especially regarding to the extracted organic N.

The extraction with 0.01 M $NaHCO_3$ was originally followed by digestion of the extract and Kjeldahl analysis (Mc Lean, 1964). This method showed a very good agreement with mineralized N from 21 days incubations and uptake of N in a pot experiment with ryegrass in a very large group of soils in UK (Jenkinson, 1968). Fox and Piekelek, (1978) proposed the modification consisting in the use of UV absorbance to detect the released organic matter, which implies a simplification of the procedure. These authors found a high correlation between organic N in the extract and UV 260. In this work as in others (Giroux and Sen Tran, 1987; Serna and Pomares, 1992, Hong et al, 1990) the authors reported a good relationship between the low wavelength Measurements (UV 200 and UV 205 nm) with N availability parameters, but somehow distrust this method because the measurement includes not only dissolved organic material but also NO_3^- in solution. In measurement of standards Giroux and Sen Tran, (1987) warn about the lack of linearity between organic matter or NO_3^- concentration and UV 205 absorbance when the values are over 1.750. On the other hand the results of UV 260 gave contradictory results, being in the study of Serna and Pomares, (1992) superior than UV 205 respect of N released by 16 week incubation and inferior compared to the uptake of N by maize.

In contrast UV 205 showed a better relationship with N uptake by sugar beet than UV 260 nm in Giroux and Sen Tran, (1987) experiment.

It has been postulated that 0.01 M $NaHCO_3$ acts as a mild extractant, comparable to PB, HW and $CaCl_2$ extraction, however in this study UV 260 was poorly correlated with all these methods, and UV 205 was highly correlated only with PB. Hong et al, (1990) reported a similarly low correlation between UV 260 and UV 200 respect of total N, moreover they found a very low correlation of both indexes with PB-N, being UV 200 highly correlated to N-NO_3 content.

In the present work both UV methods, especially UV 205 were closely related to mineralization parameters. The fact that the NO_3^- contents of the soils were in general low (7 mg N kg^{-1} in average) can have contributed to the good behaviour of UV205, consequently avoiding the reported overestimation of the available N when the soils have high NO_3^- levels (Giroux and Sen Tran, 1987).

Unlike the other extraction methods tested, the two methods that involve UV absorbance were better correlated to the N released in short-term (14 days) respect of long-term incubation. These results can be seen as an indication that 0.01 M NaHCO3 only extracts the most easily decomposable fraction of soil N. They showed also a good relationship with N absorbed by barley.

Many different methods of soil physical fractionation have been used in order to try to identify the SOM fractions that are more reactive in terms of mineralization. Physical fractionation can be performed with or without soil aggregates destruction. In the first case the particulate organic matter is separated, while when the aggregates are not destroyed the SOM fractions associated with soil minerals can be also identified (Tiessen et al., 1984). The particulate organic matter consists mainly in litter residues, which have been partially decomposed and are in consequence more likely to undergo further decomposition than physically protected SOM (Hassink, 1995). On the other hand other authors report that not only the size of the particles are useful for discriminating between SOM fractions but also the density of the fractions can be used for this purpose (Balesdent et al., 1988; Gambardella and Elliot, 1994). The light fraction

is recognized as less humified than the heavy fraction (Janzen et al., 1992; Hassink, 1995).

The amounts of N present in the fraction greater than 0.200 mm were similar to those reported by Warren and Whitehead, (1988) for 27 grassland soils of the UK. In contrast with our results, these authors found a close relationship between the N in the fraction greater than 0.200 mm and N absorbed by ryegrass, while in the same experiment the N in this fraction was not highly related to N released by incubation. Hassink, 1995 using a procedure that separated the fraction greater than 0.150 mm into 3 different density fractions, reported a very good relationship of the light fraction with N mineralized in 84 days of incubation. Interestingly in his study there were not differences between sandy and heavy textured soils. The proportion of total soil N in the fraction between 0.200 and 0.063 mm in the present study (9%) was in lower than that reported for Australian soils by Cambardella and Elliot, (1992), from 12 to 29 %.

Although the two physical fractionation indexes showed a poor relationship with all the mineralization parameters when the whole set of data were considered, they presented remarkably high linear regression coefficients with N released in 14 and 84 days of incubation in some soils. The coefficients were in Reinshof R^2=0.87 and 0.92 for fraction greater than 0.200 mm in 14 and 84 days of incubation and R^2=0.92 and 0.94 for fraction between 0.200 and 0.063 mm in 14 and 84 days of incubation respectively (Tables 2.9 and 2.10). In Bewartshäusen the determination coefficients were R^2=0.55 and 0.82 for 0.200-0.063mm fraction in 14 and 84 days of incubation respectively. Even though these indexes were able to distinguish between treatments in many of the soils, it seems that the particularities of each soil have a strong influence in the amount and composition of the particulate organic matter, being the extreme example of this behaviour the sandy soils. This fact makes these indexes difficult to interpret, but on the other hand they represent a different approach respect of the assessment of potentially available N of the soils compared to the chemical extraction procedures.

The physical fractionation has been also used for SOM quality evaluation, especially comparing different soil management practices (Janzen et al., 1992; Cambardella and Elliot, 1994 and Franzluebbers and Arshad, 1997). This approach is somehow coincident with the results from the present study, considering that differences in N

content in the higher size fractions of SOM appear to be consequence of soil management in each soil.

2.4.3 Practical considerations

From the practical point of view the different methods present different types of difficulties. As it was mentioned the HCl hydrolysis requires a long time of extraction, followed by digestion of the sample and NH_4^+ distillation, being very time consuming and consisting of many different procedures, which makes it difficult to adopt as a standard method. Similar considerations can be made respect of HW, even though the reflux time is shorter and the chemicals less hazardous. The PB procedure is rather simple, however the time of the extraction should be carefully observed because it has been found that longer extraction time result in higher amounts extracted (Gianello and Bremner, 1988). The method of determination of organic N extracted by 0.01M $CaCl_2$ has been adapted to be a standard method (Houba et al., 1987), with automatic determination of mineral and total N in the extracts. However, as it was mentioned the two steps procedure, besides the low concentration of organic N dissolved can represent a problem in some cases, depending specially on very precise laboratory equipment. The UV absorbance methods are rather simple, however they present two difficulties, first the filtration of the extract is very difficult, being necessary to centrifuge the soil solution mixture previous to the filtration. The second difficulty found in this study, which was not reported in other works, is the timing of the measurement of the absorbance, which we observed could affect the results, requiring the standardization of the time between the extraction and the measurement. The physical fractionation analysis is a time consuming procedure, since the wet sieving should be done manually, however the determination of total N in the fractions, which was made by Kjeldahl in this study, can also be performed with automatic equipment.

2.5 SUMMARY AND CONCLUSIONS

The methodology used, consisting in the comparison of the results of different methods with the amounts of N released during aerobic incubation of soil samples, was suitable for the evaluation of different indexes of N availability under controlled conditions. Unfortunately the comparison of the indexes with the field mineralization parameters was not possible in the same extent, being only possible for some soils and treatments.

Most of the indexes tested were able to forecast the the cappacity of the soils to mineralize N, represented by either N released in 14 to 84 days of aerobic incubation and N absorbed by crops in soils varying in pedological characteristics as well as in management. Considering the whole group of soils and management the methods that showed closer relationships with the mineralization parameters were PB and HW for long term released N, while UV 205; UV 260 and PB were able to assess the differences in N availability in short term released N.

On the other hand the objective to find a parameter of N mineralization potential was not accomplished since the mineralization parameters tested were not strongly related among themselves. Moreover the ranking of the N mineralization indexes varied according to the mineralization parameter used. Considering the most robust parameters (amount of mineral N released in 14 and 84 days of incubation) it was possible however to evaluate the capacity of the indexes to forecast the N mineralization in short as well as in long term.

3 EFFECT OF THE AMOUNT OF WHEAT STRAW AND SOIL MINERAL N ON MINERALIZATION PATTERNS IN AGRICULTURAL SOILS OF URUGUAY

3.1 INTRODUCTION

Crop production systems in Uruguay generally consist of winter and summer crop rotations. Winter crops (mainly wheat and barley) are sown in late autumn and harvested at the end of the spring, while summer crops (mainly sunflower, maize and soybean) are sown in late spring-early summer and harvested in autumn. These systems imply an intensive use of the soil, where the residues of one crop decompose simultaneously with the growth of the next crop of the sequence. In these conditions the N immobilized during the decomposition of previous crop residues can affect the N availability in the soil and hence N nutrition of the current crop. Under those circumstances the knowledge of the mineralization process as influenced by residues amount and composition acquires especial importance.

When crop residues are incorporated into the soil, there is generally a flush in the microbial population activity in response to the addition. This response implies the growth of the microbial biomass caused by the substrate increase, with simultaneous assimilation of C and nutrients from the crop residue (Knapp et al., 1983). The use of C from the residues as an energy source during decomposition results in production of CO_2. Therefore, the increase in the amount of CO_2 evolved from the soil, when plant residues are decomposed, can be seen as an indicator of the rate of C mineralization. Along this process the N requirements are determined by the C flow, being the N present in the residue under decomposition the primary N source. When this amount is insufficient soil mineral N is incorporated (Mary et al., 1996). Microorganisms use organic C as energy source and a portion contributes to the biomass growth. The proportion of decomposed C assimilated into microbial biomass respect of evolved CO_2 has been difficult to determine, since it depends on the characteristics and activity of the microbial population, being the most commonly reported values between 20 and 40% (Alexander, 1977).

After the incorporation of plant residues microbial growth peaks, and decreases as the decomposition proceeds, due to the depletion of easily decomposable material. In

consequence a two-phase decomposition model has been proposed, with an initial high-speed decomposition rate fallowed by a slow decomposition phase (Jenkinson, 1977).

Once the readily available C is depleted, part of the newly formed biomass is likely to starve, becoming the substrate for a new microbial population. Since the C:N relationship of the microbial biomass is low (between 5 and 15, Van Veen et al., 1985) its decomposition gives place to a release of mineral N, usually known as remineralization. This remineralization is however limited, since it has been observed that there are mechanisms in the soil aimed to protect the microbial biomass, which makes the remineralization process slower than expected given the low C:N relationship (Van Veen et. al., 1984). It is also possible that the immobilized N from the dying microbial biomass evolve to stabilized N in organic matter (Haynes and Swift, 1988, Shindo and Nishio, 2005). In coincidence Sorensen and Amato, (2002), working with ^{15}N marked pig slurry and $(NH_4)_2SO_4$ reported very low mineralization rates of previously immobilized N. Differences in remineralization patterns between soils were observed by Schnier et al., (1987) working with ^{15}N. These authors reported remineralization of 23 and 46 % of previously immobilized N in a clay and a loamy soil respectively.

Residues of cereal crops are likely to cause mineral N immobilization during decomposition, due to their high C:N ratio. When decomposition occurs alongside the growth of the following crop, this process can lead residue decomposition and plant uptake to compete for the soil mineral N (Recous et al. 1992). The source of N, however can affect the overall process of mineralization, especially by means of effects on the soil environment. Fertilizer application can promote changes in pH and salt content of the soil solution that in turn affect the growth of the microbial populations which decompose crop residues (Kowalenko et al., 1978; Fog, 1988).

Nitrogen fertilizers can be broadly classified, based on composition, in salts containing ammonium (NH_4^+), nitrate (NO_3^-) and organic forms of N. In urea, one of the most widely used fertilizers, N is in organic form, but it is rapidly hydrolysed in the soil, being NH_4^+ and CO_2 the products of the hydrolysis. Once the NH_4^+ is released in aerated soils, the nitrification process, consisting in the biological transformation of NH_4^+ to NO_3^-

occurs. As a result of the nitrification process H^+ are released into the soil, with the consequence of soil acidification (Van Breemen et al., 1984). Even though this process also occurs during mineralization of soil organic matter (SOM) and crop residues rich in N, the effect of acidification is more conspicuous when major amounts of fertilizers containing NH_4^+ are applied. The buffer capacity of the soil, related to the clay and organic matter content, opposes the pH change of the soil, being the acidification more important in soils with low buffer capacity like sandy soils with low organic matter content.

This work is aimed to determine the mineralization patterns of different amounts of wheat straw incorporated to the soil and the effect of soil mineral N content, and N fertilizer sources on those patterns.

Two pot experiments were installed in this study. The first experiment consisted in the evaluation of the time course of wheat straw (WS) decomposition, testing different WS rates with and without N addition trying to mimic different situations that occur in soils after wheat harvest. Wheat straw decomposition was evaluated through soil microbial respiration and the concomitant effect of N immobilization. The second experiment was implemented in order to compare the effect of different N sources on WS decomposition, because from the first experiment it was clear that a more detailed study of the effect of N addition on WS decomposition was needed to improve the understanding of the process.

3.2 MATERIALS AND METHODS

3.2.1 Soil and treatments

The soil was taken from the upper layer of a Typic Argiudoll, pH (H_2O) 5.7 (1:2.5 soil:water), organic C 29.1 g kg^{-1} total N 1.9 g kg^{-1}.

Two experiments were established. Experiment 1 consisted in a factorial experiment with two N levels and 4 wheat straw levels. N levels: without fertilizer application (N0) and with 80 mg N kg^{-1} of soil (equivalent to 100 kg ha^{-1}, N100). The calculation of the fertilizer amount was made considering a bulk density of 1.25 g cm^{-3} and a soil depth of

10 cm. Wheat straw amendment levels: Addition of ground wheat straw (WS) 0.8; 2.4 and 4.8 mg WS kg^{-1} of soil (equivalent to 0; 1000; 3000 and 6000 kg ha^{-1}). There were three replications per treatment.

Experiment 2 consisted in a factorial experiment with two WS levels and 4 N sources: Control, urea (U), ammonium sulphate (AS) and potassium nitrate (PN), all fertilizers were added in a rate of 100 mg N kg^{-1} of soil. Wheat straw finely ground was incorporated at a rate of 4.8 g kg^{-1} of soil to half of the pots. There were three replications per treatment.

The chemical composition of WS was: total C 411.6 g kg^{-1}, total N 5.9 g kg^{-1} and C:N70.

3.2.2 Incubation procedure

In Experiment 1 fresh soil was passed through a 5 mm sieve, and roots and stubble were removed. The soil was not let to dry for sieving and mixing in order to keep the minimal disturbance. The portion of soil, corresponding to each pot, was extended in a thin layer. After mixing with the wheat straw, the soil corresponding to each of the fertilized pots was spread with urea solution (6.4 mg N mL^{-1} water), mixed carefully and deionised water was spread and mixed. The unfertilized pots received only deionised water. The amount of water added was aimed to reach 0.28 g g^{-1} of soil, equivalent to 80% of the soil water retained at 0.01 MPa.

In Experiment 2 the incubation procedure was similar to the above described. The fertilizers were added in water solution (5.6 mg N mL^{-1}).

3.2.3 Experiment management and sampling

In experiment 1 the pots containing the equivalent to 8 kg of dry soil were partially covered and let to stand in a room with air conditioning at 23ºC, the spatial allocation of the pots was changed weekly. Soil moisture content was weekly checked by weight, and water was added when required. The procedure was similar in experiment 2, except for the amount of soil (2 kg) and the incubation temperature was 21ºC.

In experiment 1 on days 2; 7; 14; 21; 28; 35; 49; 63; 78; 91; 106 and 120 a soil sample was collected from each pot, while in experiment 2 soil samples were taken after 2; 5;

11; 18; 25 and 32 days of incubation. At each extraction date the water content of the samples (oven dry at 105ºC) were determined in order to correct possible bias from the target. For respiration rates (CO_2–C evolution) sub samples were taken only at the beginning of the incubation and CO_2–C evolution was measured on days 1; 2; 5; 9; 13; 15; 18; 21; 25; 29 and 32.

3.2.4 Chemical analysis

For mineral N assessment 20 g of the fresh samples were shaken with 100 mL 2M KCl for 1 hour and filtered. The NO_3^--N content was determined by colorimetry after reduction through a Cd column (Griess-Ilosvay reaction; Mulvaney, 1996). The NH_4^+-N content was determined by colorimetry following the Berthelot method (Rhine et al.,1998).

In order to measure CO_2 evolution a 50 g fresh soil sample from each pot was weighed into a 50 mL plastic beaker and put into a 1 L glass jar with a 5 mL vial of 0.25 M NaOH. A control jar without soil was also included. The jars were sealed and incubated at 23ºC for 24 hours. Then the jars were opened and the excess NaOH titrated with 0.1 M HCl after addition of $BaCl_2$. The amount of CO_2 evolved from the soil was calculated by subtracting the CO_2 trapped in the control jar. A similar procedure was followed in experiment 2, except that a continuous measurement of soil respiration was made in order to collect all the evolved CO_2. For this purpose after each titration the water content of the soil was checked, corrected when necessary, and the vial with 5 mL of 0,25 M NaOH was replaced.

In experiment 2 soil pH was measured (1:2.5 soil:0.01 M $CaCl_2$) with a pH Orion Research 701 electrode. In addition the electric conductivity (EC) of the soil suspension (1:2.5 soil:water) was measured with an Orion 122 specific conductivity meter.

3.2.5 Statistical analysis

In experiment 1 analysis of variance for, NO_3^--N, NH_4^+-N, mineral N and CO_2-C evolved were performed following a factorial design (0 and 80 N levels and four WS levels) with three replications. Differences among treatment effects were tested through lsmeans procedure (pdiff) (Appendix 3.1; 3.2; 3.3 and3.5). Due to significant interaction between factors the trends of WS rates with and without N addition were analyzed separately

through orthogonal contrasts. Net mineralization rates were calculated for each period as the slope of linear regression of NO_3^--N, NH_4^+-N and mineral N on time for each treatment from the 7th week onwards, after the net N immobilization had been completed.

In experiment 2 the results of NO_3^--N, NH_4^+-N, mineral N, C evolved, pH and electrical conductivity were analyzed following a 2×4 factorial (two WS levels and four N sources) with three replications. Differences among treatment effects were tested through lsmeans procedure (pdiff) (Appendix 3.5; 3.6; 3.7; 3.8 and 3.9).

The statistical analyses were carried out using the GLM procedure (SAS Institute, Inc. 1985). The results presented are average of three replications

3.3 RESULTS

3.3.1 EXPERIMENT 1

In this experiment decomposition of different amounts of wheat straw with and without N fertilizer addition was studied. The effect of these treatments on soil N availability as well as on soil microbial activity was evaluated.

3.3.1.1 Nitrogen mineralization - immobilization

The dominant form of mineral N measured was NO_3^-, except for the first two samplings, when fertilized treatments showed higher NH_4^+ levels , as a consequence of the ammonification of urea (Appendix 3.1). Considering this fact and the lack of significant differences between treatments in the NH_4^+ content after the second week, the results presented are those corresponding to mineral N content (NO_3^--N + NH_4^+-N).

Mineral N accumulation followed a relatively constant increasing trend from the first week onwards in unamended treatments (fertilized and unfertilized) and in N80-WS0.8. In general mineral N in WS amended soils showed initially a decrease followed by an increasing trend similar to the unamended treatments (Fig. 3.1).

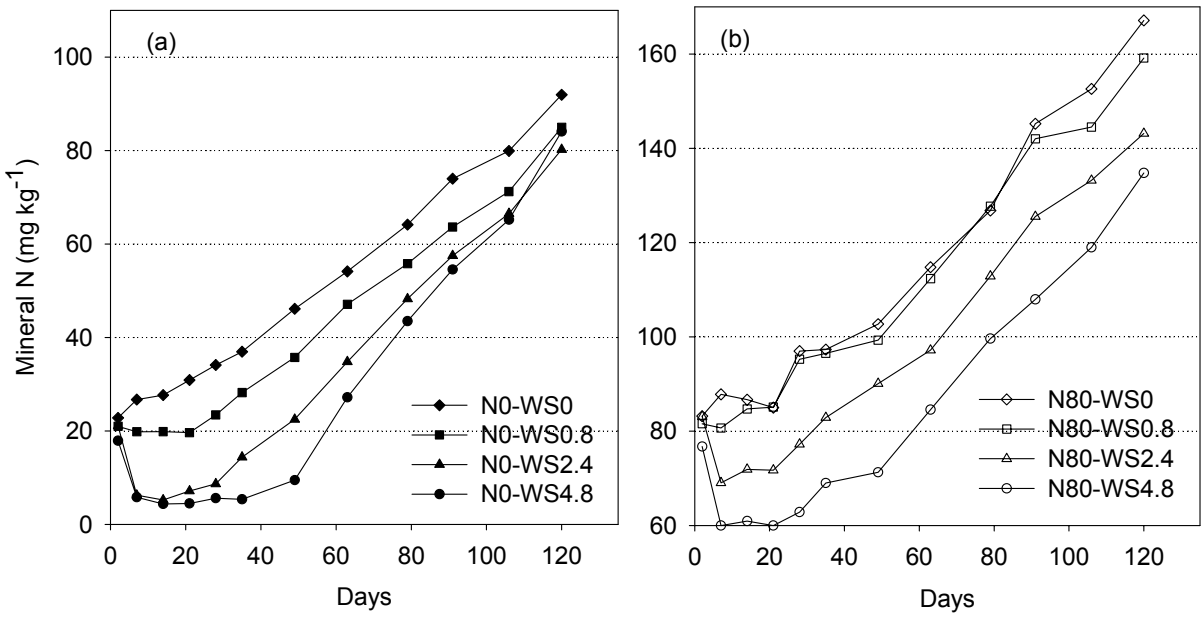

Figure 3.1. Time course of mineral N in a clay soil unamended (0) and amended with wheat straw (0.8; 2.4 and 4.8 mg kg^{-1} rates) (a) without and (b) with N fertilization (80 mg kg^{-1} rate). (Note the different scale in the y-axis).

The amounts of mineral N in fertilized treatments exceeded those of unfertilized soils in all sampling times (Appendix 3.3), being the difference between fertilized and unfertilized unamended soils (N80-WS0 and N0-WS0) 60 mg kg^{-1} at the first sampling (2 days), without further increase in the following sampling dates. The amount of N added was equivalent to 80 mg kg^{-1}; hence an important part of the mineral N from the fertilizer was unaccounted for.

The effect of the WS addition on the mineralization-immobilization process was influenced by N addition, indicated by the significant interaction between the effect of N and WS on mineral N content at days 7; 14; 21; 35; 49; 63; 91 and 120 (Table 3.1). In fertilized soils the treatment with the lowest WS rate (WS0.8) behaved in all sampling times, except at the beginning, in a similar way as the unamended treatment (not significant differences between N80-WS0 and N80-WS0.8). In contrast, for unfertilized soils the WS0.8 was significantly lower than WS0 in all sampling times, except at the end of the study, indicating the immobilization of N in N0-WS0.8.

Table 3.1. Results of the ANOVA for the effect of N and WS addition on Mineral N content and the interaction between both factors as well as the results from the analysis of orthogonal contrasts for fertilized (+N) and unfertilized (-N) treatments at each sampling date. * indicate P< 0.01

Day	Effect N	Effect WS	Interaction	Contrast Response to WS (+ N)	Contrast Response to WS (- N)
2	*	NS	NS	NS	NS
7	*	*	NS	Lin-quadr	Linear
14	*	*	*	Lin-quadr	Linear
21	*	*	*	Lin-quadr	Lin-quadr
28	*	*	NS	Linear	Linear
35	*	*	*	Linear	Lin-quadr
49	*	*	*	Linear	Lin-quadr
63	*	*	*	Linear	Lin-quadr
79	*	*	NS	Linear	Linear
91	*	*	*	Linear	Lin-quadr
105	*	*	NS	Linear	Linear
120	*	*	*	Linear	Linear

Figure 3.2 shows the net N immobilization, which was calculated as the difference between the N content of the control soil and the WS amended soils either for fertilized or for not fertilized treatments. In unfertilized soils there was a clear increase of immobilized N from day 7, reaching a maximum at 25 to 50 days. Thereafter a remineralization of N was observed. In fertilized soils a high level of immobilized N was reached already after 7 days of incubation, followed by a period with no major changes until the 70 days, after which a trend of increasing N immobilization was observed.

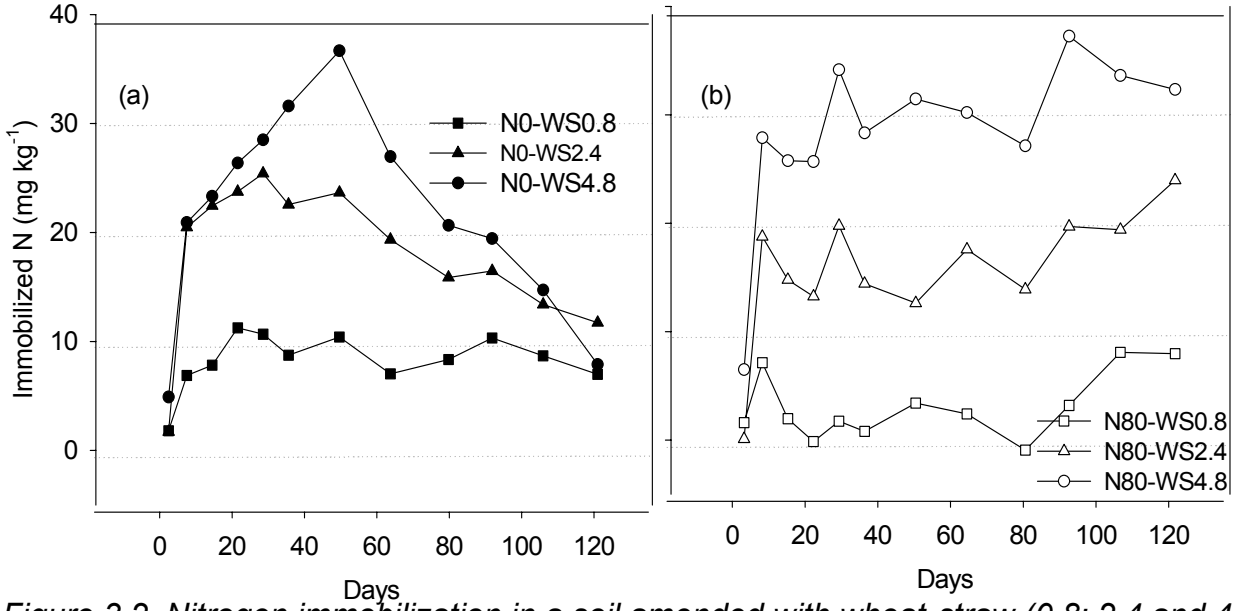

Figure 3.2. Nitrogen immobilization in a soil amended with wheat straw (0.8; 2.4 and 4.8 g kg^{-1} rates), (a) without and (b) with N fertilization (80 mg kg^{-1} rate).

3.3.1.2 Nitrogen mineralization rates

The estimation of daily mineralization rates (mg of N kg^{-1} soil day^{-1}) was made through regression analysis of soil mineral N content on days of incubation, from 49 days onwards. The starting point was chosen considering that the treatments with high amounts of WS presented a distorted N mineralization pattern due to N immobilization in the first weeks of incubation. With the aim to compare mineralization rates in different treatments, only the portion after 49 days of incubation was used.

Fertilized soils showed similar mineralization rates, regardless of the amount of WS added. On the contrary in the unfertilized soils the treatments with high amendment (N0-WS2.4 and N0-WS4.8) presented significantly higher mineralization rates than control and N0-WS0.8 (Tables 3.2 and 3.3). In the unamended treatments the slope of the regression lines (daily N net mineralization rate) was significantly higher in the fertilized than in unfertilized treatment (0.88 vs. 0.64 mg of N kg^{-1} day^{-1} for N80-WS0 and N0-WS0 respectively). The same occurred in treatments amended with 0.8 g kg^{-1} (mineralization rates of 0.83 vs. 0.65 for treatments N80-WS0.8 and N0-WS0.8 respectively). In opposition for WS2.4 and WS4.8 mineralization rates of unfertilized treatments were higher than those of the fertilized treatments with the same WS addition, although not significant differences were found.

Table 2. Regression of mineral N content on days of incubation. The regression fitted is Mineral N = b + a x, being x days of incubation and the slope of the regression line equivalent to mineralization rate. * indicates P<0.05

Treatment	Intercept (b)	Mineralization rate (a)	R^2
	mg kg^{-1}	mg kg^{-1} d^{-1}	
N0-WS 0	14.4*	0.64*	0.96
N0-WS 0.8	4.5^{NS}	0.65*	0.98
N0-WS 2.4	-15.6*	0.80*	0.97
N0-WS 4.8	-37.8*	1.01*	0.99
N80-WS 0	59.8 *	0.88 *	0.94
N80-WS 0.8	60.9*	0.83*	0.94
N80-WS 2.4	51.2*	0.78*	0.97
N80-WS 4.8	29.5*	0.87*	0.87

Table 3.3. Probability of common slope of the regression of mineral N on days of incubation. Each test compared the slope of two regression lines. Levels of probability lower than 0.05 indicate significant differences in slope.

	N 0 WS 0.8	N 0 WS 2.4	N 0 WS 4.8	N 80 WS 0	N 80 WS 0.8	N 80 WS 2.4	N 80 WS 4.8
N0-WS 0	0.741	0.003	0.000	0.000	0.003	0.007	0.016
N0-WS 0.8		0.003	0.000	0.000	0.004	0.008	0.022
N0-WS 2.4			0.001	0.186	0.588	0.749	0.425
N0-WS 4.8				0.049	0.003	0.000	0.123
N80-WS 0					0.462	0.122	0.897
N80-WS 0.8						0.423	0.688
N80-WS 2.4							0.334

The WS4.8 mineralization rate was higher than WS0.8 in fertilized and unfertilized soils, although differences were significant only in unfertilized soils. This higher mineralization rate of N0-WS4.8, made it possible to overcome the lower mineral N content after the immobilization period. This fact also explains the lack of significant differences in mineral N content between the amended treatments of N0 soils at the end of the incubation period.

3.3.1.3 Carbon mineralization

Figure 3.3 shows that in the first measurement (day 7) respiration rates of ranged from 12.8 to 38.7 mg C kg^{-1} soil day^{-1} for N80-WS0 and N80-WS4.8 respectively, and at day 120 from 10.5 to 14.3 mg C kg^{-1} soil day^{-1} for N0-WS0 and N0-WS4.8 respectively.

There was a declining trend in respiration rates (CO_2-C evolution) along the studied period in all treatments, especially in the WS amended soils, indicating a high activity of the soil microbial biomass at the beginning that diminished when the energy rich substrate decayed.

Respiration rates of the amended soils were significantly higher than those of the unamended soils at all sampling times (Fig. 3.3, Appendix 3.4). Those differences were reflected in the total cumulative CO_2 –C calculated (Fig. 3.4). However significant differences in CO_2 evolution after 35 days involved mainly the treatments with high straw rate. There was a trend of linear response in CO_2 evolution with respect to WS amount at most samplings, especially in fertilized soils (Table 3.4).

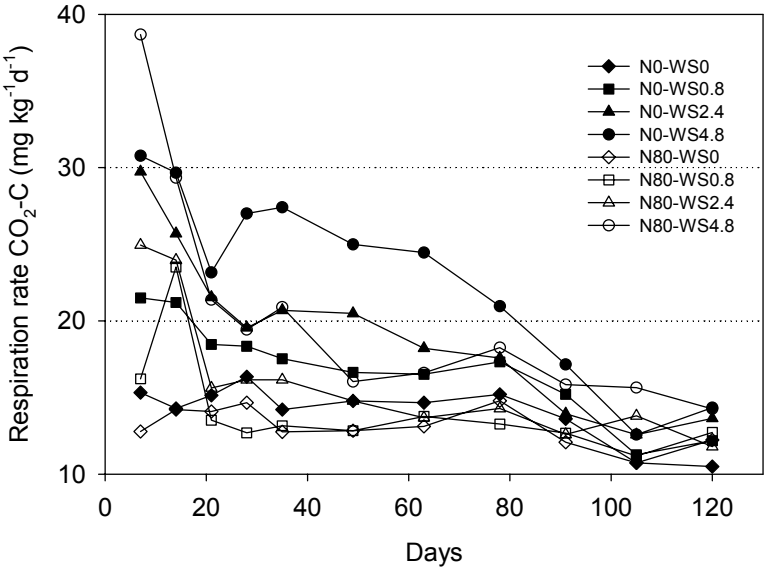

Figure 3.3. Time course of respiration rate in a clay soil unamended and amended with wheat straw (0.8; 2.4 and 4.8 g kg^{-1}), without and with N fertilization (80 mg kg^{-1}).

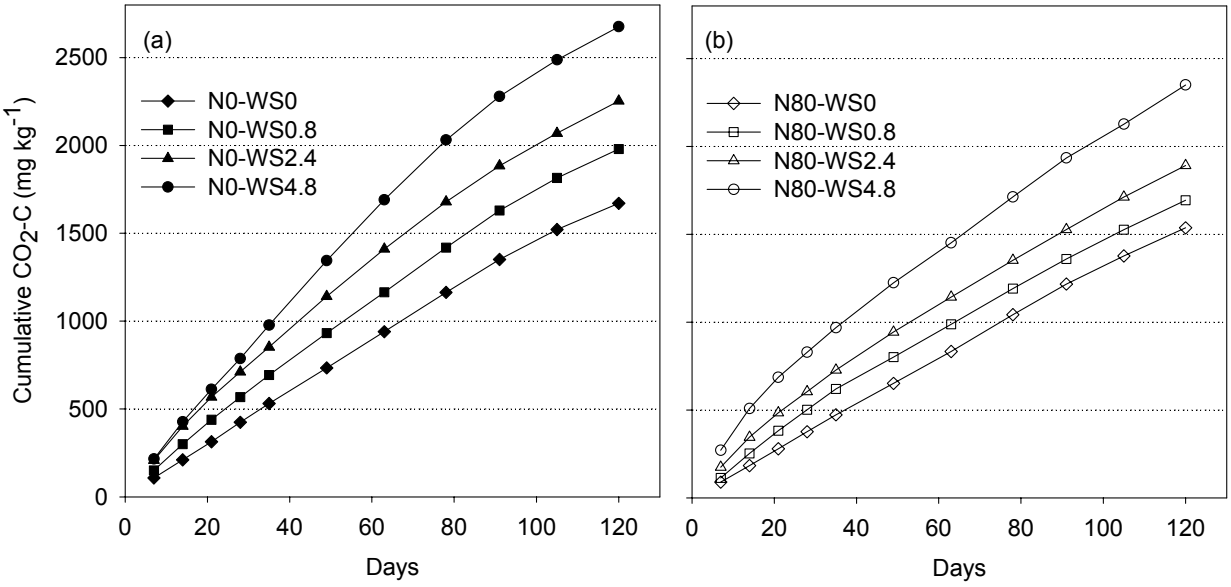

Figure 3.4. Time course of cumulative CO_2 –C in a clay soil amended with wheat straw (0.8; 2.4 and 4.8 g kg^{-1}), (a) without and (b) with N fertilization (80 mg kg^{-1}).

The effect of fertilizer application on C evolved was significant at days 35; 49; 63; 78 and 91 (Table 3.4), indicating that there were differences in microbial activity between fertilized and unfertilized treatments in the middle of the studied period (Fig. 3.5). In all those samplings fertilized soils produced significantly lower amounts of CO_2 than unfertilized soils with the same WS rate (Appendix 3.4). The most marked disagreement was observed between fertilized and unfertilized WS4.8 treatments, that showed significant differences in most sampling times, presenting similar values only at the end of the studied period. In opposition there were no significant differences between the fertilized and unfertilized unamended soils (N0-WS0 and N80-WS0), although the unfertilized control presented slightly higher values.

Table 3.4. Results of the ANOVA for the effect of N and WS addition on respiration rate (mg C-CO_2 kg^{-1}soil day^{-1}) and the interaction between both factors as well as the results from the analysis of orthogonal contrasts for fertilized (+N) and unfertilized (-N) treatments at each sampling date.

Day	Effect N	Effect WS	Interaction	Response to WS (+ N)	Response to WS (- N)
7	NS	*	*	Linear	Lin-quadr
14	NS	*	NS	Linear	Linear
28	NS	*	NS	Lin-quadr	NS
35	*	*	NS	Linear	Linear
49	*	*	*	Linear	Linear
63	*	*	*	Lin-quadr	Lin-quadr
79	*	*	*	Linear	Lin-quadr
91	*	*	*	Linear	Linear
105	NS	*	NS	NS	Linear
120	NS	*	*	Linear	Lin-quadr

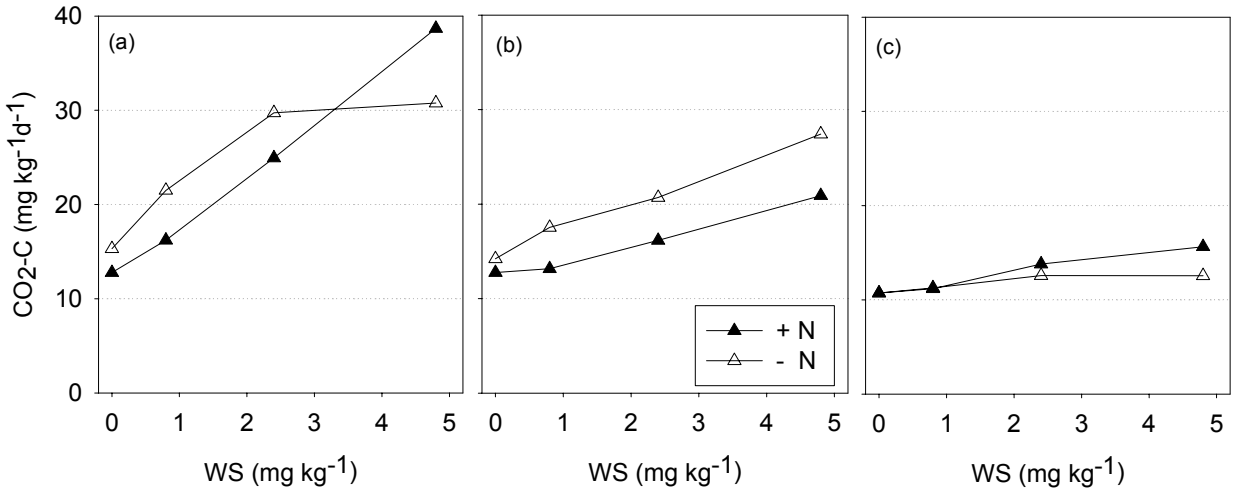

Figure 3.5. Effect of WS amendment (0.8; 2.4 and 4.8 g kg^{-1} rates) and N addition (0 and 80 mg kg^{-1} rates) on respiration rates at (a) 7 days (b) 35 days and (c) 105 days of incubation.

The treatments also influenced the respiration patterns (Fig. 3.5). In unamended soils (N0-WS0 and N80-WS0) there was only a slight decline in CO_2-C evolution rate along the incubation period. Wheat straw amended soils fertilized with urea showed a very high C evolution rate at the first sampling, followed by a steep decrease thereafter, while in amended unfertilized soils there was a gentler decline along the incubation. It is important to remark that the biomass respiration in this experiment was measured during 24 hours at given sampling times.

From the day 49 onwards the analyses showed significant interactions between the effect of N and WS on respiration rates (Table 3.4). In unfertilized soils there were differences between WS treatments until the end of the experiment, while for fertilized soils only the WS4.8 was different from the rest.

3.3.2 EXPERIMENT 2

In experiment 2 the effect of different N sources (urea, KNO_3 and $(NH_4)_2SO_4$) on wheat straw decomposition, especially related to N immobilization and soil microbial biomass activity were compared. In order to explain differences between N sources soil pH and electrical conductivity were monitored along the experiment.

3.3.2.1 Nitrogen mineralization – immobilization

Figure 3.5 shows that most of the mineral N added as fertilizer was recovered in the first sampling in the soils fertilized with PN.and U, being the difference in mineral N between fertilized and control soils 100; 90 and 103 for U, AS and PN respectively The hydrolysis of urea was fast, reaching the maximum amounts of mineral N at the 2 day sampling, however, from day 5 the total amounts of mineral N in urea treated soils were lower than those of the other N sources and, in contrast with the other fertilizers, in unamended soils decreased from day 2 to 5 of incubation.

In soils fertilized with urea and AS the nitrification process was rather slow (Fig. 3.5a). In the unamended soils with urea the amounts of NH_4^+-N were significantly higher than control and PN until day 25, while in the AS treatment NH_4^+-N was significantly higher than in the other N sources until the end of the incubation (Appendix 3.5).

Amounts of NO_3^--N increased in all treatments except for WS amended control and PN (Fig. 3.5b). While unamended control and PN presented small increases in N-NO_3^-, due to SOM mineralization, in U and AS the increases of NO_3^--N were mainly caused by the nitrification of added N (Appendix 3.6). Nitrification rate depends on NH_4^+ availability; hence it was higher in U and AS treatments compared to control and PN.

Total soil mineral N content decreased in WS amended soils, while increased in unamended soils (Fig. 3.5c). In amended soils fertilized with AS the amounts of N-NH_4^+ were significantly lower than in the respective unamended treatment from day 5 and in those with urea from day 11 (Appendix 3.7). In amended soils fertilized with PN the NO_3^--N levels were lower than those of the correspondent fertilizer treatment of the unamended soils from day 11 onwards. In NO_3^--N contents no differences were found between WS amended and unamended soils of U and AS treatments until 18 days of incubation, being higher those of the unamended soils thereafter.

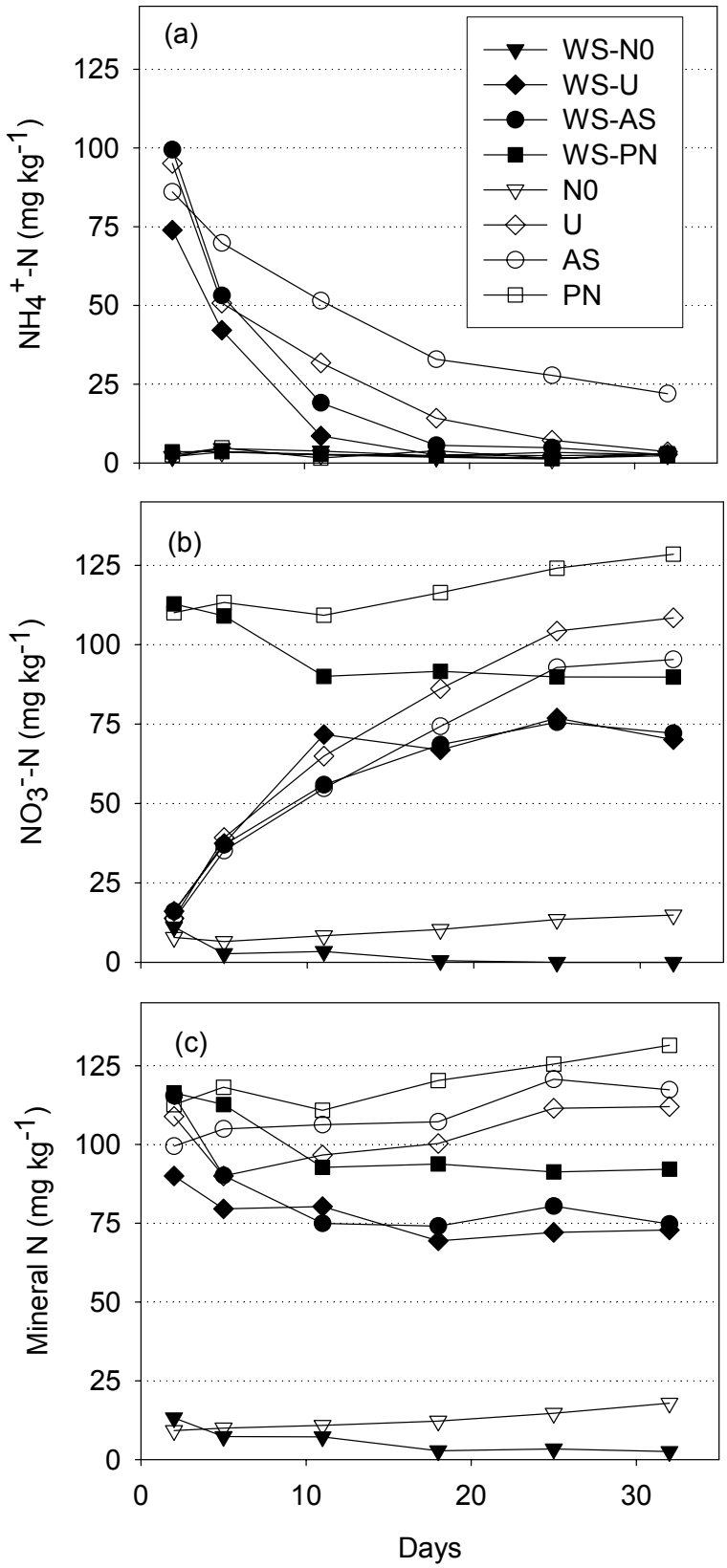

Figure 3.5. Time course of (a) NH_4^+-N, (b) N-NO_3^--N and (c) total mineral N content of the soil fertilized with urea (U), $(NH_4)_2SO_4$ (AS) and KNO_3 (PN) as influenced by wheat residues (WS) addition.

Nitrogen immobilization for each N source was calculated as the difference between mineral N content in unamended soil minus mineral N content in amended soil fertilized with the same N source. The calculated amount of immobilized N varied with N source (Fig. 3.6), showing the interaction between the effects of N and WS on total amounts of mineral N at all sampling times except for 2 and 5 days. Larger amounts of N were immobilized in the soils fertilized with AS compared to the control, U and PN in the first two weeks of the incubation. Nevertheless the maximum amount of immobilized N, for the three N fertilizers, was similar (around 40 mg N kg^{-1} of soil). Without N application, on the contrary, a lower amount of immobilized N (maximum 15.4 mg N kg^{-1}) was estimated, being the soil mineral N almost depleted by the third week of incubation (Fig. 3.5c).

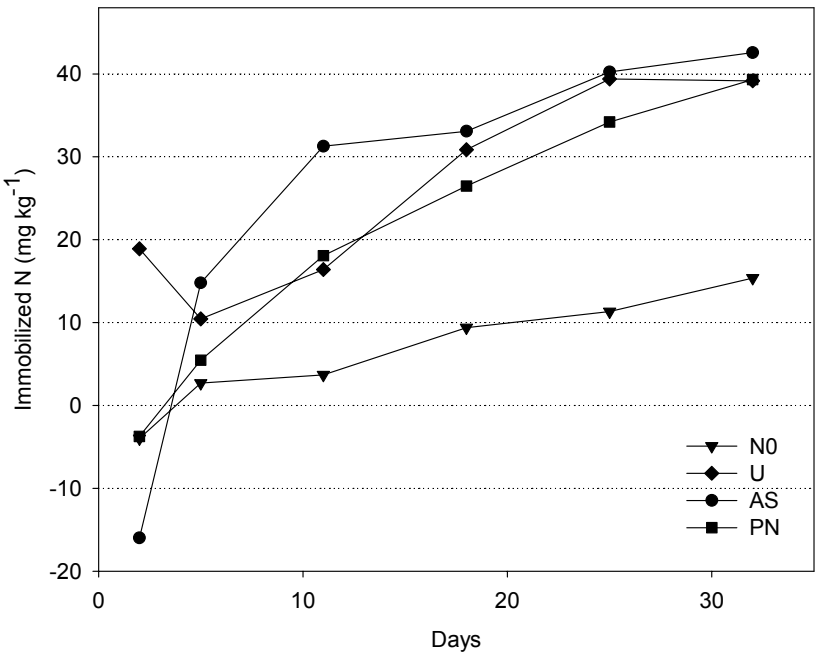

Figure 3.6. Time course of N immobilization in a soil amended with wheat straw at a rate of 4.8 g kg^{-1}, unfertilized (N0) and fertilized with urea (U), $(NH_4)_2SO_4$ (AS) and KNO_3 (PN) at a rate of 100 mg kg^{-1}.

3.3.2.2 Carbon mineralization

Wheat straw addition had a significant effect on CO_2 evolution from the second day onwards, with respiration rates nearly three times higher in the amended soils compared to unamended soils (Fig. 3.7a). Even though respiration rates decreased along the incubation, differences between amended and unamended soils were obvious

until the last sampling. The cumulative CO_2-C amounts reflected the effect of wheat straw addition (Fig. 3.7b) on soil biomass respiration.

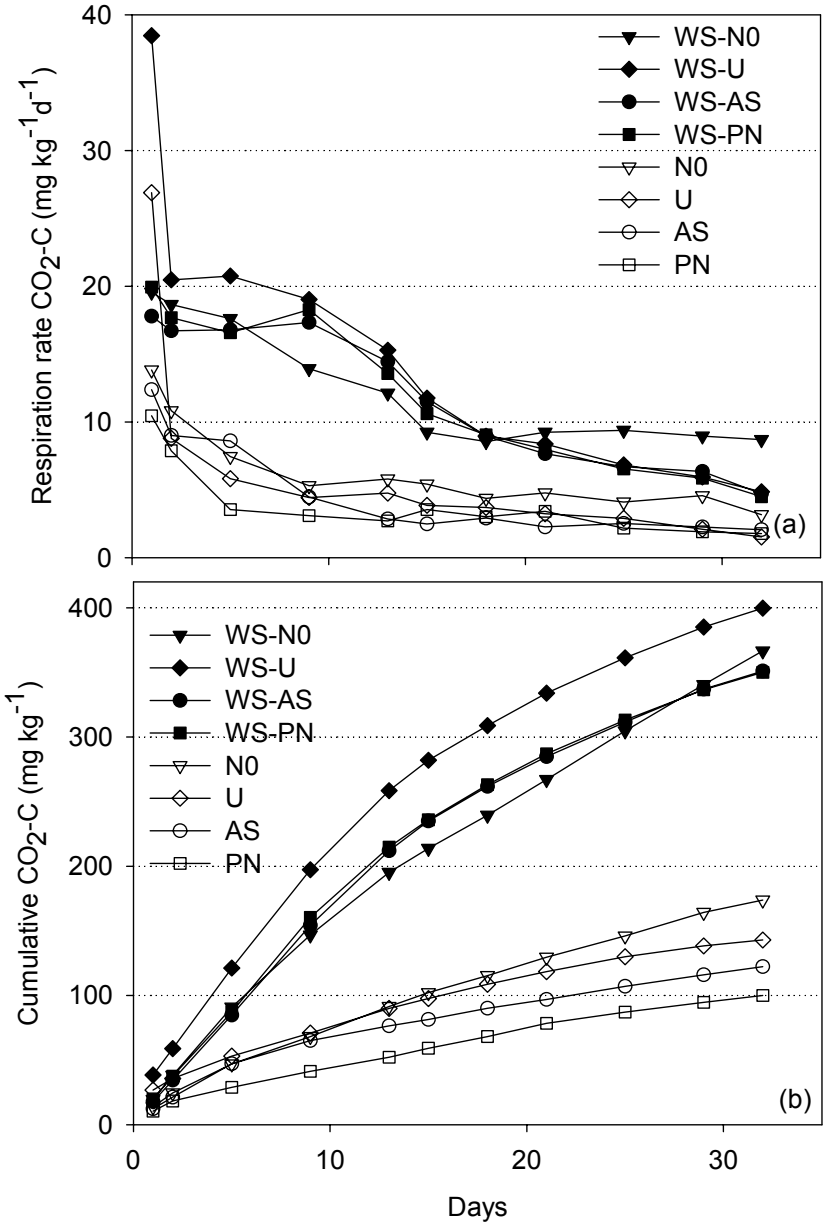

Figure 3.7. Time course of CO_2–C (a) evolution rates and (b) cumulative in soils fertilized with urea (U), $(NH_4)_2SO_4$ (AS) and KNO_3 (PN) at a rate of 100 mg kg^{-1} with and without wheat straw (WS) amendment at a rate of 4.8 g kg^{-1}.

With regard to differences among N sources, in WS amended and unamended soils there were no significant differences between N sources in CO_2 evolved along the incubation, except for urea. Urea fertilized soils showed higher rates in the first three sampling times in WS amended soils and in the first sampling of the unamended soils (Appendix 3.8).

The unfertilized unamended control (N0) presented higher respiration rates compared to fertilized unamended treatments from day 13 until the end of the incubation (Fig. 3.7a). In amended soils the CO_2 evolved from the control without fertilizer (WS-N0) was lower than the fertilized treatments during the first 14 days and did not differ significantly until the sampling at day 25, being significantly higher thereafter. These results were concordant with the significant interaction between the effect of N source and wheat straw addition on microbial activity at most sampling times.

3.3.2.3 Soil pH and electrical conductivity

The pH of the fertilizer solutions (100 mg N L^{-1}) was 7.15 for U, 5.36 for AS and 5.00 for PN. These solutions were spread over layers of humid soils and represented 6 % of the total soil water content.

By the second day sampling the only effect of the fertilizer application consisted in a rise in pH promoted by urea addition (3.8) with respect to the original pH (from 5.0 to 5.2 and 5.3 in amended and unamended soils respectively).

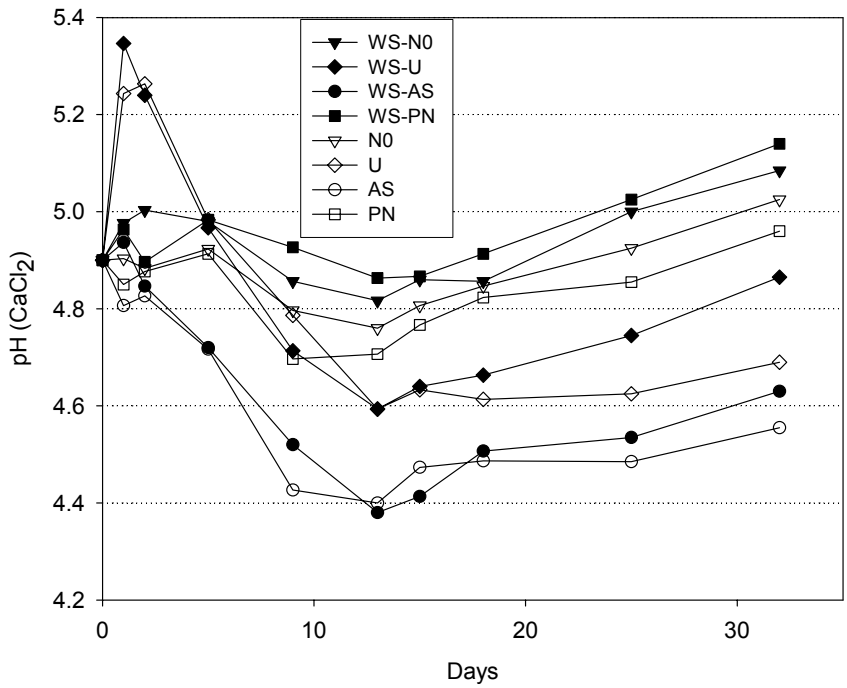

Figure 3.8. Time course of soil pH, measured in 0.01 M $CaCl_2$ suspensions (1:2.5 soil solution), in soils fertilized with urea (U), $(NH_4)_2SO_4$ (AS) and KNO_3 (PN) at a rate of 100 mg kg^{-1} with and without wheat straw (WS) amendment at a rate of 4.8 g kg^{-1}.

From the second day onwards the soils that received $(NH_4)_2SO_4$ showed lower pH than the respective WS amended and unamended control. In opposition the soils with urea showed higher pH than control in the first week, but decreased rapidly, being significantly lower than control by day 9 (Appendix 3.9). Unamended soils fertilized with PN showed a declining trend at the beginning of the incubation, being significantly lower than control by day 9.The effect of KNO_3 differed from the other N sources because pH did not suffer major changes, while the ammoniacal sources produced further decreases in pH, reaching the lowest values between 13 and 18 days after fertilizer application. After U application, pH reached a minimum value of 4.6 and, while the minimum for AS was 4.4, in both amended and unamended soils. In general amended soils showed higher pH than the respective N source treatment in unamended soils, especially from day 9 of incubation. At the end of the incubation an increasing trend in soil pH was observed in all soils, more marked in soils with wheat straw addition.

The EC of the 1:2.5 soil:water suspension was measured by day 13 of incubation in order to assess the effect of fertilizers on salt content of the soil solution (Fig. 3.9). Since these measurements were made in conditions very far from the actual water content of the soil, the results should be taken in relative terms for comparison between fertilizers, rather than absolute EC of the soil solution.

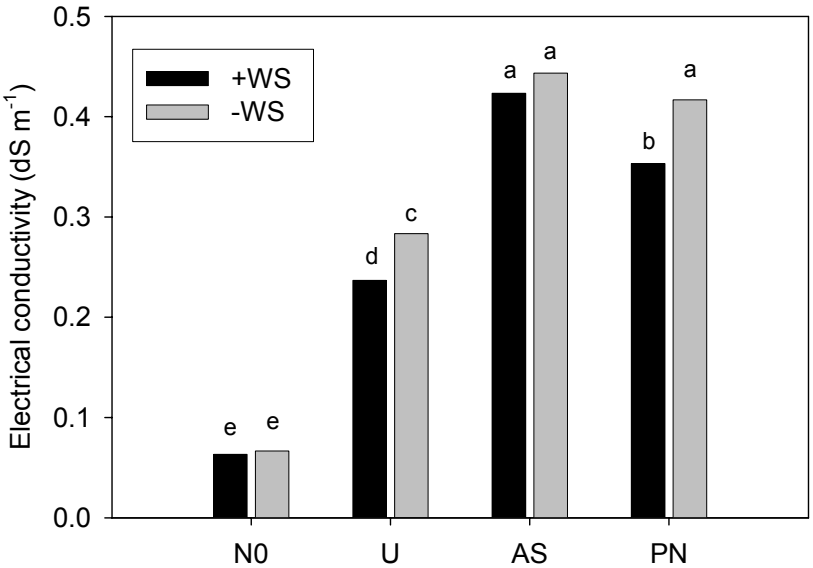

Figure 3.9. Electrical conductivity, measured in water suspensions, in soils fertilized with urea (U), $(NH_4)_2SO_4$ (AS) and KNO_3 (PN) with (+WS) and without wheat straw (-WS) amendment. Different letters indicate significant differences (P<0.05).

There was a significant increase in EC in fertilized soils with respect to the control, the highest amounts for AS followed by PN, although not significant differences between these two sources were found in unamended soils. Even though EC of the soils fertilized with urea was significantly higher than control, the values were lower than those of the other N sources. There was also a significant interaction between the effect of fertilizers and wheat straw addition, since the effect of fertilizers on the increase in conductivity was larger in unamended than in WS amended soils. However this tempering effect of residues, respect to EC increase, was significant only in U and PN fertilized soils. There was a positive significant correlation between mineral N levels and electrical conductivity (r=0.89), however this correlation was mainly determined by the low values of the soils without N addition; when those soils were removed from the analysis, the result was a weak not significant relationship (r=0.38).

3.4 DISCUSSION

The time course of soil N mineralization in the unamended treatments did not reach a plateau at any time during the 17 weeks of incubation in experiment 1, presenting a steady increase (Fig 3.1). The reason for this behaviour can be the relatively low temperature of incubation (23ºC), which influenced these results, promoting a gentle increase without a maximum.

There were two different decomposition patterns corresponding to the +N and –N treatments (Fig. 3.1), which explain the interaction between the effects of N and WS rate on soil N mineralization. In the first case, when N availability did not restrict microbial growth, the soil microbial biomass activity was driven by the amount of readily available C (WS rate). This fact was confirmed by the predominant linear dependence of mineral N and evolved C of the fertilized soils respect of the amount of WS added (Tables 3.1 and 3.4). The second pattern can be observed when N was limiting biomass growth (unfertilized treatments). In this case there were no significant differences in mineral N content between WS2.4 and WS4.8 at the beginning of the incubation (days 7; 14; 21 and 28, Appendix 3.4), both treatments with very low levels of mineral N, indicating that the decomposition of crop residues was delayed by N shortage. In accordance Mary et al. (1996), concluded that the disappearance of inorganic N due to immobilization decreased the mineralization rate, when the same amount of maize

residues was combined with different levels of available N. These results highlight the dependence of mineralization of straw on the N availability. Nevertheless by day 63 all treatments, even the highly amended without N addition, presented mineral N levels higher than those at the starting point of the incubation.

According to Mengel (1996) net N immobilization in the soils where N is non-limiting is a very fast process. In concordance, there was a remarkable amount of immobilized N only 7 days after N and WS addition (Fig. 3.2). In the first 70 days of incubation, in unfertilized soils there was a delay in maximum immobilization in high WS compared to low WS applications, reaching maximum immobilization by day 49 in treatment WS4.8, day 28 in treatment WS2.4 and day 21 in treatment WS0.8. In fertilized soils with high WS rate (WS2.4 and WS4.8) the highest immobilization occurred 28 days after application, while the low WS rate (WS0.8) showed the highest difference respect to unamended control by day 7.

Maximum amounts of net N immobilization calculated in the first 70 days of the experiment in soils without N application were 11.2; 25.4 and 36.7 mg N kg^{-1} of soil for WS0.8, WS2.4 and WS4.8 respectively, while in fertilized soils the amounts were 7.1; 19.8 and 34.2 mg kg^{-1} for WS0.8, WS2.4 and WS4.8 respectively. Hence, in this case the availability of N resulted in lower amounts of immobilized N. These results also indicate that the high N availability did not promote a “luxury” consumption of N in agreement with previous results from Mary et al.,(1996).

The remineralization of the immobilized N was different in fertilized and unfertilized soils, while in fertilized soils the immobilized N tended to remain as such; in the unfertilized soils there was a decrease in the immobilized amount. This re-mineralization of immobilized N could be due to biomass decay after a period of strong growth. The causes of biomass decay in the field are generally related to environmental conditions (changes in temperature and water content of the soil), but in the laboratory experiment these factors were not likely to play any role. The depletion of the readily available C of the wheat residue, together with the low mineral N availability caused by the immobilization process, may have induced the decay of the soil micro organisms followed by re-mineralization. Decomposition of microbial material releases large amounts of mineral N due to the high N content of the biomass (Mengel, 1996).

According to the characteristics of the experiment, it is difficult to explain the increase of immobilized N of the fertilized soils at the end of the period (Fig. 3.2). Reinerstsen et al., (1984) distinguished different pools of organic C in wheat straw, and concluded that the soluble and intermediately available C pools determine the amount of immobilized N in early stages of decomposition. This fact could explain the early-immobilized N while the later peak could be due to mineralization of the C present in recalcitrant C pools of the residues. The respiration rate of the fertilized highly amended soil showed a slight increase at the end of the incubation, which can be an indication that the biomass was still actively decomposing the wheat residue.

Soil biomass activity (CO_2 –C evolution) showed a decreasing trend (Fig. 3.7), coincident with results widely reported regarding to plant material decomposition (Bremer et al., 1991; Hassink, 1994; Millar and Baggs, 2004). Studying wheat straw decomposition, Corbelis et al., (1999) attributed the high C evolution rates in the first two weeks of incubation, to the mineralization of soluble C sources. In our study the fact that the wheat straw was ground and mixed with the soil, probably was determinant to enable the soil microorganisms to reach immediately the crop residue, and consequently accelerate the decomposition process.

The negative effect of urea addition on respiration rates observed in many of the sampling dates (Fig 3.3) is contradictory with the highest activity of microorganisms that can be expected in soils without N limitation. The explanation of this behaviour can be a shift in decomposition speed promoted by the high N availability, which led to a very high CO_2 release at the beginning, followed by a rapid fall in microbial activity in fertilized pots. The lack of N of the unfertilized treatments WS0.8 and WS2.4, that promoted a delay in the decomposition process may have resulted in lower CO_2 initial release but a more sustained microbial activity because soil micro organisms continued decomposing the easily decomposable portion of the straw longer than in the fertilized soils. The delay in consuming the available C could have influenced in the large amounts of C evolved at each sampling time in the N deficient soils. The other possible explanation implies a negative effect of N application on microbial biomass, as reported by Fog, (1988).

Supporting the second hypothesis (a negative effect of fertilizer on soil microorganisms) there was a linear relationship between the amount of immobilized N after 49 days of incubation and the cumulative evolved CO_2 –C (Fig. 3.10). The linear relationship suggests that similar proportions of N and C were assimilated by the soil microorganisms in spite of the different treatments. This fact can be seen as an indication that in the fertilized soils a smaller proportion of the added straw was decomposed, and in consequence less N was immobilized. The relationship at 49 days is presented because N immobilization in unfertilized soils reached the maximum at that time, but close relationships were also found for 7; 14; 21; 28 and 35 days of incubation (Appendix 3.10), while after the 49 days relationships between immobilized N and accumulated CO_2 became weaker. In the present experiment CO_2 –C evolution was not measured in the first week of incubation. In consequence it is not possible to know if at this early, though decisive, phase the fertilized soils presented higher respiration rates, which were not accounted for in the relationship from figure 10, or if there was a negative effect of N addition on biomass activity. The effect of N source on C and N mineralization was studied in detail in experiment 2.

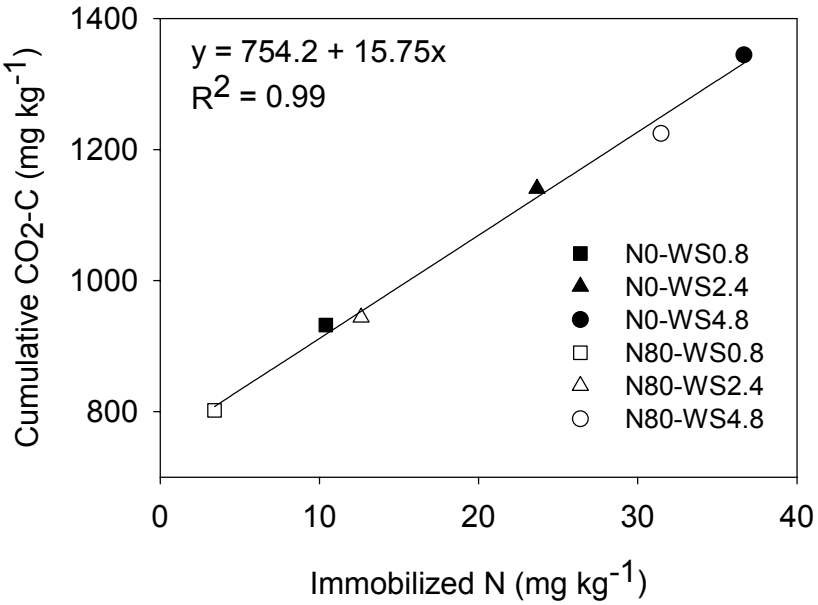

Figure 3.10. Relationship between cumulative CO_2 –C and immobilized N at 49 days of incubation. Data from figures 3.2 and 3.4.

Experiment 2 was designed in order to assess the effect of different N sources on decomposition patterns. The selected sources are widely used in extensive agriculture (urea) and horticulture (KNO_3 and $(NH_4)_2SO_4$) in Uruguay

Patterns of nitrification after U and AS application (Fig. 3.5 a and b) showed that while urea hydrolysis was very fast, in these experimental conditions nitrification was limited. The shape or the disappearance of NH_4^+ curve (exponential decrease) suggests that nitrifier population limited the process. This limitation was more pronounced after AS than after U application. The decrease in pH and the increase in electrical conductivity of the soil solution observed in the soils that received AS (Fig. 3.8 and 3.9) probably provided a less adequate environment for microbial growth, with the consequence of a slower nitrification process. The negative effect of acidity on nitrification has been widely reported (Haynes, 1986, Summer et al., 1991). Similar nitrification patterns of urea and $(NH_4)_2SO_4$ fertilizers were reported by Wickramansinghe et al., (1985). The reason for the decrease of mineral N right after urea application in both amended and unamended soils, can be either N immobilization or N loss through NH_3 volatilization. Considering the increase in soil pH following the urea application, NH_3 losses might have occurred.

The amounts of N immobilized in the WS amended soils without N addition were nearly one third of the amounts immobilized in the fertilized soils (Fig. 3.6); it is therefore clear that N immobilization in amended soils depended on N availability. The immobilization pattern observed in this experiment during straw decomposition highlights the preference for N-NH_4^+ by soil microflora, since in the U and AS treatments the principal difference between amended and unamended soils were observed in N-NH_4^+ content. Although NO_3^- was also available there were no decreases in N-NO_3^- contents until day 18 for urea and until day 25 for AS treatments, when NH_4^+ was almost depleted (Figs. 3.5a and b). The control and PN treatments showed very low NH_4^+-N levels along the incubation and the immobilized N came from the NO_3^- pool. Similar results of preferential immobilization of NH_4^+, concomitant with inhibition of NO_3^- immobilization when NH_4^+ was present, were reported by Wickramasinghe et al., (1985), Rice and Tiedje, (1989) and Recous et al., (1990). This preferential immobilization of NH_4^+ by microorganisms, has been attributed to the lower energy required for assimilation (Haynes, 1986). Working with N marked fertilizers Recous et al. (1992) reported 3 to 4 times greater immobilization rates in a field experiment when N was applied as NH_4^+ or urea compared to NO_3^-. In the present study the most marked differences corresponded to the first two weeks after N application, being the differences between N sources softened thereafter.

Wheat straw and fertilizer addition greatly affected the biological activity of the soil, measured as respiration rate (Fig. 3.7). The effect of crop residues was generally positive, increasing microbial activity in spite of the N source. The effect of N addition, on the other hand, was in some cases detrimental of microbial activity. Without straw addition lower respiration rates in fertilized respect to unfertilized soil were observed from the second day onwards (significant from day 14). Negative effects of N fertilization on soil biomass have been reported by Kowalenko et al., (1978), Maheswaran and Attiwill, (1989), Bremer et al., 1991 and Green et al., (1995) in laboratory and field experiments. On the contrary Foster et al., (1985) reported positive effects while Yang et al., (2003) did not find effects of N addition on respiration rate of the control soil. Bremer et al., (1991) studying decomposition of different plant parts and N addition observed that the negative effect of applied N on CO_2 evolution occurred mainly after 20 days of incubation, which is coincident with the results for amended soils in the present experiment.

The reasons for the negative effect of fertilization can be found in the environmental changes promoted by fertilization, especially related to the drop in soil pH and the increase in electrical conductivity of the soil solution (Kowalenko et al., 1978, Huntjens et al., 1981) and biomass inhibition by high N concentrations (Bremer et al. 1991). Fog et al., (1988) made an extensive review of different studies that reported negative effects of N addition on mineralization and concluded that the N source did not strongly influence the results. Unfortunately the inhibitory effect of the high N concentration on microbial activity is impossible to separate from the negative effect of the salt concentration in the present study.

The effect of pH on microbial growth has been reported as not very decisive when microbial activity in different soils, some of them with low pH, were compared (Summer et al., 1991). On the other hand a positive effect of pH increase on soil microbial activity was observed by Haynes and Swift, (1988) Curtin et al., (1998) and Condron et al., (1993). In a similar way Beck, (1983) reported the positive effect of soil pH increase on biomass C content in a long term experiment, where changes in pH were created by soil management, rather than soil type (pH values ranged from 5.6 to 6.3). Moreover in his work the negative effect of acidity on nitrification rate was much more marked than the

effect on microbial biomass growth. Motavalli et al. (1995) listed the possible effects of acidity on soil C dynamics, they cited reduced solubility of organic C compounds, increased amounts of biologically toxic cations such as Al^{+3} and Mn^{+2}, changes in microbial activity, enzyme activity and the composition of the microbial population, being favoured fungal compared to bacterial populations in low pH soils. Probably in fertilized soils in the present experiment the biomass growth was affected by a sudden pH decrease, which is likely to have a more marked negative effect, compared to studies where the biomass developed mechanisms of adaptation to acidity (Shah et al., 1990). It is important to remark that the studied soil is likely to present a strong buffer capacity in response to pH and EC changes due to high clay and organic C content (clay 28 %, O.C. 29.1 mg kg^{-1}). Even so, in the present study fertilizer addition promoted an important pH decrease, while EC increased from 3 to 5-fold (U and AS respectively) respect of the unfertilized soil (Figs. 3.8 and 3.9). On the other hand PN fertilized soils, whose pH values were scarcely lower than those of the control, presented significantly lower respiration rates in unamended soils (Appendix 3.8) compared to control. This fact indicates that pH change was not the unique explanation for the negative effect of fertilizer addition on microbial activity.

The effect of high salt content on CO_2 evolution, ammonification and nitrification was studied by McCormick and Wolf (1980). They found a negative effect on respiration, even with low amounts of NaCl added (250 mg^{-1}NaCl kg^{-1} soil). This effect was reduced when the soils were amended with alfalfa. In the present experiment it is possible to attribute a less damaging effect of saline content in WS amended soils to N immobilization, however this explanation is not applicable to the NaCl addition. Interestingly they also reported lower EC in the amended soils, which seems to confirm that there was an effect of the amendment in lowering saline content of the soil solution. In their work, however the moderating effect of alfalfa was insufficient to avert the negative effect of NaCl on mineralization. Comparing the effect of salinity on SOM ammoniffication and nitrification, Laura, 1977 reported a higher effect of NaCl addition on nitrification, with the consequent NH_4^+ accumulation, which was not observed in this study.

The other possible mechanism of biomass activity inhibition in N fertilized soils has been described by Fog et al., (1988). According to this process the high N level

suppresses the microbial formation of hydroxyl radicals, which are used in lignin and humic acid degradation and, in consequence, reduces the activity of lignin decomposers. The higher negative effect of N addition on respiration in the unamended soils, with humified organic matter, as well as in advanced phases of wheat straw degradation in the present study could be attributed to this mechanism.

The interaction of the effect of fertilization and wheat straw addition imply that the amended soils suffered less changes in environmental conditions (pH and electric conductivity) than the unamended soils, probably due to the removal of mineral ions from the soil solution, especially NO_3^- and NH_4^+, along the decomposition process. The decomposed wheat straw on the other hand probably released K^+ and Ca^{+2} to the soil solution, with positive effect on pH. It is possible then to confirm the contrast between the negative effect of fertilizers in CO_2–C accumulation in unamended soils and the positive effect observed in WS amended soils (Fig. 3.7b). In amended unfertilized soils it is likely that the WS decomposition process was N limited at the beginning of the incubation (respiration rates significantly lower in unfertilized control than the three N sources from day 9 to 18); hence at that time N supply was positively related to microbial activity. Nevertheless the opposite occurred thereafter, reaching similar amounts of evolved CO_2 the unfertilized control, AS and PN in amended soils at the end of the incubation. Given those results it is clear that the agronomic practice, consisting in N addition in order to speed crop residues decomposition, would be at least not effective, if not contrary to the desired objective.

As in the previous experiment a relationship between net immobilized N and evolved CO_2 was tested, however no clear relationship was found. Comparing figures 3.6 and 3.7 it is possible to notice that differences in N immobilization between N sources did not reflect in respiration rates. Straw amended soils fertilized with $(NH_4)_2SO_4$ and KNO_3 showed similar respiration rates, however N immobilization from the ammoniacal source was clearly greater. Knapp et al., (1993) who studied wheat decomposition with different N supply, found that with N addition amino acid production (N immobilization) increased, being 3.8 times higher than in (-N) treatment. At the same time they observed only 1.6 times more CO_2 evolved in +N. These results suggest that there was a change in C:N ratio in the microbial population. It is possible that in the present experiment a shift to lower C:N ratio occurred in AS treatments, which immobilized higher amounts of N per

decomposed C. The energy requirements of NO_3^- reduction on the other hand, could have also influenced, making the NH_4^+, immobilization a more efficient process.

Greater amounts of CO_2 evolved from urea fertilized soils, compared to other N sources. In general CO_2 evolution is accepted as estimation of microbial activity, in the present study however, the high amounts evolved from the urea treatment at the beginning of the incubation may have been due to the hydrolysis of urea, instead of biomass respiration. On the other hand, urea promoted larger N immobilization in the first two samplings, which can be seen as indication of active microbial population, coincident with the higher CO_2 evolution. In accordance it has been reported that the pH increase caused by urea hydrolysis can increase the solubility of organic compounds, which become available for the soil microorganisms (Foster et al., 1985).

3.5 SUMMARY AND CONCLUSIONS

Net N mineralization of soils amended with wheat straw (WS) was influenced by both the amount of WS added and the presence of fertilizer N. Nitrogen immobilization was noticeable from the 7th day sampling. At this early stage of incubation the soluble C fraction of the WS was likely to be the main source of C determining the activity of the biomass, with high amounts of CO_2 evolved from the WS amended soils. Decomposition of WS in the N-limited soils resulted in a slower process, especially when high WS amounts were added. Consequently the maximum immobilization occurred in the highly amended soils by day 28 for fertilized and by day 49 for unfertilized soils. The amount of immobilized N linearly increased with the amount of WS added, with a close relationship between amounts of CO_2 evolved and immobilized N. Microbial activity, measured as C evolution, followed a sharp decreasing trend in the first weeks of incubation in amended soils, probably due to the depletion of the easily available C. This trend was more marked in fertilized soils where decomposition was not limited by N availability, in consequence fertilized soils showed lower respiration rates after the second week of incubation. The fate of immobilized N was also influenced by N addition; while in the soil with high N availability no major changes were observed, the increase in mineral N of amended unfertilized soils in the last period of incubation suggest a remineralization process.

With regard to the effect of different N sources on SOM and WS mineralization, differences were observed in N mineralization patterns. There was a preferential immobilization of NH_4^+ over NO_3^- from soils with U and AS, which in turn showed higher amounts of immobilized N than PN fertilized soils. In terms of respiration rates, N source did not strongly affect WS decomposition, being the amounts of evolved CO_2 similar in the soils fertilized with U, AS and PN. There was a negative effect of N addition when it was applied without a C source, which resulted in lower biomass activity. Probably this effect was due to soil acidification and increase in salt concentration as well as the negative effect of high N levels on the capacity of soil microorganisms to decompose organic materials. This negative effect of fertilizer applications was counteracted by the WS addition, especially in early decomposition phase, being the accumulated amounts of evolved CO_2–C similar in fertilized amended soils, despite the N source.

4 CROP RESIDUE COMPOSITION AND MINERALIZATION PATTERNS IN AGRICULTURAL SOILS OF URUGUAY

4.1 INTRODUCTION

In agricultural production crop residue management is an important aspect in order to achieve a sustainable crop production. Crop residues replenish organic compounds, which are in continuous turnover in the soil. In intensive production systems of Uruguay not only crop residues are incorporated to the soil but also the organic matter is complemented through the use of green manures. These green manures are usually legumes, being the use of egyptian clover (*Trifolium alexandrinum, L.)* especially adequate, since this clover presents a relatively short growth period, which enables the sowing in autumn, growing during winter and spring and finally incorporation in late spring, with the aim to provide a fresh N source for the following summer crop. With respect to summer crops soybean and especially sunflower residues are much easier to manage than maize or sorghum residues. The reason for this behavior can be related to the characteristics of the crop residues or to the amount of crop residues incorporated, which is usually higher in maize and sorghum.

The composition of plant residues determines the result of the N mineralization process (net N mineralization or immobilization), being frequently the C:N ratio of the incorporated residues used for forecasting the outcome (Schomberg et al. 1994, Trinsoutrot et al. 2000). The relationship between C:N and the net effect on N mineralization enables to find critical C:N levels. According to this approach ratios higher than critical level will promote N immobilization and lower C:N ratios N release. It has been found that the critical C:N ratio lies between 15 and 33 (Black, 1968; Quemada and Cabrera, 1995; Trinsoutrot et al. 2000). Other studies suggest that the N concentration of the plant material has a closer relationship with the mineralized N than C:N (Frankenberger and Abdelmagid, 1985).

Most crop residues present C:N ratios larger than 30, moreover the residues of cereal crops like wheat, barley, maize and rice usually present a very low N concentration. Under those circumstances the expected net effect will be net immobilization of mineral soil N, which in turn, depends on mineral soil N availability. According to Recous et al.

(1995) mineral N availability determines the kinetics of decomposition of cereal crop residues, for this reason the lack of soil mineral N can mask the differences in chemical composition.

Decomposition patterns are not exclusively controlled by nutrient availability; moreover the composition of the materials strongly influences degradation (Stott et al., 1983; Janzen and Kucey, 1988). It has been observed that lignin is the most resistant fraction in respect of microbial decomposition (Kirchmann and Berqvist, 1989; Fox et al.,1990), consequently lignin rich residues would decompose slower than those with low lignin content. Other studies suggest that the polyphenol content of the plants plays an important role on decomposition rates, being decomposition of phenolic compounds extremely slower compared to other components of plant materials (Kelley and Stevenson, 1987; Palm and Sanchez, 1991; Janssen, 1996), From studies comparing decomposition of different plant material it can be concluded that lignin and polyphenols, with structure characteristics similar to those of humic acids, present a strong resistance to microbial attack compared to cellulose and soluble organic compounds (Stott et al., 1983; Haynes, 1986). On the other hand a positive influence of the soluble C and N on decomposition rates have been reported (Bremer et al., 1991; Schomberg et al., 1994).

The decomposition kinetics is likely to be influenced by different components of the material along the whole decomposition period. It has been suggested that in the initial stages of mineralization the C:N ratio is the best indicator of mass loss and N release, while the lignin content becomes more important in the last stages of decomposition (Taylor, et al, 1989; Quemada y Cabrera, 1995).

Considering the available information the hypothesis to test was the occurrence of different mineralization patterns in crop residues varying in composition. In this aspect the capacity of cereal and oil crop residues for N immobilization as well as the ability of the green manures to release N to the soil during decomposition were evaluated. In consequence in the present work two experiments were made in order to study differences in decomposition patterns of crops residues from some of the most common crops in Uruguay. In the first experiment residues from two cereal crops (wheat and maize) and an oilseed crop (sunflower) were compared. Since all these crop residues

present low N content, the influence of N addition on decomposition was also tested. In the second experiment the influence of the chemical composition of the plant materials on decomposition patterns was studied. In this experiment not only crop residues, but also legume green manures, as well as different plant parts were compared.

This work is aimed to determine the mineralization patterns of residues from different crops incorporated to the soil and the effect of mineral N availability on those patterns.

4.2 MATERIALS AND METHODS

4.2.1 Soil and treatments

The soil was taken from the upper layer of an Arenic Hapudalf, $pH_{(H2O)}$ 5.3 (1:2.5 soil:water), organic C 10.2 g kg^{-1} total N 1.1 g kg^{-1}.

Two experiments were established: Experiment 1 consisted in a factorial experiment with two N levels and 4 crop residues. N levels: without fertilizer application (N0) and with 80 mg N kg^{-1} of soil (the amount equivalent to 100 kg ha^{-1}, N80). The calculation of the fertilizer rate was made considering a bulk density of 1.25 and a soil depth of 10 cm. Crop residues: No addition (R0), addition of ground residues: wheat straw (*Triticum aestivum, L*, WS), sunflower (*Helianthus annus,L*, Sun) and maize (*Zea mays, L*, M). The amounts of residues added were 2.4 g kg^{-1} of soil equivalent to 3000 kg ha^{-1}. Wheat straw and shoots of sunflower and maize were collected after harvest of the different crops, dried at 60ºC and ground. Characteristics of the crop residues are presented in Table 4.1.

Experiment 2 consisted in the comparison of green manures and crop residues. The plants tested were egyptian clover (*Trifolium Alexandrinum, L.*) young (C-y) and mature (C-m) shoots and young roots (C-r), Sunflower (*Helianthus annus*, *L.*) leaves (Sun-L) and stems (Sun-S), Soybean (*Glycine max, L.*) leaves (Soy-L) and stems (Soy-S) and Maize shoots (*Zea mays*, *L.* M). Sunflower, soybean and maize were harvest residues, although in the case of sunflower the crop was not completely dry yet. Sunflower residues came from a different field respect of those used in experiment 1, while maize residues were from the same origin. Clover plants were taken from clover crops grown as green manures. Young and mature clover corresponded approximately to 70 and

100 days after sowing respectively. Clover roots were washed and visible nodules removed. For practical reasons in this work all plant materials are in many cases referred to as residues, although this word is not strictly applicable to clover plant parts. All plant materials were ground. The amount of residues added was 2.4 g kg^{-1} of soil equivalent to 3000 kg ha^{-1} of dry matter. Characteristics of the plants tested are presented in Table 4.1.

Table 4.1 – Composition of plant materials

Plant material	C	N	C:N	Lignin	Cellulose	Hemi-cellulose	Soluble Polyphenol	Soluble C
	(g kg^{-1})			(g kg^{-1})				
Experiment 1								
Wheat straw	411	5.9	70	69	416	282	41	34
Sunflower shoots	381	7.2	53	88	319	111	51	62
Maize shoots	401	3.9	103	38	418	357	37	33
Experiment 2								
Clover-young shoots	389	27.2	14	28	146	118	62	79
Clover-mature shoots	394	24.8	16	65	232	119	79	100
Clover – young roots	398	22.9	17	71	260	127	40	82
Sunflower – leaves	399	11.9	34	24	172	62	73	66
Sunflower – stems	381	6.2	61	80	175	77	42	139
Soybean – leaves	456	16.2	28	81	177	132	115	106
Soybean – stems	474	5.8	82	129	418	150	23	53
Maize – shoots	401	3.9	103	38	417	357	37	33

4.2.2 Incubation procedure

In Experiment 1 fresh soil was passed through a 5 mm sieve, and roots and stubble were removed. The portion of soil, corresponding to each pot (8 kg), was extended in a thin layer. After mixing with the crop residues, urea in water solution (N 6.4 mg N mL^{-1}), was spread in the N80 treatments, the soil was mixed carefully again and deionised water added and mixed. The unfertilized pots received only water. The amounts of water added, as well as water+urea solution, were aimed to reach 0.19 g g^{-1} of soil, equivalent to the water retained at 0.01 MPa.

In Experiment 2 in order to avoid mineral N shortage the soil was pre-incubated with the aim to reach a high mineral N content (70 mg N kg^{-1}soil at the beginning of the study). The soil was passed through a 5 mm sieve, and roots and stubble were removed. The portion of soil, corresponding to each pot (2 kg) was extended in a thin layer. After mixing with the crop residue corresponding to each treatment, deionised water was added and mixed carefully again. The amount of added water was aimed to reach 0.17 g g^{-1} of soil, equivalent to 90% of water retained at 0.01 MPa.

4.2.3 Experiment management and sampling

The pots were partially covered and let to stand in a room with air conditioned at 21 ºC, the spatial allocation of the pots was changed weekly. The soil moisture content was weekly adjusted by weight, and water was added when required.

In experiment 1 soil samples for analysis of mineral N, respiration rates, and soil water content measurement were taken after 5; 12; 18; 33; 47; 61; 82; 103; and 131 days of incubation. At each sampling a portion of soil was taken from different parts of the pot and soil was gently mixed again. Soil from these samples was incubated for 24 hours for respiration rates measurement.

In experiment 2 soil samples were taken for analysis of N and soil water content measurement after 1; 8; 16; 23; 30; 44; 58 and 79 days of incubation as described for experiment 1. For respiration rates (CO_2–C evolution) subsamples were taken only at the beginning of the incubation and rates were measured in the same samples on days 1; 2; 4; 8; 11; 15; 18; 22; 25; 30; 32 and weekly afterwards, with the last sampling after 96 days of incubation. In this case respiration rates were measured without mixing the soil.

4.2.4 Chemical analysis

Total N content of the plant residues was measured following the Kjeldahl procedure. Total C was determined through oxidation of organic C with $K_2Cr_2O_7$ in concentrated H_2SO_4 at 150ºC for 1 hour (Nelson and Sommers, 1986) followed by colorimetric determination at 600 nm. Lignin, cellulose and hemicellulose after Van Soerst (1979). Polyphenol and soluble C 1 g of plant material were extracted at 100ºC with 100 mL distilled water for 30 minutes. After filtration soluble C was measured as described for total C and polyphenol content was determined by colorimetry in the presence of Folin Denis reagent (King, 1967).

Mineral N determinations and soil respiration in experiments 1 and 2 were performed as described in chapter 3.

Due to the characteristics of the incubation procedures the results of the mineral N analysis are cumulative, since a no leaching incubation was performed. The results from the C analysis, on the other hand, represent rates, since measurements were sequential. In this case, from the measured respiration rates the cumulative evolved CO_2–C from each treatment was calculated.

4.2.5 Statistical analysis

Experiment 1. Analysis of variance for NO_3^--N, NH_4^+-N, mineral N and evolved CO_2–C were performed following a factorial design (0 and 80 N levels, without residue and three crop residues) with three replications. Differences among treatment effects were tested through lsmeans procedure (pdiff) (Appendix 4.1; 4.2; 4.3 and 4.4). Due to significant interaction between factors the effects of the residues with and without N addition were analyzed separately. Net mineralization rates were calculated for each period as the slope of linear regression of NO_3^--N, NH_4^+-N, and mineral N on time for each treatment from 47 days onwards, after the net N immobilization was apparently completed.

Experiment 2. One way analysis of variance for NO_3^--N, NH_4^+-N, mineral N and evolved CO_2–C was performed following a randomized design with three replications. When data failed the homogeneous variance test, a logarithmic transformation was made. Differences among treatment effects were tested through lsmeans procedure (pdiff) (Appendix 4.5 and 4.6). Linear, polynomial and “step wise” regression analyses were performed to test relationships between mineral N and CO_2–C evolved respect to plant material composition. The statistical analyses were carried out using the GLM procedure (SAS Institute, Inc. 1985).

4.3 RESULTS

4.3.1 EXPERIMENT 1

In this experiment mineralization patterns of wheat, sunflower and maize residues were compared. With this aim C and N mineralization time courses were examined. The influence of crop chemical characteristics as well as N addition on those decomposition patterns was also tested.

4.3.1.1 Carbon mineralization

Figure 4.1 shows the cumulative CO_2–C evolved from the different treatments. The cumulative CO_2–C evolution was calculated from the daily respiration rates measured and the length of the period between samplings.

The highest amounts CO_2 evolved from the crop residues amended soils and the lowest from control soils. At the beginning of the incubation respiration rates ranged from 3.5 mg C kg^{-1} soil day^{-1} for treatment N80-R0 to 10.4 mg C kg^{-1} soil day^{-1} in N80-M, and at the end of the incubation from 2.9 to 4.3 mg C kg^{-1} soil day^{-1} for the same treatments (Appendix 4.1). There was a declining trend in soil respiration (measured as CO_2–C evolution) along the studied period in all treatments, specially in the amended soils, indicating a high activity of the soil microbial biomass from the beginning of the incubation period, which diminished when the energy rich substrate decayed. In consequence the accumulated CO_2–C patterns showed a greater increase in the first 7 days of incubation, which takes a flatter shape towards the end, especially in amended soils (Fig. 4.1).

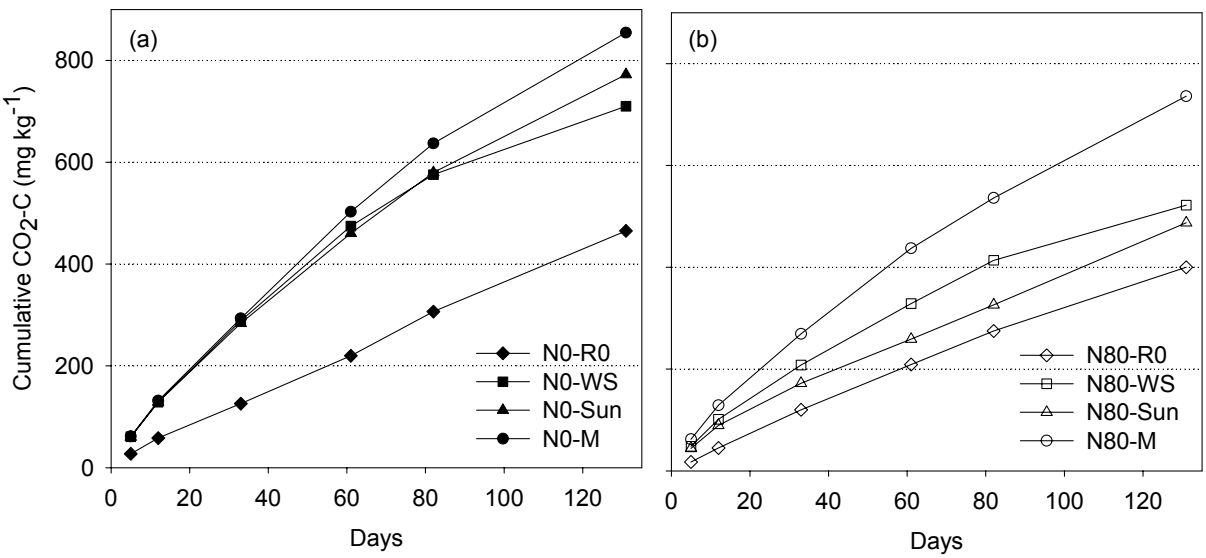

Figure. 4.1. Time course of cumulative evolved CO_2–C in a sandy soil unamended (R0) and amended with crop residues of wheat (WS), sunflower (Sun) and maize (M), (a) without N fertilizer (N0) and (b) with N fertilization (N80).

There were no significant differences among crop residues in unfertilized soils (Table 4.2) , while fertilized soils amended with maize showed higher respiration rates than in those amended with wheat straw or sunflower residues, except for the last two sampling times. Although the soils amended with wheat straw presented higher respiration rates than those of soils with sunflower residues, differences were not significant.

Table 4.2. Results of the ANOVA for the effect of N and crop residue addition on soil mineral N content and the interaction between both factors as well as the results from the analysis of orthogonal contrasts for fertilized (+N) and unfertilized (-N) treatments at each sampling date. * indicate P<0.05.

Day	Effect N	Effect Residue	Interaction	Contrast M vs. WS and Sun (-N)	Contrast M vs. WS and Sun (+N)
5	NS	*	NS	NS	*
12	*	*	*	NS	*
33	*	*	NS	NS	*
61	*	*	NS	NS	*
82	NS	NS	NS	NS	NS
131	NS	NS	NS	NS	NS

There was a significant negative effect of fertilizer application on rate of C evolved at many sampling times, being respiration rates of fertilized soils lower than those of the unfertilized soil with the same amendment, especially for wheat straw and sunflower. However N fertilizer application did not affect CO_2–C evolution at the beginning and at the end of the incubation (Table 4.2). In consequence there were differences in microbial activity between fertilized and unfertilized treatments especially in the middle of the studied period, and those differences were reflected in the accumulated amounts, being those of fertilized treatments lower compared to unfertilized soils both in control and amended treatments.

4.3.1.2 Nitrogen mineralization – immobilization

Figure 4.2. shows the effect of urea fertilization on time course of NH_4^+ and NO_3^- in soil. In soils without fertilizer N the dominant form of mineral N measured was NO_3^-, however the fertilized treatments showed high NH_4^+ levels during the first 21 days of incubation, indicating that in this soil the nitrification of NH_4^+ is rather slow (Appendix 4.2). In consequence the fertilized soils showed NH_4^+ levels significantly higher than the unfertilized soils until the end of the experiment. The amount of NO_3^--N in the control soil without fertilizer steadily increased along the incubation, in a similar way as the

amount of NO_3^--N of the fertilized control after the 28 days sampling. Considering that in most of the incubation the NH_4^+ represented only a small proportion of total mineral N in the following sections total mineral N content will be considered instead of NH_4^+ and NO_3^- separately.

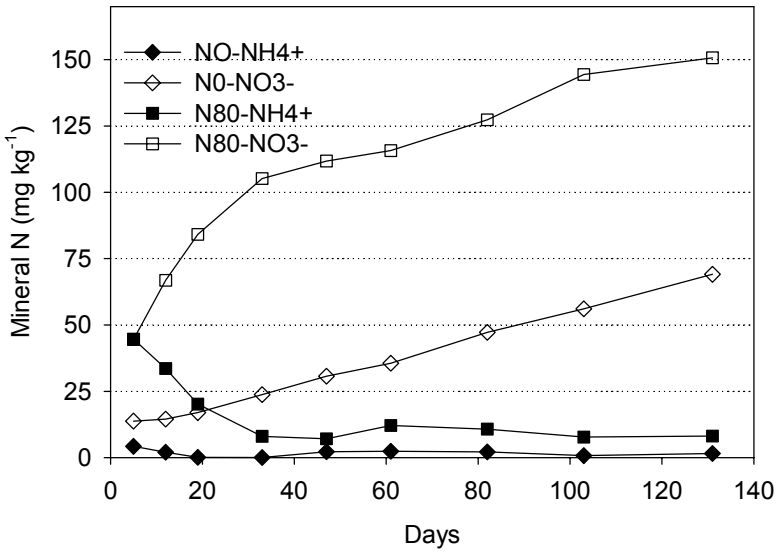

Figure 4.2. Time course of NO_3^--N and NH_4^+-N content in a sandy soil with (N80) and without (N0) N fertilization.

In figure 4.3 the time course of mineral N accumulation in the soil unamended (R0) and amended with crop residues of wheat (WS), sunflower (Sun) and maize (M) with and without N addition is presented. In soil without N addition there was an increase in mineral N content of the soil at the beginning of the experiment only in R0, all other soils showed similar or lower values by day 12 compared to the first sampling. While in the unamended control mineral N steadily increased, the soils with crop residues showed a decreasing trend during the first 18 days of incubation. At that time the immobilization process led to almost complete depletion of the soil mineral N and only after 47 days of incubation the mineral N started to increase.

The effect of the crop residues on soil mineral N content was different with and without N addition. In unfertilized soils there were not differences among residues in mineral N levels during the first 33 days of incubation (Appendix 4.4). Unfertilized soils amended with WS and Sun showed significantly larger amounts of mineral N than M thereafter. Even though N0-WS presented higher N levels than N0-Sun differences were not significant. In fertilized soils differences among crop residues were clear from day 12,

with a similar trend: N80-WS and N80-Sun presented significantly higher mineral N levels than N80M. There were also significant differences between N80-WS and N80Sun by days 47; 61 and 82, when WS amended soils presented higher amounts of mineral N. At days 47 and 82 treatment N80-WS did not significantly differ from the fertilized unamended soil (N80-R0).

In fertilized soils mineral N accumulation followed an increasing trend from day 12 onwards in all treatments including those with crop residues (Fig. 4.3b). The amounts of mineral N in fertilized soils exceeded those of unfertilized soils in all sampling times, being the difference between the unamended soils with and without N application 74 mg kg^{-1} at the first sampling (5 days), which represented a 92.5 % of the added N.

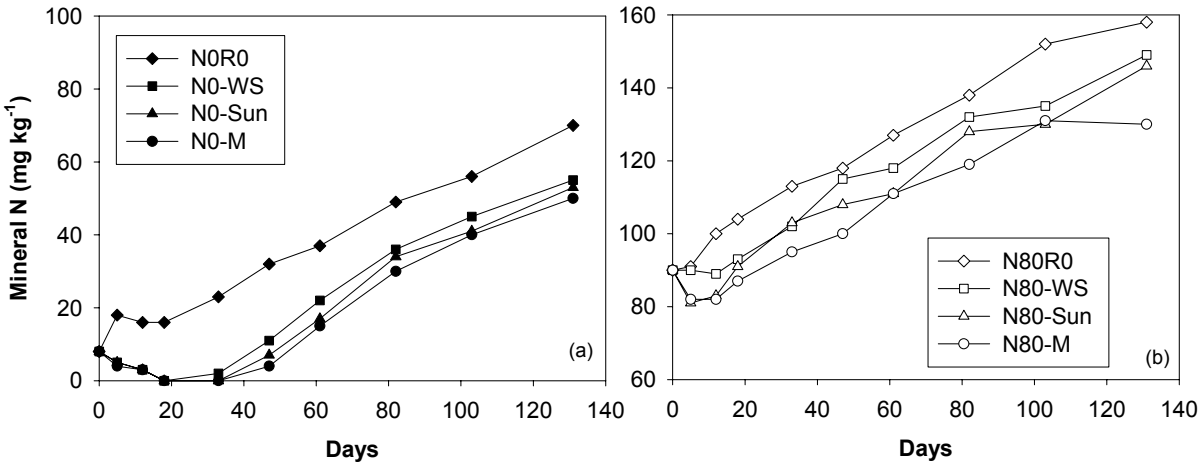

Figure 4.3. Time course of mineral N accumulation in a sandy soil unamended (R0) and amended with crop residues of wheat (WS), sunflower (Sun) and maize (M) (a) without and (b) with N fertilization. (Note the different scale in the y-axis).

The statistical analysis showed significant effects of both fertilizer N and crop residues on soil mineral N content at all sampling times (Table 4.3). The effect of the amendments on the mineralization-immobilization process was influenced by N addition, indicated by the significant interaction between the effect of crop residues and fertilizer N at 18; 33; 47 and 61 days samplings.

Table 4.3. Results of the ANOVA for the effect of N and crop residue addition on soil mineral N content and the interaction between both factors as well as the results from the analysis of orthogonal contrasts for fertilized (+N) and unfertilized (-N) treatments at each sampling date. * indicate P<0.05.

Day	Effect N	Effect Residue	Interaction	Contrast M vs. WS and Sun (-N)	Contrast M vs. WS and Sun (+N)
5	*	NS	NS	NS	NS
12	*	*	NS	NS	NS
18	*	*	*	NS	*
33	*	*	*	NS	*
47	*	*	*	*	*
61	*	*	*	*	NS
82	*	*	NS	*	*
103	*	*	NS	NS	NS
131	*	*	NS	NS	*

The calculated net N immobilization (difference between crop residues amended soils and controls, either for fertilized or not fertilized treatments) is presented in Fig. 4.4. In the unfertilized soils differences in N immobilization among crops could be observed only after 47 days of incubation, with larger N amounts immobilized in the soil with maize residues followed by sunflower and wheat residues. In fertilized treatments M showed higher net N immobilization than the others at most sampling times, with a trend of lower amounts of N immobilized in WS than in Sun.

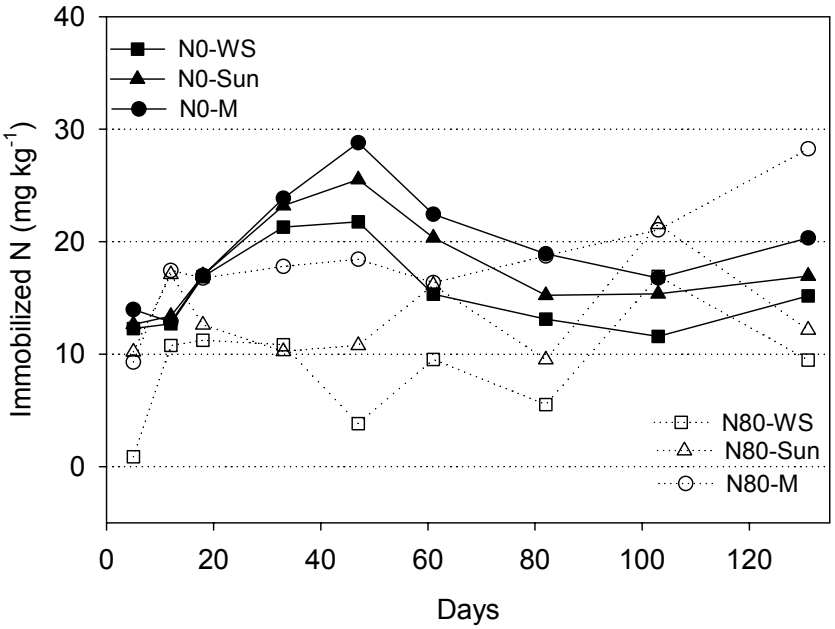

Figure 4.4. Time course of net N mineralization in a soil amended with wheat straw (WS), sunflower (Sun) and maize (M) with (N80) and without N fertilization (N0).

The estimation of daily mineralization rates (mg of N kg^{-1} soil day^{-1}) was made through linear regression analysis of soil mineral N content, from day 47 to the end of the incubation (Table 4.4). The starting point of the analysis was chosen considering that, as shown in Fig. 4.3, the immobilization effect was apparently completed by day 47 in unfertilized soils, The comparison of the slope of the regression lines as estimation of net N mineralization rates is presented in Table 4.5.

Table 4.4. Linear regression of mineral N content on days of incubation. The regressions fitted are: Mineral N (mg kg^{-1}) = b + a x, being x days of incubation. The slope of the regression line corresponds to the mineralization rate in mg of N kg^{-1} soil day^{-1}.* indicate P<0.05.

Treatment	Intercept (b)	Mineralization rate (a)	R^2
N0- R0	11.3*	0.46*	0.99
N0-WS	-10.0*	0.52*	0.97
N0-Sun	-15.5*	0.55*	0.97
N0-M	-18.3*	0.55*	0.97
N80-R0	97.5 *	0.49 *	0.97
N80-WS	97.2*	0.40*	0.81
N80-Sun	87.2*	0.46*	0.92
N80-M	87.6*	0.37*	0.88

Table 4.5. Probability of common slope of the regression analysis of mineral N content on days of incubation. Each test compared the slope of two regression lines. Levels of probability lower than 0.05 indicate significant differences in slope.

Treatment	N0-WS	N0-Sun	N0-M	N80-R0	N80-WS	N80-Sun	N80-M
N 0-R0	0.104	0.037	0.008	0.409	0.350	0.650	0.147
N 0-WS		0.599	0.426	0.484	0.005	0.316	0.001
N 0-Sun			0.549	0.237	0.024	0.144	0.005
N 0-M				0.138	0.016	0.078	0.003
N 80-R0					0.152	0.751	0.045
N 80-WS						0.236	0.756
N 80-Sun							0.097

In crop residues amended soils the fertilized treatments showed lower mineralization rates than unfertilized soils, while in the control soils N0R0 and N80-R0 the opposite trend was observed. In the mineralization rates of the unamended control soils however not significantly differences between N0 y N80 were found (0.46 and 0.49 mg N kg^{-1} soil day^{-1} for control soils with and without N addition respectively).

4.3.2 EXPERIMENT 2

In this experiment plant materials, covering a wide range of chemical characteristics were compared in order to find relationships between these characteristics and

decomposition patterns. With this aim intensive sampling of the soils amended with the crop residues, with posterior mineral N determination, as well as frequent CO_2-C measurements were performed during 80 days of incubation.

4.3.2.1 Plant material composition

The plant materials tested in experiment 2 showed a wide range of N, cellulose, hemicellulose, lignin, soluble C and polyphenol contents (Table 4.1). The highest N concentrations corresponded to the three clover plant parts, followed by Soy-L, Sun-L and Sun-S and Soy-S, while M presented the lowest N concentration. In terms of cellulose content C-y, Sun-L and Sun-S and Soy-L presented the lower values while Soy-S and M the highest; M also presented markedly higher hemicellulose levels than the rest of plant materials. In contrast M, C-y and Sun-L showed the lowest lignin content, while Soy-S presented the highest, followed by Soy-L, Sun-S and C-r. The highest amounts of polyphenol were observed in Soy-L, with important amounts in C-r and the lowest in Soy-S and M. The soluble C was highest in Sun-S, followed by Soy-L and C-m, while M presented the lowest levels.

The clover shoots presented differences in composition attributable to plant age, being mature shoot richer in cellulose and lignin than young shoots, while the N content of the younger shoots was higher. Young clover roots showed high lignin content but a lower polyphenol content than clover shoots while the N content was lower than shoots. There were important differences in leaves and stems in both sunflower and soybean, with leaves presenting higher polyphenol and N concentration and lower lignin content than stems. There was not a clear trend in soluble C between leaves and stems.

Correlation matrix of the chemical components of the tested plant materials is presented in Table 4.6. Most of the components were not significantly correlated. Only the positive correlation between cellulose and hemicellulose and the negative correlation between cellulose and soluble C reached significant levels.

Table 4.6. Correlation between chemical characteristics of crop residues. In parenthesis probability levels

	Total N	Lignin	Cellulose	Hemi-cellulose	Soluble Polyphenol	Soluble C
Total C	-0.31(0.45)	0.69 (0.06)	0.42 (0.29)	0.09 (0.82)	0.08 (0.84)	-0.26 (0.53)
Total N		-0.30 (0.47)	-0.55 (0.15)	-0.38 (0.34)	0.43 (0.29)	0.24 (0.55)
Lignin			0.41 (0.30)	-0.11 (0.80)	0.25 (0.53)	0.18 (0.66)
Cellulose				0.74 (0.04)	-0.63 (0.09)	-0.70 (0.05)
Hemi-cellulose					-0.30 (0.46)	-0.65 (0.08)
Soluble Polyphenol						0.38 (0.65)

4.3.2.2 Carbon mineralization

Figure 4.5 shows the calculated cumulative evolved CO_2–C from the soils amended with different plant materials, as proportion of the added C. These results represent the difference between cumulative CO_2–C amounts produced in soils with plant material and those of the control unamended soil, as percentage of the added C. The crop residue amended soils released significantly more CO_2–C than control from the first day of sampling onwards (Appendix 4.5). Rates of CO_2–C evolution from the control were similar to those of the amended soils only at the last week of incubation (nearly 100 days).

There was a declining trend in the CO_2–C evolution rate along the studied period in all treatments, especially in the amended soils (Appendix 4.5). This trend was reflected in the pattern of accumulated C, with a sharp increasing trend in the first 14 days of incubation followed by a slower increase thereafter (Fig. 4.5).

Respiration rates of the soils with different amendments showed differences since the beginning of the incubation, being the amounts of C evolved from Sun-S amended soils significantly higher than any other treatment in the first two days of incubation (Appendix 4.5). At this time respiration rates of the clover-amended soils (C-y, C-m and C-r) were also significantly higher than those of Sun-L, soybean (leaves and stem) and M, being M significantly lower than any other amendment. In the second week C-r showed the highest respiration rates, followed by the C-y and C-m and Sun-S. By day 21 soils amended with maize residues presented significantly higher respiration rates than the other treatments, followed by Soy-S. In consequence the mineralization pattern of those two treatments showed a different shape, with an increasing trend at the middle of the incubation period, when the other treatments presented much flatter C accumulation

patterns. In terms of total C evolved, on the other hand differences during the first 14 days of incubation were decisive, and M and Soy-S could not reach the levels of Sun-S and egyptian clover parts. The treatments that incorporate leaves (Sun-L and Soy-L) presented rather low cumulative C, being Sun-L higher than Soy-L, due to significantly higher respiration rates in the second week.

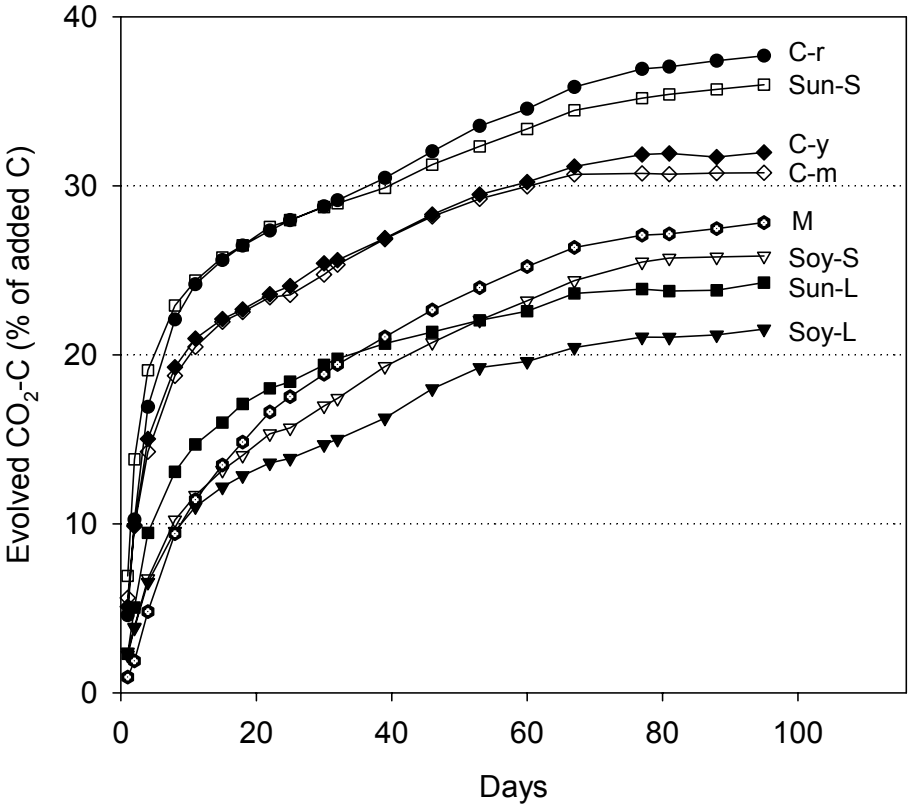

Figure 4.5. Time course of cumulative CO_2–C, as a percentage of the added C, in a sandy soil amended with egyptian clover (young and mature plants C-y and Cm, and roots, C-r), sunflower leaves (Sun-L) and stems (Sun-L), soybean leaves (Soy-L) and stems (Soy-S) and maize (M). The cumulative CO_2–C for each crop is the difference between the respiration from crop residue amended soil and unamended soil.

The different trends in respiration rates along the incubation are summarized in Table 4.7 where the respiration rates of the different treatments were averaged in time periods. It can be observed that the egyptian clover and sunflower residues presented higher respiration rates in the initial period, being the following CO_2–C amounts comparatively small. For soybean and maize residues a more balanced pattern can be observed, although the total amount of respired CO_2 was lower.

Table 4.7. Respiration rates (average per period) of egyptian clover (young and mature plants C-y, Cm, and roots, C-r), sunflower leaves (Sun-L) and stems (Sun-S), soybean leaves (Soy-L) and stems (Soy-S) and maize (M). The evolved CO_2–C for each crop is the difference between the respiration from residue amended soil and unamended soil.

	1 day	2-4 days	5-15 days	16-30 days	31-46 days	47-60 days	60-77 days
				CO_2-C mg kg^{-1} d^{-1}			
C-y	48.9	35.3	6.1	2.0	1.5	1.3	1.0
C-m	54.0	30.9	6.6	1.7	2.3	1.2	0.5
C-r	43.9	42.9	7.5	2.1	1.9	1.7	1.4
Sun-l	22.2	23.7	5.6	2.2	1.3	0.8	0.8
Sun-S	63.3	43.6	5.5	1.8	1.3	1.4	1.1
Soy-L	24.5	16.1	5.6	1.8	2.1	1.3	1.0
Soy-s	27.4	16.6	6.6	2.8	2.6	2.0	1.6
M	9.0	11.6	7.5	3.5	2.4	1.8	1.1

In order to establish relationships between the amounts of evolved CO_2-C in different stages of decomposition and chemical characteristics of the studied plant materials, simple and “step wise” regression analyses were performed. The results from these analyses are presented in Fig. 4.6 and Table 4.8.

Table 4.8. Regression of respiration rates and cumulative evolved CO_2-C on chemical characteristics of the crop residues. The “step wise” procedure estimates the contribution of each characteristic to the R^2 of the multiple regression.

	Respiration rate			Cumulative CO_2-C		
Day	Characteristic	Coefficient	Contribution to R^2	Characteristic	Coefficient	Contribution to R^2
1	Soluble C	0.43	0.60	Soluble C	0.43	0.60
	Polyphenol	-0.40	0.16	Polyphenol	-0.40	0.16
	C:N	-2.99	0.12	C:N	-2.99	0.12
8	No significant			Soluble C	1.11	0.39
				Polyphenol	-1.56	0.24
				C:N	-1.25	0.22
				Hemicellulose	0.12	0.03
				Lignin	-0.21	0.02
22	Cellulose	0.0018	0.85	Soluble C	0.92	0.34
	C:N	0.07	0.15	Polyphenol	-1.54	0.31
	Soluble C	-0.005	0.02	C:N	-1.27	0.23
				Hemicellulose	0.19	0.06
60	Polyphenol	-0.0097	0.86	Polyphenol	-1.77	0.32
	Cellulose	0.0024	0.10	C:N	-1.36	0.41
	Soluble C	0.0029	0.02	Soluble C	0.74	0.12
				Hemicellulose	0.32	0.11

Table 4.8 shows that different chemical characteristics of the plants influenced respiration rates and cumulative CO_2-C amounts. At the beginning of the incubation the first characteristic to explain differences among respiration rates was soluble C, while cellulose and hemicellulose reach the significance level in the following period. Conversely polyphenol content was negatively related to respiration rates. A positive

effect of N concentration (expressed as negative coefficient of C:N ratio) and negative effect of lignin content were also observed, also their influence was clearly lower than the influence of soluble C, cellulose en polyphenol.

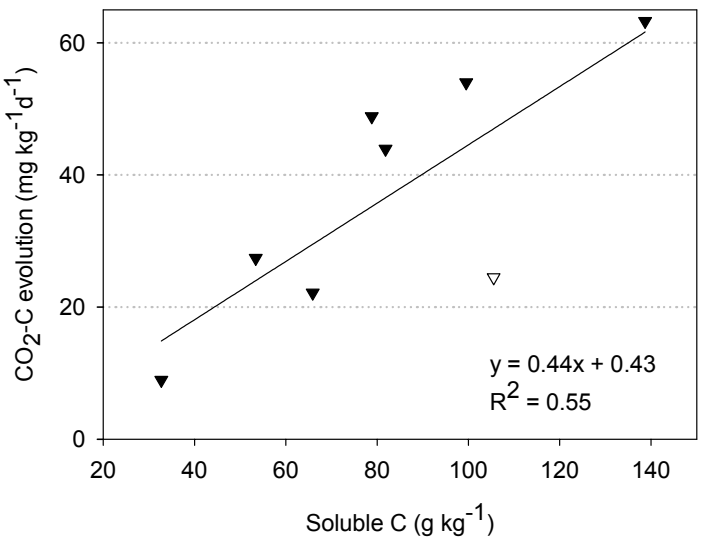

Figure 4.6. Relationship between CO_2–C evolution rate in the first day of incubation and soluble C of the plant materials.

Figure 4.6 shows the dependence of the initial respiration rates on soluble C content, the outline (white triangle) correspond to Soy-L, when this material was not included in the regression the determination coefficient increased considerably (R^2=0.86).

4.3.2.3 Nitrogen mineralization - immobilization

In this experiment the soil was pre-incubated in order to obtain a natural high mineral N content, without fertilizer addition. The aim of this procedure was to avoid any interference of N fertilizer on soil biomass activity, as occurred in the studies presented in chapter 3 and experiment 1 in this chapter.

In Fig. 4.7 the net mineralized N in the soils with different plant materials is presented. Net mineralized N was calculated as the difference between mineral N content of the soil with residue minus the mineral N content of the control at each sampling time. The mineral N content of the soil at the beginning of the incubation was 70.4 mg kg^{-1} (NO_3^--N 67.5 and NH_4^+-N 2.9 mg kg^{-1}). The unamended control showed a steady increase in mineral N content, being the mineralization rate 0.39 mg kg^{-1} soil day^{-1}.

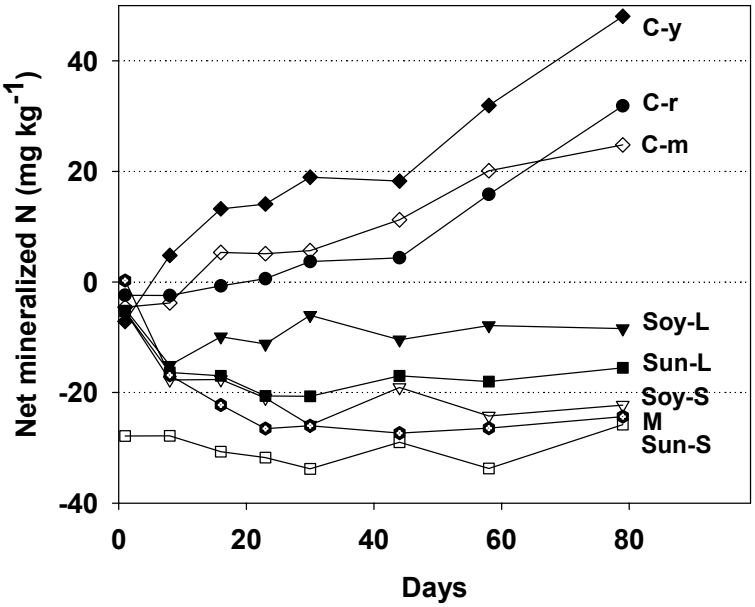

Figure 4.7. Time course of net N mineralization in a soil amended with egyptian clover (young and mature plants C-y and C-m, and roots, C-r), sunflower leaves (Sun-L) and stems (Sun-S), soybean leaves (Soy-L) and stems (Soy-S) and maize (M). Negative values indicate net N immobilization. Net mineralized N was calculated as the difference between mineral N content of the amended soil and the control at each sampling time.

By the second day of incubation all treatments showed some extent of N immobilization (Fig. 4.7), being the amount of N immobilized in the soil with Sun-S clearly higher than the other treatments (Appendix 4.6). In general terms the soils amended with clover residues presented net N mineralization, although this was not obvious in the first two samplings. By day 8 only the C-y promoted net N mineralization, while C-m and C-r showed increased net N mineralization from day 12 onwards. All other plant residues (sunflower, soybean and maize) presented net N immobilization during the 79 days studied, being higher in stems than in leaves in both sunflower and soybeans. The addition of M produced net N immobilization, but this effect was hardly perceptible at the first sampling and significant from day 8 onwards. Soy-S and Sun-L showed in a lesser extent similar immobilization pattern as M, with increasing immobilization in the first 16days of incubation. There was a different trend in net mineralization and net immobilization, while the amounts of mineralized N increased along the incubation, the amounts of immobilized N tended to remain constant from 30 days onwards. The results from the analysis of variance comparing the Mineral N amounts in the soils amended with different plant materials are presented in Appendix 4.6.

In order to establish relationships between the estimated amounts of net immobilized and mineralized N after 79 days of incubation and chemical characteristics of the studied plant materials, regression analyses were performed. Results from some of the most commonly mentioned relationships are presented in Fig. 4.8. No linear relationship was found between C:N ratio and the calculated amount of net mineralized N (Fig. 4.8a), while a significant linear relationship was found with the proportion of added N (Fig. 4.8b). The best fit for the relationship between N concentration and the amount of net mineralized N corresponded to a polynomial equation (Fig. 4.8c).

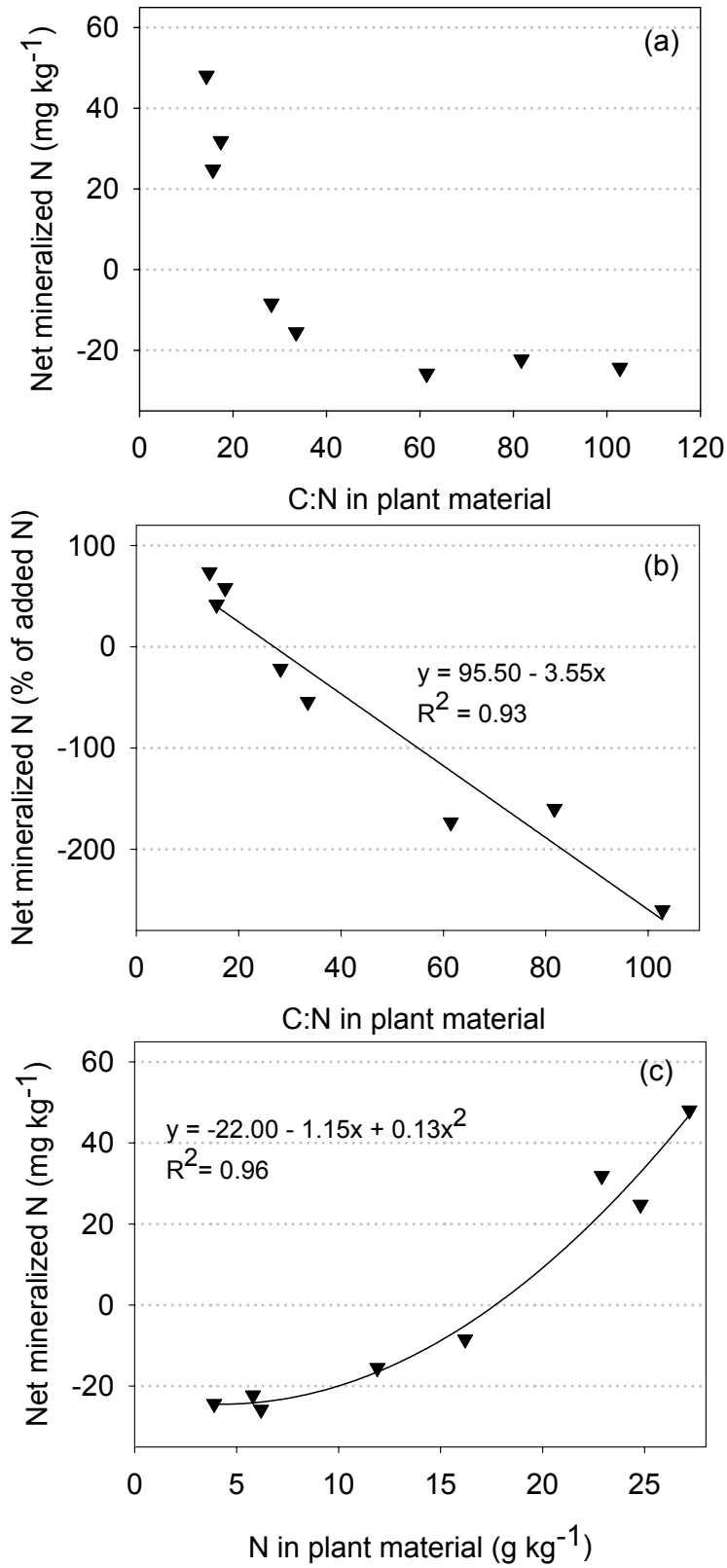

Figure 4.8. Relationship between (a) the net N mineralized in 79 days of incubation and C:N ratio of the plant material (b) relationship between the percentage of added N mineralized and C:N ratio of the plant material and (c) the net N mineralized in 79 days of incubation and N concentration of plant material.

The relationship between the net mineralized – immobilized N at the end of the incubation and other proposed indexes are presented in Fig. 4.9. These indexes considered lignin and polyphenol content of the plant residues, which are regarded as detrimental to microbial activity, and in consequence, negatively related to net N mineralization. No close relationships between those indexes and net N mineralization in 79 days of incubation were found.

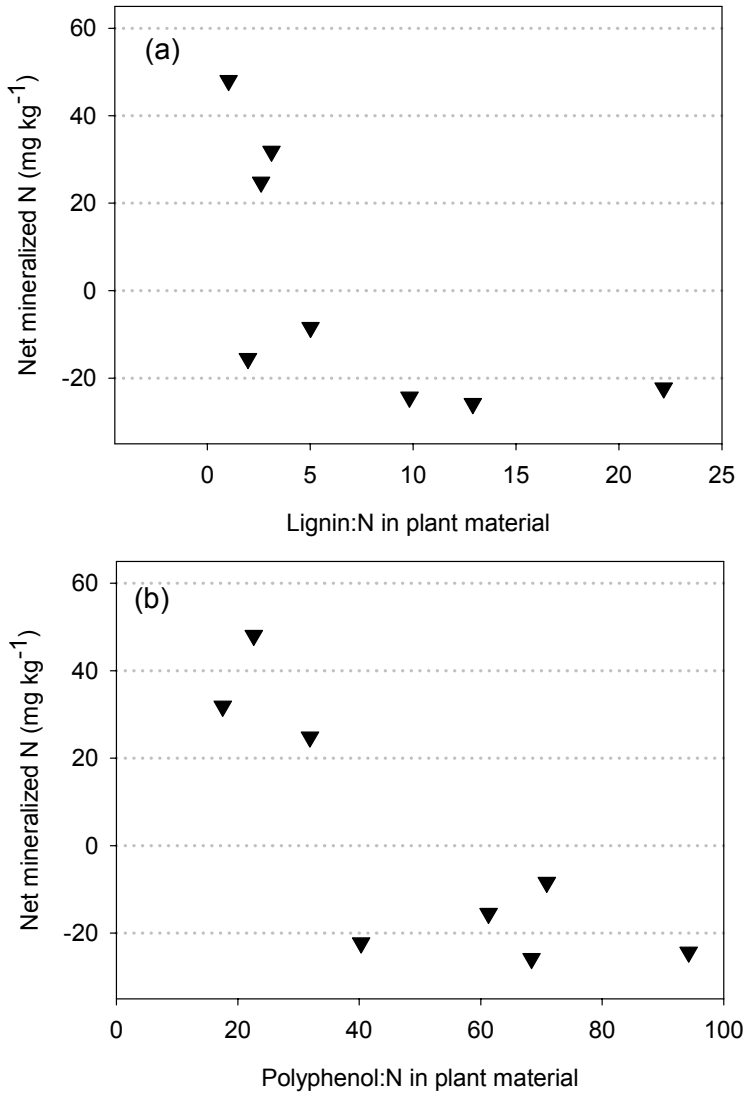

Figure 4.9. Relationship between the net N mineralized in 79 days of incubation and (a) lignin:N and (b) polyphenol:N ratios of the plant material

Results from the "step wise" regression of different crop characteristics on net mineralized N in different stages of the incubation are presented in Table 4.9. It is possible to observe that for all periods the most important characteristic was C:N ratio, being also important the soluble C content at the beginning of the incubation, while

hemicelluse and polyphenol content were related to net N mineralization in the following periods.

Table 4.9. Regression of net mineralized N on chemical characteristics of the crop residues. The "step wise" procedure estimates the contribution of each characteristic to the R^2 of the multiple regression.

Net mineralized N			
Day	Characteristic	Coefficient	Contribution to R^2
1	C:N	-0.30	0.44
	Soluble C	-0.20	0.30
16	C:N	-0.51	0.58
	Hemicellulose	0.96	0.19
79	C:N	-1.28	0.61
	Hemicellulose	1.94	0.15
	Polyphenol	-19.75	0.15

4.4 DISCUSSION

4.4.1 Carbon decomposition patterns of plant materials

In experiment 1 the evolution of cumulative CO_2–C amounts (Fig. 4.1) were lower than those from experiment 1 in chapter 3, which presented comparable treatments. Probably differences were due to the lower organic C content of the sandy soil. In accordance with the previous experiment, respiration rates decreased along the studied period, especially in crop residues amended soils (Appendix 4.1).

Differential effects of the crop residues however were clear only in the fertilized soils (Fig. 4.1 a and b). This result confirms that the decomposition kinetics of high C:N crop residues was strongly influenced by the N availability, and that the differences due to crop residue composition were clear only when N availability was non-limiting (Trinsoutrot et al. 2000). Considering this result differences in respiration rates among crops will be discussed only for fertilized treatments.

Maize residues promoted higher biomass activity than WS and Sun residues. The maize residue was especially rich in hemicellulose, less resistant than cellulose to decomposition, which, together with the low polyphenol and lignin content, was probably responsible for a greater lability of maize to decomposition. Negative effects of lignin and polyphenol on residues decomposition have been extensively reported (Baldwin et

al., 1983; Fox et al., 1983; Van Lauwe et al. 1996; Millar and Baggs, 2004) and will be discussed in more detail, in experiment 2, which present a wider range of lignin and polyphenol contents in the studied materials than experiment 1. There was an early positive effect of Sun on microbial activity, on the other hand, which was short lived, being the respiration rates of the soils with Sun from day 12 the lowest of the amended soils. This effect can be related to the relatively high soluble C content of Sun compared to the other materials. Reinertsen et al (1984) also attributed initial differences in respiration rates to soluble C availability, when N is non-limiting. The following behavior, with very low respiration rates, can be explained by the higher lignin and polyphenol contents of sunflower, which might have prevented microbial attack after the soluble C source was depleted.

In coincidence with the results from experiment 1 in the previous chapter there was a negative effect of urea addition on respiration rates, being those from the fertilized treatments significantly lower than the correspondent unfertilized treatment from day 12 to 61 (fig 4.1, Table 4.2). Negative effects of N addition on respiration rates have been reported by Kowalenko et al., (1978); Fog, (1988); Bremer et al. (1991) and Green et al., (1995). In contradiction with the results of experiment 2 in Chapter 2, the negative effect was more important in crop residues amended soils that in the control soil. The sandy soil utilized in the present experiment is likely to have influenced in this result, since it offers a lower buffer to pH and saline content changes than the soil utilized in the experiments of Chapter 2. In consequence the negative effect of N addition on microbial activity was probably enhanced. According to Bremer et al. (1991), on the other hand, one of the effects of N addition is the increase in the production of recalcitrant compounds, which can also explain the lower decomposition rates of fertilized soils amended with crop residues. In coincidence Fog, 1988 in her review of the effects of high N availability on decomposition, mentions the positive effect of N on cellulose decomposition, in contrast with the negative effect of lignin. This fact can explain the relatively low effect of N addition on hemicellulose rich M in the present study compared to the other materials, especially Sun (fig. 4.1).

In experiment 2 there were differences among plant materials in decomposition patterns along the incubation (figs 4.5 and 4.6). In the first 7 days of incubation the highest respiration rates corresponded to the Sun-S soils followed by the three clover residues.

In contrast M, Soy-S, Soy-L and Sun-L showed a very slow decomposition at this early incubation period. In the following period Sun-S decomposition was markedly slower and M faster, while clover parts showed a smoother decline in respiration rates. In the second month of incubation the highest amounts of CO_2 evolved from Soy-S, while differences disappeared at the end of the incubation. Bremer et al, (1991) comparing decomposition patterns of lentil green manure, lentil straw and wheat straw reported similar patterns to some of the plant materials of the present study. Lentil green manure, which presented the highest soluble C and N contents showed a very fast decomposition, but the respiration rates dropped also rapidly, being the lowest in 14 days of incubation (decomposition pattern comparable to Sun-S). Respiration rates of wheat straw resembled those of maize and Soy-s in the present study, with initial low respiration rates, which in contrast did not suffer a sharp decrease during the 90 days of incubation.

The proportion of evolved CO_2-C of the different materials at the end of the incubation showed a relatively wide range, from 22% in Soy-L to 38% in C-r, which can be calculated as 33% to 57% of C decomposed, if a third of the respired C is estimated to be assimilated by the soil microorganisms. In agreement Ajwa and Tabatabai, (1994) reported two fold CO_2-C from decomposed alfalfa respect to maize in 30 days of incubation. These results indicate that an important proportion of the materials did not suffer decomposition during the incubation, probably consisting in recalcitrant compounds, but also that this proportion is different in the different materials.

In the following sections the influence of the chemical composition of the different materials on those decomposition patterns will be examined.

Respiration rates were strongly related to soluble C content in the first period of incubation (Fig. 4.6). When multiple “step wise” regression tests between plant components and accumulation rates in the first 7 days of incubation were performed, in general the first variable to explain decomposition rates was soluble C, with positive effect, followed by polyphenol content with minus sign (Table 4.8). This effect can also be observed in the linear regression presented in Fig. 4.8, since the outline represents soybean leaves, which presented very high polyphenol content. Removing Soy-L from the analysis the linear regression between soluble C and respiration rate in the first day

of incubation increased to r^2=0.86. The rapid use of soluble C as energy source for soil microorganisms has been reported by Reinertsen et al., (1984); Kelley and Stevenson, (1987) and Bremer et al. (1991). As decomposition progressed, however hemicellulose and cellulose became more important (Table 4.8), at this time soybean stems and maize, both fiber rich materials, presented the highest decomposition rates (Table 4.7).

In the last period of incubation the most important single variable to explain differences between treatments was polyphenol content (Table 4.8). However this result should not be taken as an indication of the negative effect of polyphenol only in the last stages of incubation, on the contrary, the negative effect of polyphenol on respiration rates was marked during most of the incubation. In agreement Millar and Baggs, (2004), studying agroforestry residues observed a strong suppression of CO_2 evolution from the residues with the highest extractable polyphenols, which was noticeable at early stages of decomposition. According to Kelley and Stevenson, (1987) who studied decomposition of phenolic compounds, part of their C is incorporated into the humic fraction, without being decomposed; hence with lower CO_2 evolution. It has also been postulated that polyphenols produce direct inhibition of microbial activity (Baldwin et al., 1983, Videla and Hood, 2001). On the other hand it should be recognized that when polyphenol content is assessed a wide range of compounds are grouped. As Huntjens et al., (1981) confirmed studying decomposition patterns of different phenolic compounds, there were differences in mineralization. It is possible that, when a variety of species are compared, differences in polyphenol composition are responsible for differences in their influence on mineralization patterns.

Surprisingly lignin had no important effect on respiration rates at any time. This behavior is opposed to the findings of Kirchmann and Berkqvist (1989), who reported a close relationship between non-lignin C and accumulated CO_2–C (r^2=0.92). In contrast Janzen and Kucey (1988), studying wheat, lentil and rape residues did not find a significant relationship between respired C and lignin content. In the present experiment Sun-S and C-r, with relatively high lignin content presented the highest CO_2–C evolution during the first 14 days and Soy-S, also with high lignin content was superior in the final period (Fig 4.5). Sunflower and soybean stems showed higher lignin content than leaves; hence it could be expected slower decomposition rates from these materials, which was not observed. Quemada and Cabrera (1995) comparing leaves and stems of

legumes found faster decomposition in leaves in the first period and higher CO_2 evolution in stems after 16 days of incubation. In the present study the high soluble C content of Sun-S seems to have been more influential than the detrimental effect of lignin, promoting microbial growth when added to the soils.

Differences between clover roots and shoots were to some extent also unexpected since roots presented significantly higher respiration rates than the same age shoots, from days 2 to 15 of incubation, when most of the CO_2 was evolved. Even though lignin and cellulose content of the roots was higher, polyphenol content was lower, which can explain the lower respiration rates of shoots. On the other hand according to Stott et al., (1982) decomposition of lignin increases in the presence of a readily available C source, which could have been provided by soluble C in Sun-S. Vanlauwe et al., (1996) compared decomposition patterns of roots and leafs of three agroforestry legume species and found contradictory results, with one of the three species studied with higher decomposition rates in roots, other with lower CO_2 evolution and similar in the third.

Differences between clover shoots of different age were not very marked, although young clover shoots showed lower lignin, polyphenols and cellulose content than mature plants. These results are contradictory to those reported by Kirchmann and Bergqvist, (1989) who, comparing white clover tops of different age, reported lower respiration rates in older plants, related to the higher lignin content.

Many authors have found close negative relationships between C:N ratio and C mineralization rates (Janzen and Kucey, 1987). Studying this relationship, Bremer et al., (1991) reported differences in decomposition patterns of wheat straw incubated in soil and in glass wool, while those of N rich lentil residues were similar, despite the incubation medium. They attributed the different behavior to the need for external N supply of the wheat straw, while lentil residues N content was sufficient, concluding that C:N ratio may limit decomposition velocity only when it is higher than 40. In this study, however, the relationship between C:N ratio and mineralized C at the beginning of the incubation was rather poor (Table 4.7). In opposition in the middle of the incubation period there was a significant positive relationship between respiration rates and C:N ratio, at this time respiration rates were also related to cellulose and hemicellulose

contents, which represented most of the C in plant material. The small effect of C:N ratio on respiration rates was probably consequence of the incubation conditions, since plant materials were finely ground and carefully mixed with the soil. These conditions are likely to make plant decomposition more independent from its own nutrient supply (for example N content) than in situations where large pieces of crop residues are decomposed. The cumulative CO_2-C at the end of the incubation, on the other hand showed a rather close relationship with C:N ratio, probably influenced by the high amount of CO_2-C evolved from clover residues.

4.4.2 Effect of plant material composition on net N mineralization

In both experiments the soil was moist ground and only a small amount of water was added; hence incubation conditions were not very far from the natural conditions. This could be the reason why an important mineralization flush did not occur in experiment 1 (Fig. 4.3), since this initial flush is mainly consequence of previous soil management (Seneviratne and Wild, 1985; Cabrera, 1993). The light soil texture can also be a cause of minimal disruption of structure during soil preparation for the experiment. In contrast the fine textured soil used in experiments in chapter 3, which needed a great amount of water to reach the desired water content, and suffered structure disturbance when ground, presented a flush of mineralization at the beginning of the experiment.

The crop residues studied in experiment 1 presented a very low N content (Table 4.1); hence the expected result from the decomposition process was net N immobilization. This N immobilization was very fast, being noticeable from the sampling after 5 days of incubation (Fig. 4.3). Even though in both fertilized and unfertilized soils there was a clear net N immobilization, in soils with crop residues without N addition, more time was required to reach the maximum immobilization than in fertilized soils, indicating that the mineralization process was limited by N availability. These results are in agreement with those of Mary et al. (1996). The slower pace of decomposition when N limited microbial activity becomes clear analyzing the time required for the amended treatments to reach the initial mineral N content, which was only 18 days for the fertilized soils and 47 days for unfertilized soils. There is an apparent contradiction however, since respiration rates from fertilized soils were lower than those from unfertilized soils, as well as the maximum amount of immobilized N. From these results and those of Chapter 2 it is

possible that the N addition led to a smaller microbial biomass pool, which was limited by factors other than C and N availability, while in unfertilized soils a larger microbial population was limited by N availability.

According to the literature the amount of soil mineral N immobilized during decomposition of crop residues depend in a large extent on C:N relationship (Vigil and Kissel, 1991;Jama and Nair, 1996; Janssen, 1996) In consequence, in this experiment it was expected to find a higher N immobilization with addition of cereal crop residues, which showed higher C:N ratio compared to sunflower. The results are somehow contradictory because the maize addition promoted higher immobilization, as expected, while the sunflower residue immobilized more N than wheat (C:N ratios of 53 and 70 respectively). Even though no significant differences were found between mineral N content of soils with WS and Sun, except for the 61 days sampling, the expected result was a less negative effect of Sun on soil mineral content. Moreover the NH_4^+-N levels of the Sun treatment in fertilized soils were significantly lower than WS during most of the incubation (Appendix 4.4), with differences ranging between 8 and 10 mg NH_4^+-N kg^{-1} in the first 21 days. This form of N is preferentially immobilized (Recous et al, 1988). It is possible that the more resistant structure of the wheat straw (higher cellulose and hemicellulose content) delayed its mineralization (and thus immobilization). In addition sunflower presented a higher soluble C content (37 and 71 g kg^{-1} for WS and Sun respectively), and this readily available C would have promoted rapid biomass growth, with the correspondent N immobilization. This positive effect of soluble C on N immobilization has been also observed by Reinertsen et al, (1984) studying wheat straw decomposition and will be more extensively examined for experiment 2. Respiration rates however do not completely support this hypothesis, although Sun presented slightly higher respiration rates than WS by day 5 of incubation, they decreased rapidly thereafter in fertilized soils, while were similar or higher than those of WS in unfertilized soils. On the other hand the fact that the first respiration rate estimation was made after 5 days of incubation, when an important amount of N was already immobilized, do not allow to rely on calculated cumulative CO_2-C as estimation of decomposed C at this early stages of decomposition.

Analyzing mineralization rates from day 47 (Table 4.4), it can be observed that in the unfertilized treatments, N mineralization rates of soils amended with crop residues were

higher than those of the unamended control. Conversely in fertilized treatments N mineralization rates of crop residues amended soils were lower than those of the unamended control. Since the amounts of immobilized N of unfertilized soils were larger than those of fertilized soils, these results seem to indicate that net mineralization was enhanced by previously immobilized N. In agreement the effect of previously immobilized N on N mineralization was studied with labeled N by Jensen, 1993, who reported faster turnover rate of the immobilized N compared to the whole organic N pool. A similar behavior with remineralization from the highly amended soil at the end of the incubation was also observed in experiment 1 from Chapter 3. On the other hand, Kelley and Stevenson, 1987 observed than newly immobilized N became less available to mineralization even after short incubation periods. However they also found differences in the fate of the immobilized N, depending on the type of C in the added substance, being the N immobilized by glucose and phenolic glycoside addition less likely to remain immobilized than the N immobilized after catechol addition. The remineralization pattern in the present study can be observed in Fig.3.4; interestingly while in fertilized soils the immobilized N tend to remain in organic form, in unfertilized soils a proportion is remineralized. The fact that the whole system in unfertilized soils is in a great extent determined by N shortage could have caused microbial biomass death with subsequent remineralization. Schnier et al., (1987) reported differences in remineralization in different soil types, in the present study different N availability conditions caused different remineralization patterns.

In experiment 2 there were a variety of materials with a wide range in all the measured characteristics (Table 4.1). Since the amount of mineralized and immobilized N during decomposition is usually related to the C:N ratio and N content of the plant materials this aspect was the first to consider in order to interpret N mineralization results in experiment 2 (Fig. 4.8). In 79 days of incubation, the young plants of clover presented the highest net N mineralization, in accordance with their lowest C:N ratio. Mature plants presented lower net mineralization than young plants, similar to those of young clover roots. Nevertheless when all materials are considered a clear relationship was not found between the amounts of net mineralized N in 79 days of incubation and C:N ratio of the plant material (Fig 4.8a). On the other hand a linear relationship between net mineralized N and C:N ratio could be adjusted when the high C:N materials (M, Sun-S and Soy-S) were removed from the analysis. In consequence the curve that relates net

mineralization with C:N relationship presents two different trends: a linear relationship with C:N below 40 and a flat trend at higher C:N ratios. The critical C:N calculated from the linear relationship was 25.3 (r^2=0.87), which compares with those of the bibliography (Trinsoutrot et. al, 2000). However the C:N ratio was not adequate to forecast the amount of immobilized N at C:N higher than 40, because similar amounts of N were immobilized in soils amended with crop residues whose C:N ratios ranged between 62 and 103. When the relationship between C:N ratio and the net mineralized N as a percentage of the N added in the residue was tested (fig 4.8b), there was a significant linear relationship between both estimations. In agreement the critical C:N ratio calculated from this relationship (26.9) was rather close to the one calculated from the linear portion of (fig 4.8a).

Plant N concentration, on the other hand, showed a polynomic relationship with mineralized N. From the relationship presented in Fig. 4.8c it is possible to calculate critical N content that determined the net N mineralization or net N immobilization produced in soil during decomposition of the plant material. The calculated critical N concentration was 17.7 g kg^{-1}, which is also very close to the critical N level of 1.73% reported by Frankenberger and Abdelmagid, (1985). Although the relationship was not linear, it improved the forecast capacity respect of C:N for plant materials with low N content.

Comparing the relationships discussed above, it is important to consider that the N concentrations of the low-N materials were rather similar (3.9; 6.2 and 5.8 g kg^{-1} for M, Sun-S and Soy-S respectively), in correspondence with the small differences found in the amounts of N immobilized in 79 days of incubation (24.4; 25.8 and 22.3 mg kg-1 for M, Sun-S and Soy-S respectively). In contrast the C:N ratios were highly sensitive to small changes in N concentration, since the C concentration was many folds larger than N concentration, resulting, as a consequence in huge differences in the C:N ratios between those materials. In the relationship from Fig. 4.9b an improvement respect of the formed was observed, but this was very much a similar effect, because since the amounts of added N were so small, they represented a tiny fraction of the immobilized N (immobilized N represented 260 % of the N added in maize shoot). Many studies present relationships between net mineralized N and C:N ratios for materials with C:N ratios lower than 50 (Frankenberger and Abdelmagid, 1985; Fox et al., 1990; Jama and

Nair, 1996, Kuo and Sainju, 1998); from the results of the present experiment it seems that it is not adequate to extrapolate those relationships to the range of very high C:N ratios, since linear relationships do not apply. On the contrary, for materials with high C:N ratios the amounts of immobilized N seem to be more closely related to the decomposition pace (estimated through CO_2 evolution) than to the N content of the added material. This aspect becomes clear considering the large amounts of N immobilized in soils amended with Sun-S at the beginning of the incubation, which presented also the highest respiration rates.

As discussed in the previous section the decomposition process is also affected by other components of the plant materials, especially lignin and polyphenol content, which have a retarding effect on crop residue decomposition. In consequence relationships for both of them with mineralized N have been developed and reported as more closely related to N mineralization than C:N or total N (Kirchmann and Bergqvist, 1989; Palm and Sanchez, 1991, Lehmann and Schroth,1993). In experiment 2 these relationships did not improve the forecast of N mineralization made by N content (Fig. 4.9), neither [lignin+polyphenol]:N, proposed by Fox et al., (1990). Considering that these materials, especially polyphenols, promote a slower decomposition process it is possible that they are related to N release in a way that for materials with similar N content, slower decomposition results in lower N release. In addition, Oglesby and Fownes (1992) suggest that polyphenols exert an specific nitrite bonding, preventing mineral N release. Many of the results in the literature about these relationships imply the comparison of plant materials with similar characteristics, for example Kirchmann and Bergqvist (1989) worked with white clover differing in plant parts and maturity while Fox et al., (1990) and Oglesby and Fownes (1992) with different legumes. When different plant types like cereals, legumes and sunflower are compared, as in this study, it is likely that plant composition factors, other than the tested, affect the mineralization patterns; hence making more difficult to find general trends. In agreement Constantinides and Fownes, (1994) studying decomposition of many different plants found that the relationship of mineralized N and these indexes improved when only fresh legumes were included in the correlation analysis.

The soluble C content of the plant material also influenced the immobilization process (Table 4.9), in agreement with Reinertsen et al, (1984). This component could explain

the fast N immobilization observed when the sunflower stems were incorporated, given their high soluble C content (139 g kg^{-1} soluble C). During the first month of incubation Sun-S promoted higher N immobilization respect to Soy-S and M (soluble C content 53 and 33 g kg^{-1} for Soy-s and M respectively), although Sun-s showed a lower C:N relationship than both of them (C:N ratio 61; 82 and 103 for Sun-S, Soy-S and M respectively). Differences between Sun-S and M became not significant after one month of incubation, indicating that the effect of soluble C on biomass growth, and consequently N immobilization, is important especially in recently incorporated plant residues (Knapp et al, 1983; Cheshire et al., 1999).

Comparing the patterns of net N mineralization and net N immobilization, differences were found; while N release steadily increased during the studied period, immobilized N tend to remain constant. These results indicate that N assimilation by the microbial biomass reached a steady state in a rather early period of incubation and did not continue to increase thereafter, probably due to the depletion of the readily available C forms. Under this hypothesis the subsequent slow decomposition provided energy for biomass maintenance, which explains why the N release continued as decomposition of the N rich materials proceeded.

4.5 SUMMARY AND CONCLUSIONS

From the comparison of decomposition patterns of crop residues and green manures it is possible to conclude that no single component was able to explain the differences observed. In addition in different periods along the incubation, different components strongly influenced decomposition. The likely substrates for the microbial biomass in each period were identified; while in the initial period soluble C played a decisive role, structural C compounds (cellulose and hemicellulose content) became more important in the following decomposition period. The phenolic compounds, in contrast, represented a negative influence in plant material decomposition along the whole incubation period.

Decomposition of plant residues of wheat, sunflower and maize were mainly influenced by N availability. Comparing respiration rates when N was in less than adequate supply no differences between species were noticeable until three months of incubation. A

similar pattern was observed in N immobilization, with differences in species being perceptible only when the N restriction was removed (either through fertilization or by soil organic matter mineralization).

Net N mineralization of the studied plant materials was strongly influenced by their N content, being the calculated critical level 17.7 mg N kg^{-1}. In consequence only the three clover plant parts promoted N release; all other materials showed decreases in mineral N content in comparison with the control soil. Mineralization patterns however were dependent from decomposition patterns. Nitrogen immobilization followed the CO_2 evolution trends with a very fast N immobilization for the high soluble C material, and a more steady increase in mineral N immobilization in the material with the lowest initial respiration rate.

5 EFFECT OF TEMPERATURE AND SOIL WATER CONTENT ON SOIL C AND N MINERALIZATION

5.1 INTRODUCTION

Mineralization of soil organic matter (SOM) largely depends on climatic conditions. The increase in temperature and soil moisture have a positive influence on the rate of mineralization, due to their effect on biomass growth and activity (Standford and Smith, 1972; Standford and Epstein, 1974; Haynes, 1986; Lochmann et al., 1989; MacDonald et al., 1995; Zaman and Chang, 2004).

At a given temperature the time course of soil organic N mineralization is generally represented by a first order kinetics equation (Richter et al.,1982). The evolution of N mineralization can be expressed by the exponential form proposed by Standford and Smith, (1972):

$$N_t=N_0\,[1-\exp(-kt)] \qquad (1)$$

Where:
N_t is the amount of N mineralized at time t,
N_0 is the potentially mineralizable N at t_0
k is a rate constant

However the results obtained in different studies are not always coincident with this mineralization model, in many cases the shape of the mineralization curve can be better represented by a linear equation of mineral N over time (Tabatabai and Al-Khafaji, 1980; Addiscott, 1983; Beck, 1983), by a power function (Broadbent, 1986, Marion and Black, 1987) or by a two consecutive reactions model, which consider two organic matter pools with different mineralization rates (Molina et al., 1980; Richter et al., 1982, Benbi and Richter, 2002).

The power function can be expressed as:

$$N_t=a\times t^b \qquad (2)$$

Where:

N_t is the amount of N mineralized at time t,
a and b parameters

It has been observed that the conditions of incubation strongly affect the results, and in consequence the model of the relationship (Nuske and Richter, 1981). Therefore factors like soil disturbance, type of organic matter, length of incubation and temperature are decisive in the shape of the mineralization curve obtained. Soil disturbance can promote a mineralization flush in the initial stages of incubation followed by a stabilized phase thereafter (Seneviratne and Wild, 1985; Cabrera and Kissel, 1988 a). The organic matter pool under decomposition determines whether there is a homogeneous substrate with a steady mineralization rate, or more than one pool that mineralize at different rates (Nuske and Richter, 1981; Dendooven et al., 1997). Also the size of the mineralizable pool can influence mineralization patterns, according to Mary et al., (1999) the curve follows zero order kinetics when a small fraction of the organic N is mineralized and first order kinetics when a larger fraction is mineralized. Length of incubation and temperature are influencing factors that can be associated. Low temperature incubations do not generally reach a maximum, except for very long incubation periods, while when soils are incubated at high temperatures an asymptotic curve is generally obtained (Marion and Black, 1987).

When mineralization rates are studied in a temperature range, it is possible to establish a model that express how temperature affects mineralization. An exponential increase in mineralization rate as temperature increases has been observed. The most commonly applied models for describing this relationship are Arrhenius equation and Q_{10}, although Ellert and Bettany., (1992) found a polynomial (quadratic) model as more appropriate.

Arrhenius equation can be expressed as:

$$k = A\, e^{(-B/T)} \qquad (3)$$

Where:

k is mineralization rate
T is absolute temperature (K)
A and B the parameters of the equation

The equation from which Q_{10} can be calculated is:

$$Q_{10} = (k_2/k_1)^{[10/(T2-T1)]} \quad (4)$$

Where:

k_2/lk_1 are mineralization rates at two different temperatures T_2 and T_1.

These functions are valid between a given range of temperatures, generally from 0 to 40ºC, because at extreme temperatures limitations have been observed. Negligible mineralization is expected below 0ºC, while at temperatures higher than 40ºC the mineralization rates tend to remain constant or even decrease (Ellert and Bettany, 1992 Recous et al., 2001). The explanation for this behavior is that at high temperatures enzymes may be deactivated or decomposed (Fang and Moncriff, 1998).

Q_{10} indicates the increase in mineralization rate produced by an increase of 10 ºC. In many studies Q_{10} values around 2 have been found (Standford et al., 1973) meaning that the mineralization rates doubles for each 10ºC increase. This relationship is consistent with the relationship found for many biological processes. Nevertheless it has been observed that the characteristics of the SOM influence the relationship. Standford, et al., (1973) calculated Q_{10} values around 2 studying a wide range of soils. The characteristics of soil organic matter are thought to influence Q_{10} since SOM originated under trees is more ligniferous, and hence more resistant to microbial attack, than SOM originated under grasses (Campbell et al, 1984). These authors analyzed the effect of climate on Q_{10} and found that soils from warm climates tended to show lower values, while higher values were observed in soils from cold climates. Agren and Bosatta, (2001) explained the differences between soils from cold and warm regions because in warm regions the SOM has a higher proportion of low quality substrates, hence more resistant to further decomposition, therefore when temperature increases mineralization rate increases less than in soils from cold regions. Campbell et al, (1984) concluded that it is encouraging that soils could be grouped by soil zones with respect to Q_{10}. This view of the dependence of mineralization on temperature will allow to extend the use of the Q_{10} found in a region to other soils of this region that were not previously tested.

Water availability plays a key role in microbial growth and in consequence determines SOM mineralization. In contrast to temperature, water availability depends not only on

climatic conditions but also on soil type and condition, since the water retention capacity of the soils depends on those characteristics. The soil water content can be expressed in different ways; the most commonly used are gravimetric soil water content, percentage of pores filled with water and soil water potential. Soil water potential is mainly composed by osmotic and matric potential (Papendick and Campbell, 1981). Water potential is strongly related to water availability for crops, since plants absorb water according to the water potential. In consequence water availability for crops is in general expressed in terms of water potential; in this way it is possible to compare soils in terms of water availability. Water availability for the microbial population, on the other hand has been regarded in a different way, since microorganisms are able to use water retained in pores where roots are unable to grow (Skopp et al. 1990). Goncalves and Carlyle (1994) recognize the advantage of the use of water potential for soil comparison, but point out that, when moisture is non-limiting, soil water content itself is important due to its influence in transport processes. In his review of several models that simulate soil moisture effects on N mineralization, Antonopoulos, (1999), reported relationships involving soil moisture contents as well as water tension in the different models.

The negative effect of soil drying on microbial biomass is mainly related to the increase in solute concentration. According to Kieft et al., (1986) the response to decreasing external water potential involves the accumulation of intracellular solutes in order to maintain turgor. On the other hand this adaptation is limited, and may lead to cell death when the soil is very dry. But soil water content not only affects cell osmotic relationships, its effect on microbial mobility is also important (Harris, 1988). The oxygen availability, frequently expressed as percentage of air filled pore space, has been also detected to influence the relationship between soil water content and microbial biomass activity, especially at high soil water contents (Skopp et al., 1990). In agreement Howard and Howard, (1993) comparing a number of soils found that, even though the maximum mineralization occurred with different water contents, when moisture was expressed as percentage of soil water holding capacity the maxima became more similar. From the reviewed literature it can be concluded that there are differences in the ability of soil microorganisms to adapt to changes in soil water potential (Harris, 1981; Kief et al., 1986).

In a classical study Standford and Epstein, (1974) studied the effect of soil water content on N mineralization in 9 different soils and found linear relationships between the net N mineralized and water percentage. This relationship was adequate for water potentials in the middle of the tested range, while the linear relationship did not apply at water potentials in the upper and in the lower range. Other authors reported a logarithmic relationship between mineralization rates and the water potential of the soils (Reichman et al., 1966, Orchard and Cook, 1983 and Rodrigo et al, 1997).

It has been found an enhancing effect of drying and rewetting on SOM mineralization (Orchard and Cook, 1983; Harris, 1988). It is important however to consider the experimental conditions studying the process. According to Jager and Bruins, (1975), the effect of drying was far lower for soils dried at 30ºC than at 85ºC, being the CO_2 evolution after rewetting soils dried at 30ºC similar to undried soils. Frier et al. (2002), found that the change in composition of microbial communities, due to drying and rewetting, was stronger in a forest soil, which in natural conditions was less likely to dry, than in a grass soil frequently subject to drying and rewetting cycles. Kieft et al., (1987) isolated the effect of rewetting from the effect of drying. They found that the soil biomass released 17 to 32% of its C following 2.8 and 6.9 MPa potential increases respectively in one soil, and from 27 to 58% after equal water potential changes in another soil. Differences between soils were attributed to soil water regimes, being the second soil less likely to suffer drying in natural conditions. The reviewed information in consequence suggests that this effect is less marked in soils that are frequently dried and rewetted.

Even though some studies showed an interaction between the effect of soil temperature and water content (Lochmann et al., 1989; Howard and Howard, 1993, Zaman and Chang, 2004) the effect of both factors in SOM mineralization models is usually represented by independent functions (Rodrigo et. al, 1997). Like the mentioned authors Zak et al., (1999), reported interaction between the effect of temperature and soil water content, being the differences between water contents (ranging from –0.01 to –1.85 MPa) larger at high temperature (25ºC) and minimal at 5ºC. They also found differences between CO_2 evolution patterns and those of N mineralization, being the interaction between the effects of temperature and moisture stronger in CO_2 evolution than in N mineralization. In opposition Fang and Moncrieff, (2001) did not observe

interaction between moisture and temperature effects, postulating that this interaction is important only at extreme soil water contents (dry and wet soils). In agreement a similar lack of interaction between soil moisture and temperature of incubation was reported by Kladivko and Keeney, (1987) working with soil moisture levels between –0.03 and -0.3 MPa incubated at 10 and 35ºC.

To test the hypothesis that SOM decomposition, especially related to net C and N mineralization, depends on temperature and soil water content, and that it is possible to quantify these relationships in agricultural soils of Uruguay, two separated experiments were established. In the first experiment the effect of temperature, and in the second experiment the effect of moisture in two contrasting soils were tested. Soil organic matter decomposition and N mineralization were evaluated through soil microbial respiration and net N mineralization respectively.

5.2 MATERIALS AND METHODS

5.2.1 Soils and treatments

The soils 1 and 3 correspond to the same site, but samples were taken at different times. The soils presented different textures, while soil 1 was clay textured, soils 2 and 4 were sandy soils. Characteristics of the soils are presented in Table 5.1.

Table 5.1. Characteristics of the soils of the study

Soil	Site	pH (H_2O)	organic C	total N
			($g\ kg^{-1}$)	
1 and 3 -Typic Argiudoll	Paysandu	5.7	29.1	1.9
2 - Arenic Hapudalf	Soriano	5.3	10.2	1.1
4 - Mollic Hapludalf	Rio Negro	5.1	9.3	0.9

In Experiment 1 soils 1 and 2 were incubated at different temperatures: 5; 12; 21; 31 and 40ºC.

In Experiment 2 the clay soil (soil 3) was incubated at different soil water contents, corresponding to the water retained at -0.01; -0.10; -0.14; -0.90; -1.00; 2.00 MPa for C mineralization evaluation and at -0.03; -0.12; -0.28 and 1.81 MPa for N mineralization evaluation. Soil 2 was incubated at 0.01; 0.03; -0.08; -0.28 and -0.67 MPa for both C

and N mineralization evaluation. In order to calculate the soil matric potentials a curve relating soil water potentials and soil water content was fitted for each soil. The potentials used to fit the curve were -0.01; -0.03; -0.10; -0.30; -1.00 and –1.5 MPa.

5.2.2 Incubation procedures

Incubation procedures were similar to those described in Chapter 2. Briefly in experiment 1 fresh soil was passed through a 5 mm sieve, and roots and stubble were removed. The portion of soil, corresponding to each temperature treatment (8 kg), was extended in a thin layer and deionised water was spread and mixed. The amount of water added was aimed to reach 0.28 g g^{-1} of soil, equivalent to 80% of the soil water potential at -0.01 MPa. The water content was checked twice a week for 31 and 40ºC and once a week for 5; 12 and 21ºC.

The incubation procedure in experiment 2 was similar. Briefly fresh soil was passed through a 5 mm sieve, water content was determined (oven dry at 105ºC for 24 hours) and the water needed to reach the desired water contents was sprayed over a fine soil layer. The soils from each treatment were mixed and kept at room temperature overnight. Three pots were filled with the soil from each water content (equivalent to 1.5 kg of dry soil) and incubated at 21ºC for N mineralization and three samples were incubated for respiration rate measurement. Water content of the soil was checked by weight twice a week for N evaluation treatments and at each CO_2–C determination and water added when required, through drops on the soil surface.

5.2.3 Experiment management and sampling

In Experiment 1 soil 1 was incubated for 175 days (25 weeks) and soil 3 for 125 days (18 weeks) for N mineralization evaluation at 5; 12; 21; 31 and 40ºC. For C mineralization soil 1 was incubated for 265 days (38 weeks) at high temperatures (31ºC and 40ºC) and for 366 days (52 weeks) at low temperatures (5ºC and 12ºC) while soil 2 was incubated for 210 days (30 weeks) and 322 days (46 weeks) for high and low temperatures respectively. Unfortunately there was a failure in the temperature control for the 21ºC treatment, so incubations at 21ºC for CO_2–C evolution were shorter than the rest (171 days in soil 1 and 121 days in soil 3).

For mineral N analysis a soil sample was collected from each pot, once a week from the high temperature treatments and every two weeks for the low temperature treatments. A portion of soil was taken from different parts of the pot and soil was gently mixed again. At each sampling date the water content of the samples (oven dry at 105ºC) were determined in order to correct possible bias from the target. For respiration rates (CO_2–C evolution) sub samples were taken only at the beginning of the incubation and CO_2–C evolution was measured twice a week for the first 170 days and weekly thereafter without mixing the soil during the whole experiment.

In Experiment 2 soil 2 was incubated for 63 and 49 days for C and N mineralization respectively (for N mineralization a 2 weeks pre-incubation period was not evaluated) and soil 4 was incubated for 72 days. A soil sample was collected from each pot, once a week for mineral N analysis. A portion of soil was taken from different parts of the pot and soil was gently mixed again. At each sampling date the water content of the samples (oven dry at 105ºC) were determined in order to correct possible bias from the target. For respiration rates (CO_2–C evolution) sub samples were taken only at the beginning of the incubation and CO_2–C evolution was measured twice a week for the first 30 days and weekly thereafter, but no mixing of the soil was done as in the N mineralization study.

5.2.4 Chemical analysis

Mineral N determinations and soil respiration in experiments 1 and 2 were performed as described in chapter 3. The evolved CO_2–C was continuously measured.

5.2.5 Statistical analysis

In both experiments analysis of variance for mineral N and CO_2–C evolved were performed following a completely randomized design with three replications. When data failed the homogeneous variance test, a logarithmic transformation was made. Differences among treatment effects were tested through lsmeans procedure (pdiff) (Appendix 5.1 to 5.12) Time courses of net N and C mineralization as well as the relationships between soil temperature and moisture respect of rates N mineralization and respiration and were examined using linear and non-linear regressions. The statistical analyses were carried out using the GLM and REG procedures (SAS Institute,

Inc. 1985). For curve fitting the Sigmastat statistical program was used (Sigmastat for Windows. V 2.03. Access Softek Inc.).

5.3 RESULTS

5.3.1 Temperature and C mineralization

The cumulative CO_2–C evolved from the soils incubated at different temperatures reflected a clear effect of temperature on SOM mineralization (Fig. 5.1).

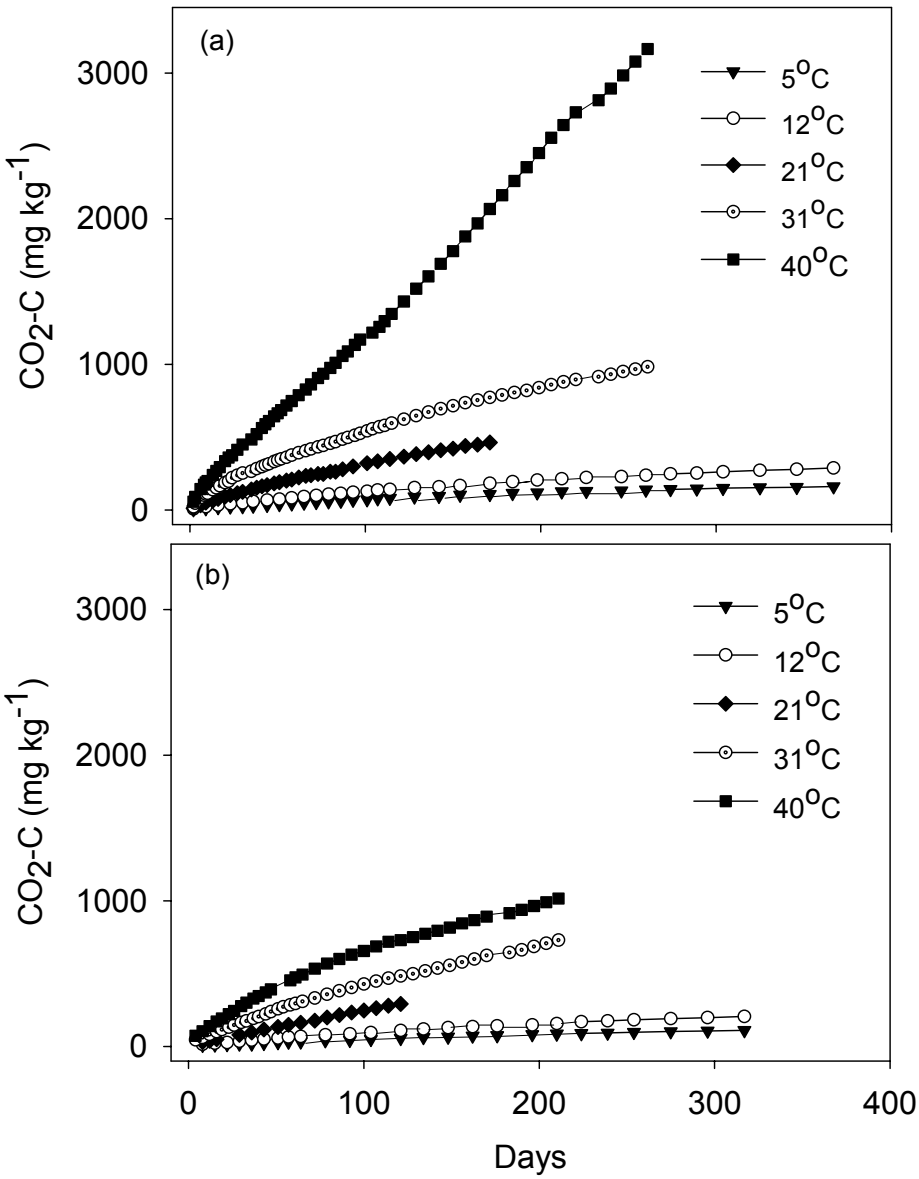

Figure 5.1. Cumulative CO_2–C evolved from (a) a clay soil (soil 1) and (b) a sandy soil (soil 3) incubated at 5; 12; 21; 31 and 40ºC.

Respiration rates in both soils showed significant differences between temperatures, except for the 5ºC and 12ºC that not always reached statistical significance although larger amounts of C evolved from the soils incubated at 12ºC respect to 5ºC at all sampling times (Appendix 5.1 and 5.2). In a similar way C mineralization rates of 21ºC; 31ºC and 40ºC in the sandy soil were not always significantly different, especially after 100 days of incubation. In contrast temperatures 31ºC and 40ºC differed all along the incubation in the clay soil.

In the clay soil (soil 1) there were differences in patterns of cumulative CO_2-C evolution among temperatures (Fig. 5.1). The highest temperature (40ºC) followed a linear trend, while the CO_2 evolved from the treatments incubated at lower temperatures presented a trend of decreasing increments, especially in the first month of incubation. The patterns of cumulative CO_2-C evolution at different temperatures in the sandy soil (soil 2) resemble those of the medium and low temperatures in soil 1.

In general the cumulative CO_2-C did not show clear signs that it was reaching a plateau, even in the low temperature treatments. This fact was probably one of the reasons why the first order kinetics model (equation 1) was not adequate to explain the relationship of cumulative CO_2-C over time. The power function (equation 2) was well related to the measured values for all but 40ºC of soil 1, and for all temperatures the polynomial quadratic function presented a good fitting. Linear relationships were adequate for low temperature treatments. Nevertheless the aim of the study was not only to find relationships, but also to find relationships that can be used for comparisons of soils and treatments.

In the present study CO_2–C evolution rates as well as net N mineralization rates were calculated from the linear portion of the time course of the cumulative CO_2–C and mineral N curves (Fig. 5.1 and 5.3). This procedure was used because, as mentioned, first order kinetics did not present a close relationship with the actual results and could not be fitted in many cases, while all mineralization curves presented a relatively extended period of linear increase. Kladivko and Keeney, (1987) and Recous et al., (1994), also used slope of the linear regression fitted for Q_{10} calculation for N mineralization. In this case difficulties arise in determining the selected period, which correspond to a linear increase of cumulative CO_2–C and mineral N with time. The

longest possible period was selected, based on r^2 of the linear relationship. As expected the period of linear response was shorter for the high temperatures than for the low temperatures time courses (Table 5.2), since at the end of the incubation at high temperatures the curves presented a flatter shape.

Table 5.2. Regression of cumulative CO_2–C and mineral N on days of incubation in two soils incubated in the temperature range 5 to 40ºC (Data from Figs. 5.1 and 5.3).

	Temperature	**5ºC**	**12ºC**	**21ºC**	**31ºC**	**40ºC**
C - Soil 1	C-CO_2 (mg $kg^{-1}d^{-1}$)	0.50	0.92	2.37	4.32	10.69
	Period	day 35-247	day 35-198	day 35-171	day 17-104	day 17-104
	R^2	0.98	0.99	0.99	0.99	0.99
C - Soil 2	C-CO_2 (mg $kg^{-1}d^{-1}$)	0.42	0.74	2.34	4.27	7.74
	Period	day 29-162	day 29-162	day 30-121	day 12-72	day 2-37
	R^2	0.99	0.99	0.99	0.99	0.99
N - Soil 1	min N (mg $kg^{-1}d^{-1}$)	0.13	0.20	0.41	1.02	1.99
	Period	day 7-175	day 7-175	day 7-175	day 7-126	day 7-91
	R^2	0.94	0.97	0.99	0.99	0.99
N - Soil 2	min N (mg $kg^{-1}d^{-1}$)	0.16	0.21	0.48	0.99	1.69
	Period	day 13-76	day 13-76	day 13-76	day 13-76	day 6-27
	R^2	0.99	0.99	0.99	0.99	0.98

The calculated CO_2–C mineralization rates ranged from 0.50 (mg $kg^{-1}d^{-1}$) at the lowest temperature to 10.69 (mg $kg^{-1}d^{-1}$) at the highest temperature in the clay soil (soil 1) and from 0.42 to 7.74 (mg $kg^{-1}d^{-1}$) for 5 and 40ºC respectively in the sandy soil (soil 2).

These calculated respiration rates showed a close exponential relationship with temperature (Fig. 5.2) that can be expressed as:

Soil 1: CO_2-C rate (mg kg^{-1} d^{-1}) =0,336 $e^{(0,0860 * temp^oC)}$ R^2=0,99

Soil 2: CO_2-C rate (mg kg^{-1} d^{-1}) =0,297 $e^{(0,0849 * temp^oC)}$ R^2=0,98

From these equations, relating the changes in soil respiration rates with temperature, Q_{10} values were calculated: Q_{10} =exp (0.086*10) and Q_{10} =exp (0.0849*10) for soils 1 and 2 respectively. The calculated Q_{10} from these data were 2.36 for soil 1 and 2.33 for soil 2.

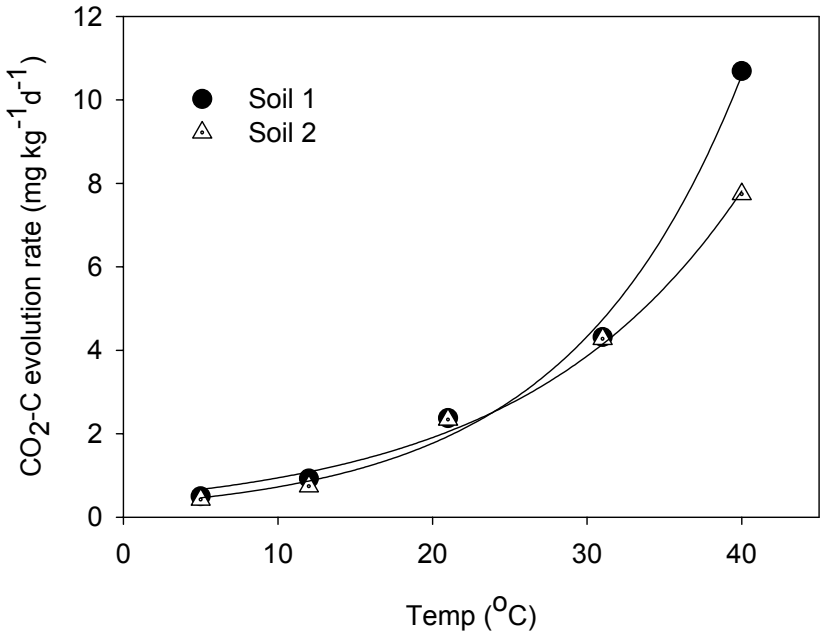

Figure 5.2. Relationship between calculated rates of CO_2–C evolution and temperature of incubation of a clay (soil 1) and a sandy soil (soil 2).

Calculations of Q_{10} between two temperatures (applying equation 2) showed lower Q_{10} values for respiration rates at high temperatures (21-31ºC) in the sandy soil and no clear pattern in the clay soil.

5.3.2 Temperature and N mineralization

Net N mineralization was strongly influenced by temperature of incubation in both soils (Fig. 5.3). The shape of the mineralization was also different in both soils, because in the sandy soil an asymptotic trend can be observed in the two high temperature treatments, while in the clay soil this tendency was only suggested.

There were significant differences among temperatures in mineral N release, except for 5ºC and 12ºC (Appendix 5.5 and 5.8). Even though differences between those treatments did not reach statistical significance at most sampling times, there was a clear trend of higher amounts of N released from the 12ºC compared to 5ºC treatment. Net mineralized N at 21ºC presented differences between soils; while in the clay soil mineral N from 21ºC was not significantly higher than 12ºC until the 28 days of

incubation, in the sandy soil significantly larger amounts were produced from the day 14 onwards.

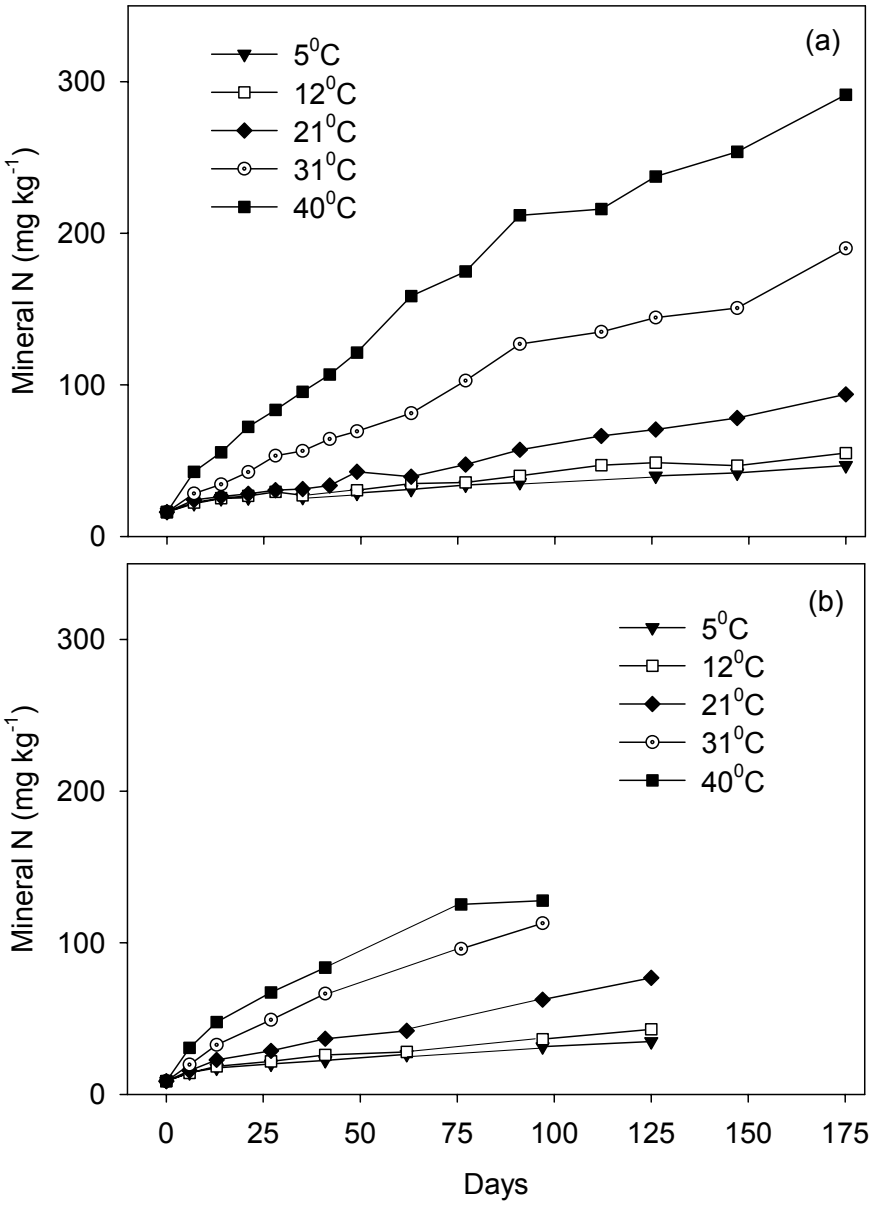

Figure 5.3. Mineral N content of (a) a clay soil (soil 1) and (b) a sandy soil (soil 2) incubated at 5; 12; 21; 31 and 40ºC.

The main mineral N form in both soils was NO_3^- at all temperatures, however the 40ºC treatments presented significantly higher amounts of NH_4^+, from the day 27 onwards in soil 2 and from 91 days in soil 1, but the proportion was never higher than 25 % of the total mineral N (Appendix 5.3 and 5.6). It is likely that the pH decrease that was

observed in the soils at 40 ºC might have had a suppressive effect on NH_4^+ nitrification. The other possible mechanism was suppression of nitrification due to enzyme denaturation at high temperatures as reported by Recous et al., (2001). The results presented correspond to mineral N (NO_3^--N + NH_4^+-N).
The amounts of net mineralized N at each temperature in the clay soil (soil 1) were clearly larger than those from the sandy soil (soil 2) when similar periods are considered, especially comparing the high temperature incubations.

In a similar way to cumulative CO_2–C evolution, in N mineralization the first order kinetic model could not be fitted. At low temperatures (5ºC and 12ºC) the power function was well related to the measured values, and for all temperatures the polynomial quadratic function presented a good fitting. Zero order kinetics was also relatively well fitted to the soil mineral N content, especially at low temperatures.

As for the cumulative evolved CO_2-C, net N mineralization rates were estimated from the linear portion of the time course (Table 5.2). The calculated net N mineralization rates ranged from 0.13 (mg $kg^{-1}d^{-1}$) at the lowest temperature to 1.99 (mg $kg^{-1}d^{-1}$) at the highest temperature in the clay soil (soil 1) and from 0.16 to 1.69 (mg $kg^{-1}d^{-1}$) for 5 and 40ºC respectively in the sandy soil (soil 2).

The estimated daily N mineralization rates showed an exponential relationship with temperature (Fig. 5.4) that can be expressed as:

Soil 1: Nmin rate (mg kg^{-1} d^{-1}) =0,0821 $e^{(0,0797 * temp ºC)}$ R^2=0,99
Soil 2: Nmin rate (mg kg^{-1} d^{-1}) =0,103 $e^{(0,0710 * temp ºC)}$ R^2=0,99

From these expressions, that relate mineralization rates to temperature for the whole range of temperatures Q_{10} values were calculated. Q_{10} =exp (0.0797*10) and Q_{10} =exp (0.0710*10) for soils 1 and 3 respectively. The calculated Q_{10} values for the temperature range 5-40ºC were 2.22 in soil 1 and 2.03 in soil 2.

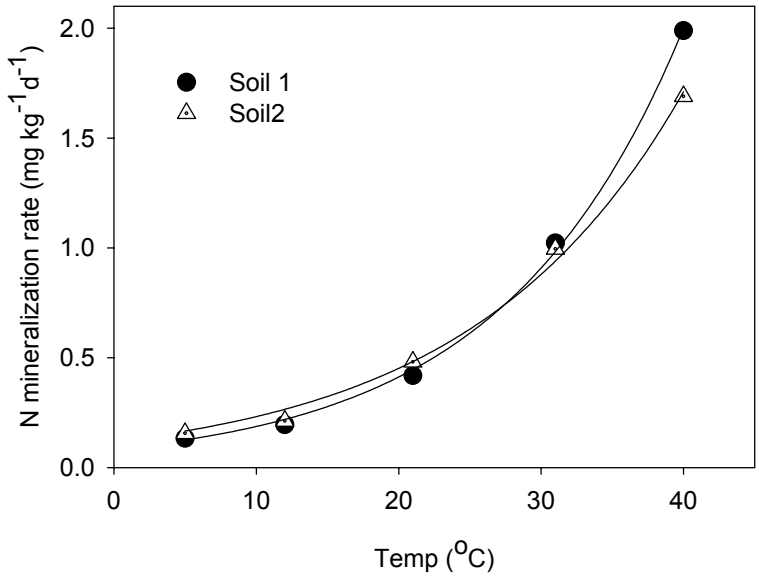

Figure 5.4. Relationship between calculated N mineralization rates and temperature of incubation of a clay and a sandy soil.

The calculated Q_{10} for N mineralization rates of the temperature pairs (5-12ºC; 12-21ºC; 21-31ºC and 31-40ºC) yielded lower values for the extreme temperatures (between 5ºC and 12ºC and between 31ºC and 41ºC) than in the middle of the temperature range in both soils.

5.3.3 Soil moisture and C mineralization

Time course of cumulative CO_2-C evolved from the soil is presented in Fig. 5.5. In the clay soil (soil 3) the curves show the characteristic shape of soil incubation at constant temperature, with a flush of C at the beginning of the incubation period, followed by a nearly constant rate from the third week onwards. In the sandy soil (soil 4) an initial decomposition flush was not detected. In both soils the highest respiration rates corresponded to the highest soil water content and they decreased as the water content decreased.

In the clay soil (soil 3) the amounts of C evolved from the soil with the highest water level were significantly larger than the rest at all except for the 5; 9 and 63 days samplings, while no significant differences were observed between the -0.10 and -0.14 MPa treatments (Appendix 5.9). Respiration rates of the -0.10 and -0.14 MPa were significantly higher than –0.90 MPa except for the last four samplings. No significant differences could be found between the two low water levels (-1.00 and –2.00 MPa) at

any sampling time, both presented lower respiration rates than –0.90 MPa especially at the beginning of the study. In the sandy soil (soil 4) the two high water levels (-0.01 and –0.03 MPa) did not differ between themselves, except for day 6 sampling (Appendix 5.10), being superior compared to the -0.08 MPa only at given sampling times (16; 20; 57 and 71 days). No significant differences were found between the two low soil water levels (-0.28 and –0.67 MPa) along the incubation, which were significantly lower than -0.08 MPa at most sampling times. Some differences are not clearly seen in the figures, since they show accumulated C amounts, while the statistical analysis was performed on the measured respiration rates (Appendix 5.9 and 5.10).

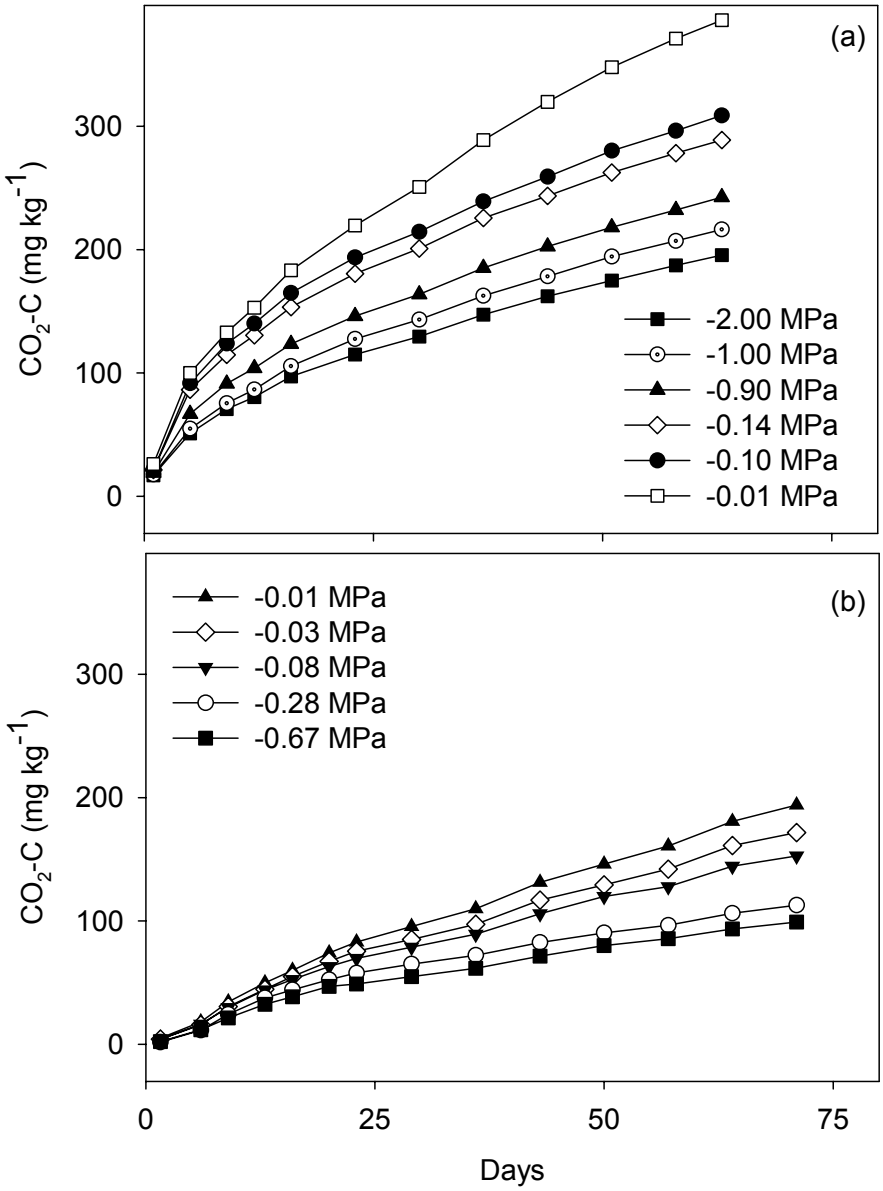

Figure 5.5. Accumulated CO_2-C evolved from (a) a clay soil (soil 3) and (b) a sandy soil (soil 4) incubated at different water potentials.

Except for the initial flush, the patterns of evolved CO_2 of the soils incubated at different soil water contents were similar in both soils, although the evolved amounts of CO_2 differ between them, being the accumulated amounts of the heavy textured soil (soil 3) nearly twice those of the light textured soil (soil 4) at the highest moisture level when similar periods are compared.

The power function and the polynomial quadratic function were well fitted to the data set. Unlike the previous cases an asymptotic model, proposed by Jones, (1984) for N mineralization, was adequate to describe the time course of CO_2–C evolution in both soils and at the whole range of moistures tested (equation 5; Table 5.3).

The fitted equation can be expressed as

$$N_t = a + b\,[1-\exp(-kt)] \qquad (5)$$

Where:

N_t is the amount of N mineralized at time t,
k is a rate constant
a and b are the parameters of the equation.

Equation 5 was developed with the objective of better describing the initial flush of mineralization, however the shape of this model is rather similar to the first order kinetics used by Standford and Smith, (1972) (Equation 1).

Table 5.3. Parameter estimation of the equations fit on the accumulated CO_2–C on days of incubation in two soils incubated at different soil water contents. Soil water levels are expressed as water potential and gravimetric water content.

		Soil water levels					
	Parameters	**-2.00 MPa 0.16 g g^{-1}**	**-1.00 MPa 0.18 g g^{-1}**	**-0.90 MPa 0.19 g g^{-1}**	**-0.14 MPa 0.26 g g^{-1}**	**-0.10 MPa 0.28 g g^{-1}**	**-0.01 MPa 0.37 g g^{-1}**
Soil 3	a (mg kg^{-1})	20.96	21.20	25.16	30.15	31.22	36.87
	b (mg kg^{-1})	211.82	238.06	250.50	284.12	301.29	442.71
	k (days^{-1})	0.026	0.026	0.029	0.034	0.035	0.024
	R^2	0.99	0.99	0.99	0.98	0.98	0.99
		-0.67 MPa 0.09 g g^{-1}	**-0.28 MPa 0.12 g g^{-1}**	**-0.08 MPa 0.14 g g^{-1}**	**-0.03 MPa 0.16 g g^{-1}**	**-0.01 MPa 0.18 g g^{-1}**	
Soil 4	a(mg kg^{-1})	0.85	0.53	1.32	-2.29	-0.08	
	b(mg kg^{-1})	125.00	136.00	236.17	300.05	357.06	
	k (days^{-1})	0.021	0.024	0.014	0.012	0.011	
	R^2	0.99	0.99	0.99	0.99	0.99	

The parameters of the fitted equations were not consistent however, because k, which is the estimation of the rate constant, did not follow the trend of the observed respiration rates, being the lowest corresponding to the highest moisture level in both soils. The parameter b which can be seen as an estimation of the potentially mineralizable C pool, on the other hand increased with the tested water levels.

As in previous cases, in order to compare trends, daily CO_2–C evolution rates were calculated from the linear portion of the time course curves (Table 5.4).

Table 5.4. Regression of cumulative evolved CO_2–C on days of incubation in two soils incubated at different soil water contents Soil water levels are expressed as water potential and gravimetric water content.

		Soil water levels					
	Parameters	**-2.00 MPa 0.16 g g^{-1}**	**-1.00 MPa 0.18 g g^{-1}**	**-0.90 MPa 0.19 g g^{-1}**	**-0.14 MPa 0.26 g g^{-1}**	**-0.10 MPa 0.28 g g^{-1}**	**-0.01 MPa 0.37 g g^{-1}**
Soil 3	C-CO_2 (mg kg^{-1}d^{-1})	2.23	2.52	2.71	3.1	3.26	4.74
	Period	day 16-51	day 16-51	day 16-51	day 16-51	day 16-51	day 16-51
	R^2	0.99	0.99	0.99	0.99	0.99	0.99
		-0.67 MPa 0.09 g g^{-1}	**-0.28 MPa 0.12 g g^{-1}**	**-0.08 MPa 0.14 g g^{-1}**	**-0.03 MPa 0.16 g g^{-1}**	**-0.01 MPa 0.18 g g^{-1}**	
Soil 4	C-CO_2 (mg kg^{-1}d^{-1})	1.07	1.18	1.79	2.07	2.37	
	Period	day 20-71	day 20-71	day 20-71	day 20-71	day 20-71	
	R^2	0.99	0.99	0.99	0.99	0.99	

Even though in the clay soil (soil 3) higher respiration rates were observed and calculated by regression analysis, compared to the sandy soil (soil 4), differences were not large. The calculated CO_2–C evolution rates were referred to soil water tension and gravimetric soil water content (Fig. 5.6 a and b). These figures show good agreement between the fitted equations and the amounts of mineralized C, indicating that microbial activity was strongly and consistently influenced by soil moisture. Interestingly the gravimetric soil water levels at the lowest water potential in soil 4 corresponded to the gravimetric soil water content of the lowest water potentials in soil 3, highlighting the differences between these two soils.

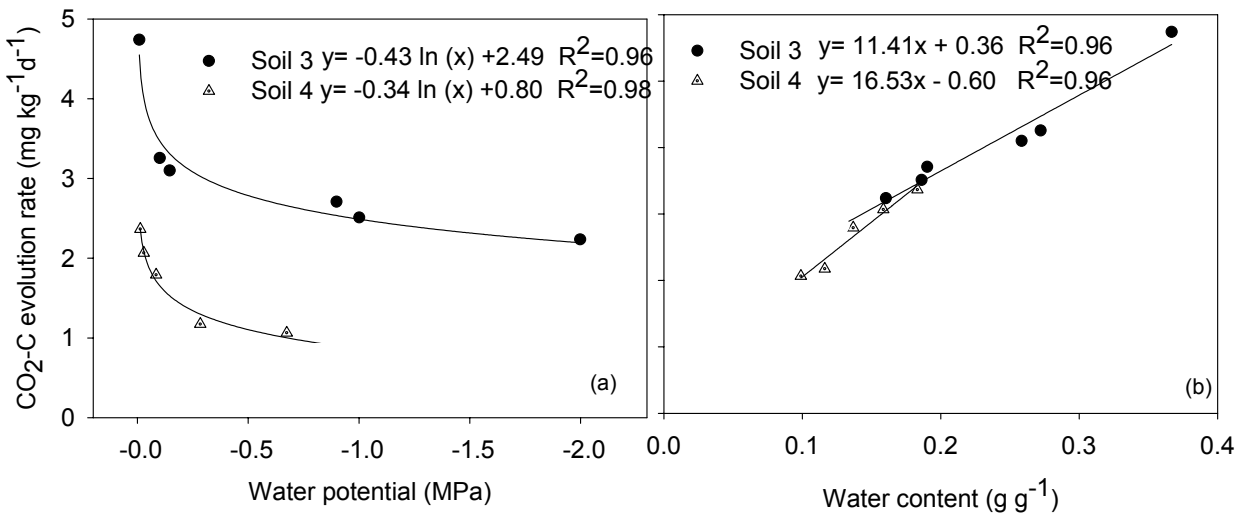

Figure 5.6. CO_2–C evolution rates related to (a) soil water potential and (b) soil water content.

5.3.3 Soil moisture and N mineralization

The figures presented in this section correspond to net mineralized N, calculated as the difference between the mineral N amount after the incubation period and the mineral N content after 1 week of pre-incubation. At that time the soils were mixed again, distributed in three pots per water content and a subsample was taken in order to determine the soil water content, which differed from the aimed water tension. In the clay soil (soil 2) the treatment with the highest water content (-0.01 MPa water potential) showed clear effects of denitrification, with fluctuating results. In consequence this treatment was not considered since the objective of the study was the effect of moisture on N mineralization, which could not be estimated using this methodology at high water contents. In the sandy soil the tested water potentials implied a lower range, since the initial soil water content corresponded to -0.67 MPa.

Net N mineralization of the soils under study was related to soil moisture, being the amounts of net mineralized N from the high water level treatments the largest, while the low water levels yielded smaller amounts of mineral N (Fig. 5.7). Nevertheless the low water content treatments presented increases in mineralization rates along the incubation. In the clay soil even the driest treatment (-1.81 MPa), being below than permanent wilting point, showed a significant net N mineralization. Both soils showed different mineralization patterns, being in the clay soil (soil 3) the increases in net N

mineralization greater in the first three weeks than in the following period and rather steady in the sandy soil (soil 4).

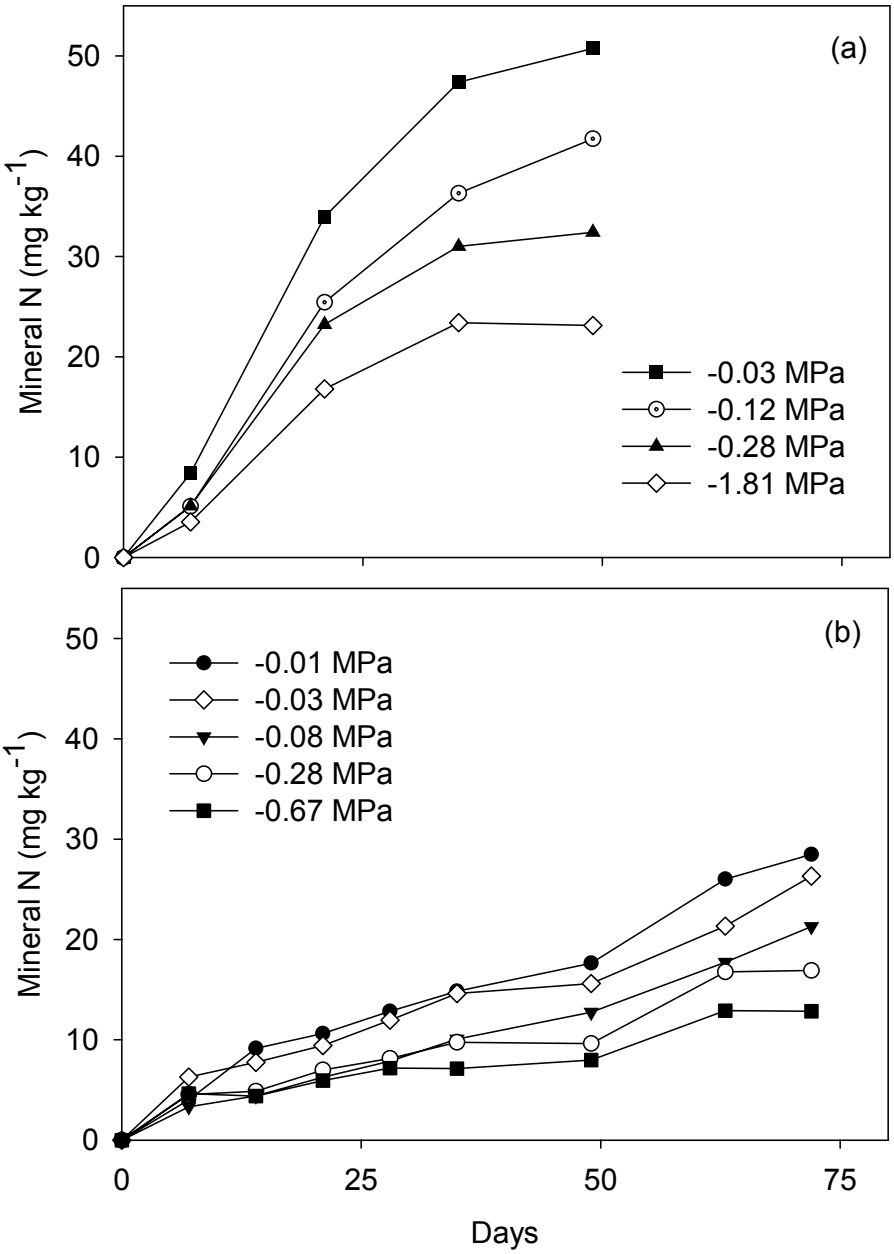

Figure 5.7. Net mineralized N in (a) a clay soil and (b) a sandy soil incubated at different water tensions.

In the clay soil the amounts of mineralized N from the -0.03 MPa treatment were significantly higher than the rest from day 35 onwards (Appendix 5.11). Differences between -0.12 and -0.28 MPa were significant only in the last sampling, being both

significantly higher than the driest treatment (-1.81 MPa) from day 35 onwards. In the sandy soil differences were less marked, being the two high water levels significantly different among themselves only at day 63, although both presented higher N mineralization than -0.08 MPa from the second week onwards (Appendix 5.12). Treatment -0.08 MPa presented larger amounts of net mineralized N than -0.28 MPa after 49 days of incubation. There were not significant differences between the two low moisture treatments (-0.28 and -0.67MPa) until the two last samplings.

In the present work there was a clear effect of soil water content on net N mineralization rates, however, as it has been previously reported there are difficulties at the time of comparing soils (Miller and Johnson, 1964). In this case the first problem arises because N mineralization followed different patterns in the heavy textured and in the light textured soils. In the sandy soil (soil 4) net N mineralization rates corresponding to the period of stabilization of the mineralization process were easily calculated, (linear portion of the relationship between mineral N content and days of incubation). In contrast in the clay soil (soil 2) this relationship was not linear, which allowed the fitting an asymptotic model (equation 5). The obtained parameters are presented in Table 5.5.

Table 5.5. Parameter estimation of the equations fit on the net N mineralized on days of incubation in a soil incubated at different soil water contents. Soil water levels are expressed as water potential and gravimetric water content. The equation fitted was Nt=a + b [1- exp (-k*t)].

		Soil water levels			
	Parameters	**-1.81 MPa 0.17 g g^{-1}**	**-0.28 MPa 0.23 g g^{-1}**	**-0.12 MPa 0.27 g g^{-1}**	**-0.03 MPa 0.32 g g^{-1}**
Soil 3	a (mg kg^{-1})	-12.10	-1.18	-11.29	-15.54
	b (mg kg^{-1})	36.19	52.75	59.22	70.92
	k (days^{-1})	0.076	0.025	0.046	0.059
	R^2	0.99	0.99	0.99	0.97

The calculated rate constants (k) did not show a clear relationship with soil water content, being the highest for the driest treatment (Table 5.5). The parameter b, which is related to the potentially mineralizable pool, in opposition to k, showed a clear positive relationship with soil water content. According to these equations the mineralizable pool correspond to a very small fraction of the total N content of the soil (representing 1.9; 2.8; 3.2 and 3.7 % of the total N the for the –1.81; –0.28; –0.12 and –0.03 MPa water levels respectively).

In the sandy soil (soil 4) on the contrary the asymptotic relationship could not be fitted, with a strong linear relationship between net N mineralized and time of incubation. Table 5.4 shows the calculated mineralization rates from the linear regression analysis of soil mineral N on days of incubation.

It was not possible to compare the calculated mineralization rates from the fitted asymptotic curves in soil 3 with the mineralization rates obtained by linear regression in soil 4. Therefore the slope of the linear regression analysis of the amounts of mineral N in the soils incubated at different water contents was also utilized in the heavy soil (soil 3) as estimation of N mineralization rates, although they did not correspond to the best fit (Table 5.6). As previously mentioned the best fit for the time course of mineral N of the clay soil at different water potentials was equation 5.

Table 5.6. Regression of Net N mineralized on days of incubation in two soils incubated at different soil water contents. Soil water levels are expressed as water potential and gravimetric water content.

		Soil water levels				
		-1.81 MPa 0.17 g g^{-1}	**-0.28 MPa 0.23 g g^{-1}**	**-0.12 MPa 0.27 g g^{-1}**	**-0.03 MPa 0.32 g g^{-1}**	
Soil 3	N min rate (mg kg^{-1}d^{-1})	0.68	0.93	1.09	1.41	
	Period	day 1-35	day 1-35	day 1-35	day 1-35	
	R^2	0.98	0.98	0.97	0.98	
		0.67 MPa 0.09 g g^{-1}	**0.28 MPa 0.12 g g^{-1}**	**0.08 MPa 0.14 g g^{-1}**	**0.03 MPa 0.16 g g^{-1}**	**.01 MPa 0.18 g g^{-1}**
Soil 4	N min rate (mg kg^{-1}d^{-1})	0.14	0.20	0.28	0.30	0.37
	Period	7-72	7-72	7-72	7-72	7-63
	R^2	0.97	0.99	0.99	0.99	0.99

In the periods included in the linear regression analysis in the clay soil the pre-incubation was not considered.

Figure 5.8 shows that there was a linear relationship between N mineralization rates and gravimetric soil water content, while the relationship with water potential followed a logaritmic model.

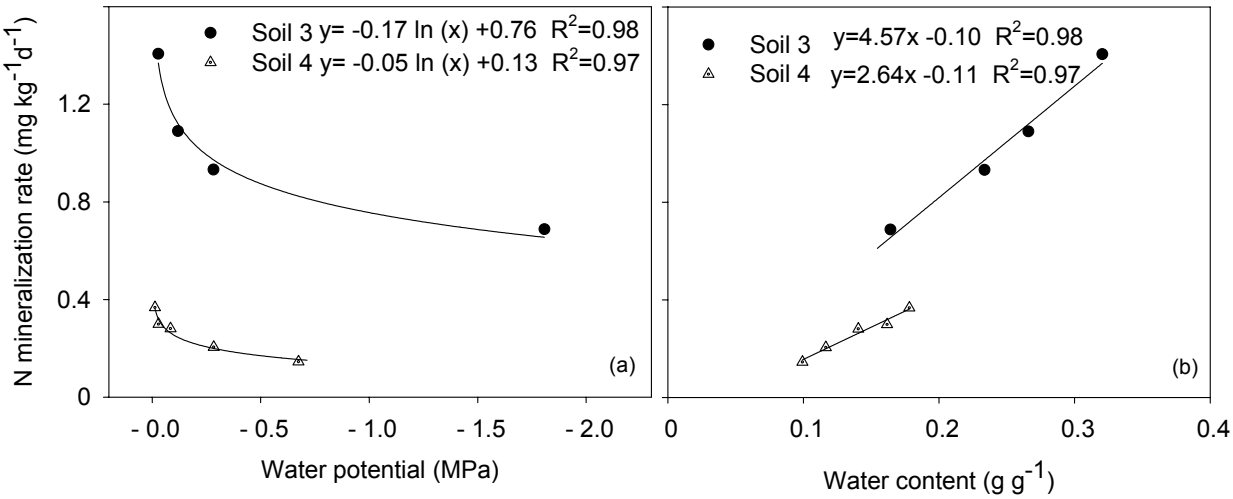

Figure 5.8. Net N mineralization rates related to (a) soil water potential and (b) soil water content.

5.4 DISCUSSION

5.4.1 Incubation procedures

The results from SOM mineralization experiments are very much influenced by the incubation procedures utilized. Standford and Smith, (1972) described an incubation procedure consisting in the mix of dry ground soil with sand, followed by the periodical leaching of the mineralized N with a N-free nutrient solution. This methodology has been extensively used thereafter, however this type of incubation, has been linked to the results obtained, especially the first order kinetics equation describing the mineralization process. In this type of experiments at the beginning of the incubation there is usually a flush of mineralization. This flush can be explained by the mineralization of labile organic compounds and the disruption of soil structure by sample processing, exposing previously protected SOM. Macduff and White, (1984) attributed this effect to redistribution of substrate and microbial populations during the sieving of the soil. When dry soils are rewetted for incubation this effect can be also attributed to the remineralization of dead biomass (Jager and Bruins, 1975; Cabrera, 1993; Fierer et al., 2002). Problems related to the drying-rewetting process as well as soil structure disturbance due to grinding and sieving are usually mentioned (Seneviratne and Wild, 1985; Benbi and Richter, 2002). In contrast Cabrera, (1993) presented a different approach, considering that the mineral N flush produced by soil drying was more representative of natural conditions than the steady increase from

fresh samples, since natural soils are exposed to drying-rewetting cycles. Also leaching has been seen as influencing the results, because it will lead to a relatively rapid exhaustion of the mineralizable N. Seyfried and Rao, (1987) compared the incubation of fresh and dried samples and two leaching procedures: nutrient solution and 0.01 M $CaCl_2$ and found higher amounts of mineralized N from the previously dried soils and from the nutrient solution leached incubations respect of the field moist and $CaCl_2$ leached soils respectively. Additionally it has been found that a certain amount of organic soluble N is leached from the soil with the mineral N, being the account of this N matter of discussion (Beauchamp et al., 1986; Benbi and Richter, 2002).

In other studies incubation has been performed with the fresh soil and without leaching (Addiscott, 1983; Howard and Howard, 1993; De Neve et al. 1996). Nuske and Richter, (1981) compared the results from leached and no leached experiments, finding lower mineralization rates in no leaching experiments. They concluded that due to the larger variability the no leaching procedure was inadequate to describe N mineralization. A review of the bibliography concerning mineralization experiments shows a number of methodologies combining the mentioned procedures.

Length of the mineralization period has been also matter of discussion. Cabrera and Kissel, (1988 b) studied the effect of the incubation length on the parameters of the double exponential model of N mineralization. They found that with longer incubations the calculated N_0 were higher and the mineralization rates lower. These authors advise to use similar times of incubation when comparisons between N mineralization potential and rate are performed. As previously mentioned, length of incubation is necessarily related to temperature of incubation, because the intensity of mineralization depends on temperature; in consequence it is still difficult to define incubation times when different temperatures are compared. The initial mineralization flush is relatively more important at low temperatures; hence in the incubations at low temperatures a long incubation period is advisable. On the other hand Fang and Moncrieff, 2001, studying the effect of temperature on soil respiration reported no effect of incubation length on CO_2 evolution, which they attributed to the use of intact soil samples.

The incubation procedure in studies concerning mineralization at different soil water content has been widely discussed (Myers et al., 1982). Standford and Epstein (1974)

in their clasical work used dry soil and added water to reach the desired water level. This procedure has been critisized, due to the strong changes produced in the soil colloids, and a drying process instead of a wetting process has been proposed (Howard and Howard, 1993). The procedure used in the present study was somehow in the middle because the soils were wetted to reach the desired soil water levels, but the departure point was not completely dry soil but a low water content (Doel et al., 1990). The initial soil water contents in the clay soil were 0.11 and 0.08 g g^{-1} for N and C incubation respectively and 0.10 g g^{-1} for both N and C incubations in the sandy soil. Water addition during incubation can be also a problematic procedure, since it is possible that the added water is not evenly distributed in the soil, especially at low water contents. This effect could not be avoided in the present study, however, due to the relatively low incubation temperature and the fact that the soils were partially covered for N mineralization and into hermetic sealed jars for C mineralization, the amounts of water required were generally small.

The other problem that is often discussed concerns to the high soil water contents and the fate of the mineralized N, since it is possible that anaerobic conditions are produced, which can promote gaseous N losses. Rodrigo et al, (1997) advised that near saturation not only NO_3^- and CO_2 should be measured but also NH_4^+ and CH_4 because increasing amounts of these substances result from microbial activity under O_2 depletion. As it was mentioned, in the present experiment in the –0.01MPa of the clay soil clear signs of denitrification were detected, which made the results variable in replications and along the incubation.

The incubation conditions, temperature and soil water content should be also selected considering the region climatic conditions (Knopp and Swank, 2002). It is possible that the soil microorganisms react in an unusual way when exposed to condition far from those occurring in the field, for example extreme temperatures.

5.4.2 Effect of temperature on C and N mineralization

In the present study the soils were not allowed to dry before the experiments, in consequence a flush was hardly observed in the net N mineralization curves (Fig. 5.3). This fact was probably one of the reasons why the first order kinetics model was not

adequate to explain the mineralization patterns in most of the temperature treatments. First order kinetics equation has been so extensively used because it enables to calculate the potentially mineralizable N (N_0) as well as the mineralization rate constant (k). However parameter estimations from this model have been criticized, especially N_0 because its calculation requires extrapolation beyond the studied period, and has produced contradictory results (Böttcher et al., 2001). In a similar way interactions between N_0 and k estimations have been reported (Kladivko and Keeney, 1987; Benbi and Richter, 2002; Böttcher 2004). In addition it has been postulated that the first order kinetics applies to experimental data in an artificial way, because it accounts for the mineralization flush at the beginning of the incubation (Seyfried and Rao, 1988; Cabrera 1993). On the other hand the power equation can be more adequate to describe N mineralization time course (Broadbent, 1986), but the parameters a and b are variable and do not allow to compare mineralization rates at different temperatures (Marion and Black, 1987). Polynomial quadratic equations have not been widely used, probably because these equations are not able to provide for theoretical explanations of the studied processes (Ellert and Bettany, 1992), however maximum can be calculated as well as mineralization rates. Mineralization rates from linear relationships are meaningful, since equals the slope of the regression line, but the limitation lies in the mineralization potential, which cannot be calculated from these equations. According to Goncalves and Carlyle (1994) the linear relationship (zero order kinetics) best represents mineralization rates when a small proportion of the pool is mineralized and first order kinetics applies when mineralization rates are high and a larger proportion of the soil N pool is mineralized.

In the present study the most characteristic decomposition pattern implied a linear increase in time, preceded by mineralization flush at the beginning of the incubation in some cases, especially at high temperatures (Figs. 5.1 and 5.3). Similar patterns were obtained by Bonde and Roswall, 1987, who attributed the constant N mineralization rates after four weeks of incubation, to a shift in microbial population. According to these authors a constant mineralization rate would be caused by a stable microbial population. In consequence the two likely explanations were fungus colonization with no growth in terms of cytoplasmic content, and a limitation of microbial growth, for example due to protozoa grazing on bacteria.

In the clay soil (soil 1) the amounts of evolved C and net mineralized N were sensibly higher than in the sandy soil (soil 2) at each temperature, indicating that the clay soil possess either a larger N mineralizable pool or a higher rate constant compared to the sandy soil. Respiration and net N mineralization rates of both soils were similar at low temperatures, while at high temperature (40ºC) the rate of the light soil was lower than that of the heavy textured soil (Figs. 5.2 and 5.4). Considering the SOM content of both soils, it is possible that in the sandy soil (soil 2) mineralization was more limited by organic matter availability, especially at high temperatures, when mineralization is extremely dynamic. For C mineralization this difference can also be consequence of the particular CO_2-C evolution pattern of the 40ºC treatment in soil 1, which did not show a decreasing trend during the whole incubation period (Fig. 5.1a). In terms of N mineralization on the other hand, Mengel, (1996) reported more rapid N mineralization in a sandy soil compared to a loamy soil. He attributed this effect to the capacity of clay and humic materials to adsorb polypeptides; hence preventing their mineralization. This mechanism can explain the slightly lower N mineralization rates observed in the present study in the clay soil at 5; 12 and 21ºC treatments compared to those of the sandy soil (Fig. 5.4). In concordance higher N mineralization rates were observed in the sandy soils compared to the other soils studied in the experiments of Chapter 2.

The linear increase in cumulative CO_2-C in the clay soil incubated at 40ºC can be attributed to an increase in the decomposable organic matter pool at high temperatures. MacDonald et al., (1995), studying organic matter decomposition in different sites found differences in the pool size estimation, which increased as the incubation temperature increased. It has been proposed that the increase in the mineralizable pool with temperature is due to changes in the microbial population (Ellert and Bettany, 1992). In agreement Zog et al., (1997) observed clear differences in microbial communities among soils incubated at 5; 15 and 25ºC, which explained the large differences found in the calculated N_0 of the equations fitted. They suggested that microorganisms adapted to high temperatures are able to metabolize substrates that are not used by microorganisms adapted to low temperatures, in this way the size of the mineralizable pool would increase at high temperature. In order to explain this behavior it has also been proposed a mechanism of increase in the efficiency of the diffusion process at high temperature (MacDonald et al., 1995).

On the other hand it is accepted that at very high temperatures there is a decreasing trend in mineralization rates, due to the incapacity of the microorganisms to adapt to such conditions (Ellert and Bettany, 1992), which is coincident with the NH_4^+ accumulation at 40ºC in the present study, probably due to the decrease in nitrification rates (Appendix 5.3 and 5.6). Nevertheless, as previously discussed, an increase of the mineralizable pool and the efficiency of nutrient diffusion in soils are likely to occur at high temperatures. There are in consequence processes in opposite directions, and probably the result in each situation depends on the characteristics of the soil biomass. The extent of microbial adaptation to high temperature environments was confirmed by Miers, (1975) who reported optimum temperature for ammonification at nearly 50ºC in Australian tropical soils.

Interestingly in the present study no effects of low temperatures on nitrification could be detected since there were not significantly different amounts of NH_4^+ in the low temperature treatments (Appendix 5.3 and 5.6). It is accepted that nitrification occurs in a narrower range of temperatures compared to ammonification, being the activity of nitrifiers depressed at low temperatures. In agreement many authors (De Neve et al., 1996), Cookson et al., 2002, Zaman and Chang, 2004) reported NH_4^+ accumulation in soils incubated at 5ºC. On the other hand, Addiscot, (1983) observed negative effects of low temperatures but in the range below 5ºC, which is consistent with the results of this study. Cookson et al., (2002) reported a different behavior of nitrifiers at the beginning and in 77 days of incubation, which they attributed to adaptation of the nitrifier population to the low temperatures, being more active after an adaptation period.

Considering the effect of temperature on N mineralization rates, the obtained Q_{10} values for N mineralization are in the range reported in previous studies. Campbell et al., (1984) reported Q_{10} between 2.17 and 2.42 for Canadian prairies, although they found higher values for the light textured soil, which attributed to the protection of SOM by soil colloids in fine textured soils. In the present study there was not a clear trend of higher Q_{10} values at lower temperatures, neither for C nor for N mineralization. These results are contradictory respect of those reported by Ellert and Bettany, (1992) and Kirschbaum, (1994), consisting in higher values in the low temperature range. On the other hand Kladivko and Keeney, (1987) reported no consistent trend in Q_{10} at different temperatures in agricultural soils in USA. Lower Q_{10} values for the high temperatures

have been interpreted as the result of the early depletion of the readily available C forms during the incubation, which force the decomposition of the recalcitrant C fractions, being this process slower (Kirschbaum, 1995). In opposition Howard and Howard, (1993), studying the relationship between temperature, moisture and respiration rates reported increased Q_{10} with temperature increases.

Comparing the Q_{10} estimations for net N and C mineralization, lower values were obtained for N than for C in both soils. Kirschbaum (1994) comparing a number of studies relating temperature and C and N mineralization found a trend of higher Q_{10} values for C than for N, although the degree of scatter in the data did not allow him to conclude that both processes have different temperature sensitivity. According to Magid et al., (2001), working in crop residue decomposition, N and C mineralization are less associated than normally assumed, being N mineralization less temperature dependent than C mineralization, especially at low temperatures. From the present study it is possible to conclude that in both soils the effect of temperature on organic matter mineralization was rather similar, despite differences in texture and SOM content of the soils, being the calculated Q_{10} in the range 2.03 to 2.36, considering both N and C mineralization. These results are coincident with Campbell et al., (1981) who found similar relationships for the soils of a given region. The similar Q_{10} found in the present study indicate that ultimately the microorganisms of both soils react in the same way when they are exposed to temperature changes in the range from 5 to 40ºC.

5.4.3 Effect of soil moisture on C and N mineralization

Even with the lowest water content (-2.00 MPa) the clay soil produced a relatively high amount of CO_2, which represented more than half of the CO_2 measured with the highest water content (-0.01 MPa). Considering that -2.00 MPa is well above wilting point, this result confirms that the soil microbial biomass is able to survive in a wide range of conditions. In agreement Stott et al., (1986) and Quemada and Cabrera, (1997), studying residue decomposition at different water potentials, reported significant CO_2-C evolution at water potentials as low as –5.00 and –6.08 MPa respectively. Other authors (Reichman et al., 1966) reported negligible mineralization under wilting point. In our study the fact that this heavy textured soil (soil 3) retained an important amount of water (16 g g^{-1}) at –2.00 MPa can explain this behavior. At soil water contents above field capacity the information is also contradictory; it has been suggested that the optimal soil

water potential for SOM decomposition and N mineralization between -0.01 and -0.05 MPa (Miller and Johnson, 1964; Benbi and Richter, 2002). In agreement in this study C mineralization was highest at the highest water contents tested (-0.01 MPa, Fig. 5.5, Appendix 5.9 and 5.10). The same trend was observed for net N mineralization (Fig. 5.7, Appendix 5.11 and 5.12), with the highest mineralization rates at soil water potentials corresponding to –0.01 MPa in the sandy soil (soil 4) and –0.03 MPa in the clay soil (soil 3), although, as previously explained, net N mineralization at the highest water content in soil 3 could not be assessed. In coincidence Mayers et al., 1982 found the highest amounts of mineralized N at water potentials between -0.01 and -0.03 MPa.

The main mechanisms of soil microorganisms in response to water stress are the production of solutes to increase internal solute concentration, and in consequence avoid water loss; the second mechanism consist of uptake of external solutes that play a similar role. The first mechanism is highly demanding in terms of energy, affecting in consequence the growth rate of the microorganisms under water stress. Differences among microorganisms in resistance to water stress are very much related to their capacity to produce these solutes (amino acids, glucose, glycerol). The characteristics of the cell wall are decisive in terms of control of water and solute exchange with the medium, therefore strong cell walls are considered an attenuating factor against drops in soil matric potential (Harris, 1981). Considering that water stress have a differential effect on microbial growth it is likely that, the water potential also affects the competitive ability of the microorganisms, leading to dominance or different groups in soils with and without water stress. On the other hand even those microorganisms with a high capacity to resist water stress are likely to suffer because of difficulties to reach the substrate (lack of continuous water films), decreased diffusion and solute transport in the soil when soil water content decreases (Pappendick and Campbell, 1981).

Mineralization patterns, for both C and N, with different soil water potentials showed differences between soils, while the clay soil (soil 3) there was a flush of mineralization at the beginning of the incubation in the sandy soil (soil 4) this flush was much less obvious. Probably this behavior was caused by differences in the amounts of water added in order to reach the different soil water contents, which were far larger in soil 3 than in soil 4. As previously mentioned a mineralization flush following soil rewetting has been extensively reported (Cabrera, 1993; Benbi and Richter, 2002). On the other hand

the lowest water level, which did not receive extra water also presented a flush at the beginning of the incubation in the clay soil, indicating that other factors, probably related to structure disturbance by soil grinding, affected the initial stages of incubation.

As a consequence of these mineralization patterns, fitting of a first order kinetics model to the data was possible. This model has been proposed to account for the mineralization flush frequently observed (Ellert and Bettany, 1988). According to Benbi et al., (2002) negative values for the intercept indicate N immobilization; interestingly for N mineralization the intercept was in a similar range for all but the -0.28 MPa treatment, indicating that a similar process could have been occurred. The incubated soil had been under pasture lately; hence it is possible that some remaining grass roots promoted N immobilization during the first stages of decomposition. The calculated k constants from the first order kinetics model did not show relationships with soil water content (Tables 5.3 and 5.5). Similar inconsistencies in k for N mineralization rates in soils ranging from –0.01 to –1.85 MPa were reported by Zak et al, (1999). The estimations of the potentially mineralizable pools on the other hand were positively related to soil water content. The increase in the potentially C and N mineralizable pool with increasing water content can be attributed to the improvement of the ability of the microorganisms to reach the substrate when soil moisture increases. This case can be seen in a similar way as MacDonald et al. (1995) findings for high temperatures; where the potentially mineralizable pool increased due to the improvement of access of the substrate by microorganisms. According to Harris, (1988) the decrease in soil moisture is likely to affect more the motile microorganisms, which can be trapped in narrow pores, while filamentous fungi, which do not need a continuous water film for their growth are less affected by water shortage. In agreement Zak et al., (1999) postulated that the decrease in the mineralizable pool caused by soil drying could be related to a change in microbial population.

Comparing the clay (soil 3) and the sandy soil (soil 4), mineralization rates at similar water potentials were clearly different, for both C and N mineralization (Tables 5.4 and 5.6). The calculated net N mineralization rates were different in both soils, which was expected regarding to differences in texture and total N content (Table 5.6). However, since in the clay soil the rates were estimated in a portion of the time course when N mineralization was not stabilized yet, the calculated values probably overestimated the

actual rates. This speculation seems to be confirmed, comparing these mineralization rates with those of soil 1, incubated at 21ºC in the temperature experiment, taken from the same site. In soil 3 N mineralization rates were more than twice the mineralization rates of soil 1 of the temperature experiment with similar soil water content (0.27 g g^{-1}) and the same temperature (Tables 5.2 and 5.6). Even though the soils for the temperature and moisture incubation experiments were taken from the same site but at different dates, the differences are exagerated to be attributed to site or seasonal variability. Respiration rates at different water contents on the other hand did not show such great differences between the sandy and the clay soil (Table 5.4), probably because they were calculated from days 16 and 20 of incubation, for soil 3 and 4 respectively, after the initial mineralization flush.

Linear relationships between gravimetric soil water content and C and N mineralization rates were observed in both soils (Figs. 5.6 and 5.8). From these linear trends it follows that close relationships with the logarithm of water potentials could also be fitted, since water potentials were calculated from the curve that relates soil water content and water potentials. These relationships are in agreement with those reported by Reichman et al., (1966), Orchard and Cook, (1983) and Rodrigo et al., (1997), relating soil water potential and mineralization rates. In coincidence, studying crop residue mineralization Stott et al., (1986) and Quemada and Cabrera, (1997) found logarithmic relationships between soil water potentials and decomposition rates. In a similar way some authors have related mineralization rates to the gravimetric soil water content, (Myers et al., 1982; Lochmann et al., 1989).

Both soils differed in the magnitude of the response of net N mineralization to soil water content, with a higher response in the clay soil (soil 3), measured as the slope of the regression of mineralization rate on soil water content (Figs. 5.8 b). Interestingly both soils showed rather similar relationships in terms of C mineralization (Figs. 5.6 b), which indicate that soil respiration depended very much on soil water content, despite differences in soil water potential. These linear relationships between mineralization rates and soil water content, with intercept near 0 indicate that if the fitted equations could be extrapolated for soil water contents below those tested, the soil biomass activity would stop only when the soil is almost dry. Although the evidence from the present experiment did not prove this hypothesis, it highlights the fact that the moisture

threshold for microbial activity, at least in the clay soil, is far below the permanent wilting point. In agreement Myers et al, (1982) that studied Canadian and Australian soils postulate –4 MPa as the limit of mineralization in soils. Interestingly the soils varied in the lower limit; while in some soils mineralization stopped at less than -1.5 MPa, others showed mineralization at –4 MPa.

Given the methodology of this study it is not possible to identify the mechanisms involved in the restraint of C and N mineralization in response to water stress. Considering that in the two studied soils the differences in soil water content produced by the treatments are arround two fold, it is unlikely that the osmotic potential play a decisive role in the observed decrease in microbial activity with lower water content. On the other hand, according to Papendick and Campbell (1981) solute diffusion is more severely limited in systems with reduced matric potential than in osmotic systems with higher water content. It is possible, therefore, that the reduced microbial activity is due to an indirect effect of substrate availability and transport than a direct effect of water potential on soil microorganisms in the present study. The transport, which would be by diffussion, is related to the soil water content, (θ), rather than to the water potential, as can be shown by the effective diffussion coefficient (D_e) of solutes in soil ($De=D_L\theta\ f/b$ Nye and Tinker, 1977) where D_L is the diffussion coefficient of the solute in water, f is the impedance factor, which is also related to θ and b is the buffer power of the solute in consideration. This hypothesis would explain the linear relationship between soil respiration and soil water content (Figs. 5.6 and 5.8). In agreement, Sabey, (1969) reported severe restrictions in nitrification with very small decreases of water potential, concluding that it is unlikely that the metabolic processes of the microorganisms can be directly influenced by those small changes in soil water potential.

5.5 SUMMARY AND CONCLUSIONS

In the studied Uruguayan soils the influence of temperature on SOM mineralization, regarding to mineral N release and CO_2 evolution, can be described by exponential relationships. The calculated Q_{10} values, for both N and C, indicate that the influence of temperature will promote a slightly more than two fold mineralization increase per each

10ºC increase. These Q_{10} estimations lie in the commonly mentioned values for mineralization in the temperature range studied (5 to 40ºC), which is coincident with the expected temperature range, given Uruguayan climate. The similar relationship between SOM mineralization and temperature in the two studied soils, despite differences in texture and organic matter content, is encouraging in view of modeling the processes with forecast aims. Obviously more soils should be studied before concluding that these values are adequate for describing the relationship in the diversity of existing soils.

Mineralization processes in the two studied soils showed a close dependence on soil moisture, with linear increases in both mineral N and respiration rates as gravimetric soil water content increased. Regarding to N mineralization the slope of the regression line of mineral N on soil water content was larger in the heavy textured respect of the sandy soil. This fact suggests that it is not possible to make a general use of the results obtained. In contrast the effect of soil moisture on soil respiration showed more similitude between the two studied soils, however it is important to consider only two soils were studied, therefore further research relating soil characteristics and the effect of soil moisture on N mineralization and biomass activity is needed.

The observed C and N mineralization patterns in a range of soil moistures indicate that there is still an important microbial activity at very low soil water potentials. This fact, together with the exponential increase of mineralization rates with temperature, suggest that in summer, when the high evapotranspiration promotes soil drying it is likely that mineral N continue accumulating. Therefore the high soil mineral N level observed after dry periods is probably not only caused by the drying-rewetting process but also by the mineralized N at low water potentials.

In the studied temperature and soil water content ranges, the effect of temperature on SOM mineralization seems to be far larger than that of the soil moisture. Interactions between those effects should be however examined.

6 CONCLUDING SUMMARY AND PERSPECTIVES

6.1 CONCLUDING SUMMARY

This work was aimed to characterize net N mineralization through the study of the most important factors that affect the process: influence of crop residue incorporation, N availability, temperature and soil moisture. The characterization of net N mineralization by means of chemical and physical N assessment indexes was also evaluated.

Crop residue amount and quality determined the amounts of net mineralized N, moreover important amounts of mineral N were immobilized when low N residues were incorporated to the soil. Mineral N availability influenced the remineralization pattern of immobilized N. Cosequently in soils with mineral N surplus net N immobilization was very fast and the amount of net immobilized N tended to remain unchanged or even increase, while in N depleted soils a trend of higher remineralization was detected after the immobilization period.

Two different aspects were important in plant material decomposition, first the material chemical composition influenced the pace of the process, and in consequence the extent of the transformation of the material in the soil. On the other hand, the net effect of N mineralization or immobilization was dependent on decomposition process as well as on the N balance of the decomposing biomass, the plant material N content and the available soil mineral N. No single chemical component of the materials was able to explain the differences in decomposition patterns of crop residues and green manures. The soluble C content was responsible for the initial biomass flush, which was followed by fast N immobilization when the N present in the residue was insufficient. In the next step of decomposition structural C compounds (cellulose and hemicellulose) determined in a greater extent the decomposition pace, while the phenolic compounds, in contrast, represented a negative influence in plant material decomposition along the whole incubation period. When the decomposing plant materials presented low N concentrations, and the soil mineral N was depleted, no differences between plant materials either in decomposition patterns or in immobilized N amounts could be detected, despite differences in plant material composition. Although N concentration of the plant material did not affect decomposition pace, was the most important

characteristic explaining the amount of net mineralized-immobilized N during decomposition.

There was a clear negative effect of fertilizer N addition on soil biomass activity, consequently this effect, more marked in some situations, negatively affected the mineralization process. The reasons for this negative effect are probably related to the observed pH decrease and increased salt concentration of the soil solution following N fertilizer addition. Moreover the results of experiment comparing N sources also suggest a direct negative effect of the high N concentration on soil biomass, since no differences in soil biomass activity were detected comparing soils that received ammoniacal or nitric fertilizers. On the other hand this negative effect of mineral N addition was partially counteracted by crop residue amendment. In the experiments that combined N addition and crop residue decomposition it was possible to observe the simultaneous positive effect of the larger N availability and the negative effect of the N fertilizer on the biomass growth. In this case N fertilization resulted in faster immobilization, but reaching a smaller amount of immobilized N in fertilized compared to N depleted soils. Similarly in the first month of incubation differences between crops in terms of N immobilization were clear in the fertilized soils in contrast to the soils without N addition, but the total immobilized N was larger in the unfertilized soils. In both cases the lower respiration rates indicated a less active biomass in the fertilized soils.

The influence of temperature on SOM mineralization, either measured as N release or soil respiration, followed an exponential model in the temperature range from 5 to 40ºC. The calculated Q_{10} values, for both N and C, indicate a slightly more than two fold mineralization rate increase per each 10ºC increase in the two studied soils, despite differences in texture and SOM. This result is coincident with studies from many other regions, and very close to the two fold increase observed in many biological processes.

There was a direct relationship between C and N mineralization and gravimetric soil water content, although differences between the two studied soils in the response to changes in water content were observed. Consequently no general model for the different soils could be fitted. Substantial microbial activity, measured either as N release or soil respiration, was observed at high water tensions, well below permanent wilting point. From these results it follows that in dry periods plant growth is likely to be

reduced while SOM mineralization continues, leading in consequence to mineral N accumulation.

Chemical as well as physical procedures were tested to forecast soil net N mineralization in a group of soils varying in pedological characteristics as well as management. Most of the indexes tested were well related to N mineralization obtained through incubation under controlled conditions, being less related to the N uptake by crops, probably because this parameter was limited by differences between crops. In this work it was clear however that it is very difficult to define the mineralization parameters for the N availability index evaluation. Furthermore the ranking of the capacity of the indexes for N availability forecast varied with the different mineralization parameters used. It was possible, however, to relate the results from the different indexes to the net mineralized N in short term or in long term incubations, but the question about which is the best parameter remained unanswered.

6.2 PERSPECTIVES

The obtained results contributed to improve the understanding of net N mineralization in agricultural soils, however some aspects should be more closely studied, while in other cases the possibility of extending the relationships found to a greater range of soils should be confirmed.

One important aspect related to crop residue decomposition that needs a closer look is the fate and characteristics of the undecompossed material. Considering that at the end of the incubation experiments of this study, very low respiration rates were measured, and nevertheless the materials were far from complete decomposition it is important to identify the characteristics of the undecompossed material as well as to determine how it relates to the stable SOM.

In terms of crop residue decomposition two important aspects were not covered in this study. The first is the effect of the physical condition of the residues, in order to determine the effect of residue size on decomposition patterns as well as the possible interactions with the chemical composition. The second important aspect related to crop residue decomposition, which was not studied, is residue placement.

Although the result of this study about effect of temperature on SOM mineralization was in tune with findings from experiments in soils from other regions of the world, the general application in soils of Uruguay should be confirmed. On the other hand the effect of temperature fluctuations needs further assessment.

There was a linear and close relationship between soil water content and C and N mineralization but there was not clear relationship between water potential and SOM mineralization. The reasons for this, weather it is because of substrate availability or mobility or microbial activity per se shoul be further investigated. Moreover from the soil moisture experiments as well as from the experiment comparing N sources questions arise about the effects of environmental conditions, especially related to changes in the soil solution, on microbial growth.

In terms of dependence of mineralization on soil moisture the other important aspect to consider is the possibility to identify soil characteristics that can explain the different relationships observed in the two studied soils. The most obvious difference between these two soils was texture, however other soil characteristics like pH could have also influenced in the observed results. From these studies relationships of more general validity between soil characteristics and the mineralization response to changes in soil water content can be established.

In the studied temperature and soil water content ranges, the effect of temperature on SOM mineralization seems to be far larger than that of the soil moisture, this result needs to be confirmed in experiments combining these two factors. Interactions between those effects as well as the drying rewetting effect should be also examined.

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Appendix 2.1. Soil N availability indexes and mineralization parameters, average of four replications. Results from the analysis of variance for each index in each soil. The indexes are: total N, mineral N at the beginning of the incubation (Min N), acid hydrolysis (HCl), extraction with phosphate-borate (PB), extraction with hot water (HW), extraction with $CaCl_2$, total and organic extracted N ($CaCl_2$-t and $CaCl_2$-o), UV absorbance after $NaHCO_3$ extraction at 260 and 205 nm (UV 260 and UV 205), N in fraction higher than 200 μ and in the fraction between 63 and 200 μ (>200 and 200-63). Results from the analysis of variance for mineralization parameters. The mineralization parameters are: amount of net mineralized N at 14 and 84 as well as between 14 and 84 days of incubation (14 days, 84 days and 14-84) and daily net mineralization. Statistical analysis were performed at each sampling dates. Differences between treatments are indicated by different letters (P< 0.05).

		total N	Min N	HCl	PB	HW	$CaCl_2$-t	$CaCl_2$-o	UV 205	UV 260	>200	200-63	14 days	84 days	Rate constant	rate
	treat	N – mg kg^{-1}							absorbance		N – mg kg^{-1}		N – mg kg^{-1}		d^{-1}	N-mg $kg^{-1}d^{-1}$
Be	N1	1089 a	4 b	720 b	18 c	56 a	13 c	8 a	0.671 c	0.359 b	25 a	80 b	27 b	42 b	0.052 b	0.271 a
Be	N2	1178 a	13 ab	789 ab	22 b	61 a	25 b	6 a	0.978 b	0.394 ab	27 a	103 ab	35 a	55 a	0.066 a	0.283 a
Be	N3	1215 a	20 a	835 a	24 a	65 a	30 a	5 a	1.178 a	0.430 a	37 a	120 a	41 a	58 a	0.074 a	0.289 a
GM	N1	709 a	5 b	496 a	10 b	45 b	8 b	4 a	0.396 b	0.208 a	102 c	104 b	9 b	27 b	0.022 a	0.245 a
GM	N2	707 a	8 ab	524a	14 ab	53 a	14 a	5 a	0.533 a	0.228 a	127 b	123 a	17 a	36 a	0.035 a	0.279 a
GM	N3	740 a	13 a	513 a	16 a	53 a	18 a	4 a	0.614 a	0.236 a	158 a	122 a	18 a	40 a	0.035 a	0.300 a
Ce	N1	807 a	8 b	541 a	17 c	40 c	13 b	5 a	0.587 c	0.277 a	39 b	38 b	13 b	27 c	0.032 a	0.204 a
Ce	N2	873 a	28 a	589 a	36 b	64 b	38 a	4 a	0.762 b	0.256 a	74 a	67 a	23 a	37 b	0.066 a	0.237 a
Ce	N3	912 a	34 a	575 a	46 a	79 a	49 a	3 a	0.937 a	0.291 a	68 a	71 a	28 a	45 a	0.079 a	0.259 a
Jue	N1	1728 a	7 a	1259 a	24 a	82 c	10 b	5 a	0.709 b	0.349 a	40 c	115 a	24 b	47 a	0.049 a	0.275 a
Jue	N2	1823 a	14 a	1225 a	28 a	87 b	18 ab	3 a	0.957 a	0.391 a	48 bc	137 a	28 ab	55 a	0.057 a	0.282 a
Jue	N3	1694 a	18 a	1160 a	26 a	91 a	23 a	2 a	1.000 a	0.361 a	59 a	128 a	32 a	58 a	0.058 a	0.304 a
Jue	N2 org	1802 a	11 a	1265 a	26 a	92 a	16 ab	4 a	0.909 a	0.376 a	51 ab	127 a	31 a	58 a	0.055 a	0.311 a
KL	N1	1538 a	6 a	954 a	22 a	92 a	14 b	10 a	0.803 b	0.426 b	31 b	116 a	35 a	54	0.088 a	0.282 a
KL	N2	1644 a	6 a	1152 a	25 a	100 a	19 ab	12 a	0.919 ab	0.462 ab	55 ab	130 a	43 a	65 ab	0.074 a	0.357 a
KL	N3	1711 a	10 a	1199 a	25 a	105 a	21 a	12 a	1.034 a	0.496 a	58 a	140 a	44 a	68 a	0.084 a	0.400 a
Ot	N1	1157 a	5 b	620 a	18 b	48 a	10 c	5 a	0.863 b	0.483 a	29 a	59 b	27 b	39 b	0.093 a	0.176 a
Ot	N2	1237 a	7 b	679 a	19 ab	57 a	14 b	5 a	0.997 a	0.480 a	39 a	111 a	31 ab	44 ab	0.093 a	0.168 a
Ot	N3	1251 a	10 a	739 a	21 a	59 a	19 a	3 a	1.096 a	0.479 a	46 a	104 a	34 a	45 a	0.108 a	0.153 a
HH	N1	1075 a	3 a	746 a	16 a	64 a	9 a	5 a	0.643 a	0.344 a	29 a	100 a	21 a	34 a	0.094 a	0.145 a
HH	N2	1095 a	3 a	778 a	18 a	65 a	11 a	6 a	0.666 a	0.357 a	35 a	97 a	23 a	35 a	0.110 a	0.142 a
R	GA	1162 b	10 a	799 b	21 b	57 b	16 a	4 a	0.832 a	0.374 a	25 b	67 b	25 b	45 b	0.050 b	0.291 b
R	SBV	1216 b	12 a	832 b	22 b	59 b	16 a	5 a	0.862 a	0.397 ab	32 b	81 b	29 b	49 b	0.050 b	0.308 b
R	GV	1369 a	10 a	949 a	25 a	74 a	19 a	7 a	0.902 a	0.426 a	72 a	115 a	41 a	69 a	0.055 a	0.410 a

Appendix 2.2. Soil N availability indexes and mineralization parameters - complete data. The indexes are: total N, mineral N at the beginning of the incubation (Min N), acid hydrolysis (HCl), extraction with phosphate-borate (PB), extraction with hot water (HW), extraction with $CaCl_2$, total and organic extracted N ($CaCl_2$-t and $CaCl_2$-o), UV absorbance after $NaHCO_3$ extraction at 260 and 205 nm (UV 260 and UV 205), N in fraction higher than 200 μ and in the fraction between 63 and 200 μ (>200 and 200-63). Results from the analysis of variance for mineralization parameters. The mineralization parameters are: amount of net mineralized N at 14 and 84 days of incubation (14 days, 84 days) rate constant k and daily net mineralization rate.

Site	treat	block	pH	total N	Min N	HCl	PB	HW	$CaCl_2$-t	$CaCl_2$-o	UV 205	UV 260	>200	200-63	14 days	84 days	k	rate
				N – mg kg^{-1}							absorbance		N – mg kg^{-1}				d^{-1}	mg $kg^{-1}d^{-1}$
Be	1	1	7.2	991	5	681	17	45	12	7	0.670	0.374	23	78	23	39	0.047	0.263
Be	1	2	7.2	1123	2	691	18	62	12	7	0.642	0.348	25	85	29	45	0.048	0.304
Be	1	3	7.2	1096	5	768	16	61	12	7	0.726	0.367	25	71	30	44	0.055	0.274
Be	1	4	7.1	1148	6	742	19	58	17	12	0.646	0.349	28	85	26	41	0.055	0.241
Be	4	1	7.0	1267	10	838	23	59	23	9	1.032	0.418	23	103	38	58	0.064	0.310
Be	4	2	7.0	1069	15	723	20	53	24	8	0.909	0.390	32	96	33	50	0.059	0.281
Be	4	3	7.2	1273	8	800	22	69	21	5	0.925	0.392	26	113	36	59	0.067	0.277
Be	4	4	7.0	1102	18	796	21	64	29	5	1.048	0.377	27	99	35	51	0.066	0.263
Be	6	1	6.9	1308	23	868	25	66	32	3	1.286	0.497	38	135	45	63	0.07	0.326
Be	6	2	7.0	1141	12	807	24	62	24	5	1.072	0.416	41	91	43	52	0.076	0.257
Be	6	3	7.0	1274	18	852	23	71	31	4	1.103	0.432	45	121	39	58	0.065	0.309
Be	6	4	7.0	1135	27	812	25	61	34	7	1.251	0.376	25	132	38	59	0.074	0.266
GM	1	1	5.7	681	5	476	9	42	7	4	0.380	0.202	102	104	8	24	0.021	0.211
GM	1	2	5.8	784	5	504	10	44	8	5	0.394	0.208	90	104	9	29	0.02	0.267
GM	1	3	5.7	651	5	488	11	47	8	4	0.358	0.194	105	101	9	26	0.028	0.214
GM	1	4	5.7	720	6	518	11	48	9	4	0.451	0.231	110	108	11	31	0.024	0.289
GM	4	1	5.5	619	10	544	15	51	18	3	0.562	0.197	142	111	21	38	0.042	0.264
GM	4	2	5.5	771	6	525	15	54	10	4	0.515	0.249	96	130	15	37	0.024	0.340
GM	4	3	5.6	725	8	481	14	53	17	6	0.555	0.236	136	127	15	34	0.042	0.241
GM	4	4	5.5	714	6	548	12	53	12	5	0.500	0.230	132	123	15	35	0.032	0.271
GM	5	1	5.6	774	9	488	14	47	16	4	0.580	0.223	171	115	15	36	0.035	0.283
GM	5	2	5.5	703	11	553	14	55	17	5	0.623	0.252	135	122	18	39	0.038	0.289
GM	5	3	5.3	704	20	506	20	59	23	3	0.667	0.226	149	133	18	44	0.028	0.354
GM	5	4	5.5	780	11	504	15	50	17	4	0.585	0.243	177	120	20	41	0.038	0.274

Appendix 2.2 (cont.)

Site	treat	block	pH	total N	Min N	HCl	PB	HW	$CaCl_2$-t	$CaCl_2$-o	UV 205	UV 260	>200	200-63	14 days	84 days	k	rate
							N – mg kg^{-1}					absorbance		N – mg kg^{-1}			d^{-1}	mg $kg^{-1}d^{-1}$
Ce	1	1	5.3	784	10	516	17	43	16	6	0.559	0.227	46	37	13	27	0.047	0.173
Ce	1	2	5.1	822	7	597	18	39	12	5	0.645	0.332	51	45	13	30	0.03	0.227
Ce	1	3	5.3	855	7	511	16	40	12	5	0.527	0.238	29	37	12	25	0.033	0.200
Ce	1	4	5.2	765	8	541	17	39	12	4	0.620	0.310	29	32	12	26	0.02	0.214
Ce	4	1	5.3	806	30	567	41	75	46	6	0.835	0.253	64	82	28	42	0.108	0.210
Ce	4	2	5.1	871	17	583	30	52	30	4	0.694	0.257	62	65	20	36	0.054	0.250
Ce	4	3	5.2	903	39	665	39	63	37	3	0.812	0.291	84	53	22	39	0.024	0.263
Ce	4	4	5.3	911	26	541	34	68	38	4	0.706	0.222	85		20	32	0.05	0.226
Ce	6	1	5.0	946	39	607	45	79	43	4	0.969	0.321	79	92	31	51	0.074	0.257
Ce	6	2	5.1	914	26	576	40	70	43	3	0.833	0.269		67	25	43	0.071	0.260
Ce	6	3	5.2	935	40	574	50	84	55	1	0.990	0.268		70	24	41	0.062	0.289
Ce	6	4	5.1	854	33	541	49	82	55	4	0.956	0.308	56	54	30	46	0.089	0.231
Jue	1	1	7.3	1932	7	1444	27	84	8	5	0.706	0.336	39	134	25	42	0.067	0.200
Jue	1	2	7.4	1479	5	1048	22	79	12	6	0.695	0.333	37	99	26	50	0.046	0.300
Jue	1	3	7.4	1961	8	1440	26	81	12	5	0.764	0.387	39	124	24	49	0.043	0.314
Jue	1	4	7.4	1540	7	1104	20	84	9	3	0.670	0.340	44	105	21	45	0.043	0.286
Jue	4	1	7.3	1971	21	1356	29	83	20	3	0.919	0.331	44	142	33	54	0.075	0.257
Jue	4	2	7.3	1655	11	1127	26	85	20	2	0.987	0.367	48	149	28	59	0.049	0.300
Jue	4	3	7.3	1760	10	1225	27	88	13	4	0.925	0.496	51	123	26	49	0.06	0.229
Jue	4	4	7.3	1904	15	1192	29	92	20	1	0.999	0.371	50	132	27	56	0.044	0.343
Jue	5	1	7.2	1409	15	978	23	90	20	2	0.935	0.341	65	125	33	59	0.052	0.329
Jue	5	2	7.3	1578	14	1008	24	88	23	5	1.055	0.397	59	115	35	65	0.053	0.371
Jue	5	3	7.3	1867	27	1230	28	91	30	2	1.054	0.360	53	126	30	51	0.057	0.271
Jue	5	4	7.3	1921	17	1423	29	94	19	1	0.955	0.349	59	146	30	55	0.066	0.243
Jue	9	1	7.3	1584	10	1017	23	92	16	5	0.874	0.411	47	104	33	62	0.06	0.314
Jue	9	2	7.3	2048	20	1521	27	93	22	3	0.999	0.343	45	138	26	49	0.049	0.271
Jue	9	3	7.4	1684	7	1155	26	94	14	4	0.867	0.381	57	123	33	64	0.049	0.386
Jue	9	4	7.4	1891	8	1365	26	91	14	4	0.895	0.368	54	145	30	55	0.061	0.271

Appendix 2.2 (cont.)

Site	treat	block	pH	total N	Min N	HCl	PB	HW	$CaCl_2$-t	$CaCl_2$-o	UV 205	UV 260	>200	200-63	14 days	84 days	14-84	rate
				N – mg kg^{-1}							absorbance		N – mg kg^{-1}				d^{-1}	mg $kg^{-1}d^{-1}$
KL	1	1	7.1	1233	5	824	19	71	14	10	0.725	0.389	26	80	29	48	0.077	0.300
KL	1	2	7.4	1828	6	1045	26	107	17	12	0.859	0.461	38	139	37	57	0.092	0.286
KL	1	3	7.3	1781	6	1059	25	105	14	10	0.835	0.446	32	154	40	58	0.079	0.271
KL	1	4	7.3	1310	6	887	20	84	14	9	0.795	0.409	29	93	34	52	0.083	0.271
KL	4	1	7.2	1595	4	1090	24	105	22	14	0.903	0.469	59	102	42	67	0.058	0.400
KL	4	2	7.3	1807	10	1230	26	107	23	14	1.047	0.495	47	149	48	69	0.057	0.371
KL	4	3	7.3	1933	7	1412	31	107	18	12	0.963	0.485	65	158	47	65	0.094	0.371
KL	4	4	7.0	1241	5	877	18	79	12	7	0.766	0.402	47	112	37	57	0.082	0.286
KL	6	1	7.4	1789	12	1218	28	103	24	14	1.045	0.484	48	136	47	68	0.066	0.343
KL	6	2	7.4	2387	17	1678	34	133	27	14	1.303	0.573	82	224	46	76	0.062	0.557
KL	6	3	7.4	1444	6	1045	21	103	18	13	0.951	0.493	56	100	40	64	0.111	0.357
KL	6	4	7.1	1224	7	856	19	81	15	9	0.838	0.435	47	100	41	63	0.085	0.343
Ot	1	1	7.0	1166	5	579	17	46	11	5	0.853	0.476	24	65	26	39	0.08	0.196
Ot	1	2	7.1	1137	5	595	17	49	10	5	0.851	0.469	31	58	25	37	0.075	0.174
Ot	1	3	7.0	1128	5	688	18	44	10	4	0.822	0.495	27	56	25	36	0.09	0.141
Ot	1	4	6.9	1196	6	616	19	53	10	5	0.925	0.492	32	56	31	43	0.101	0.194
Ot	4	1	6.9	1281	9	765	20	60	16	4	1.032	0.511	60	128	34	46	0.099	0.146
Ot	4	2	7.1	1291	5	737	20	54	14	5	0.902	0.462	35	90	31	46	0.091	0.197
Ot	4	3	7.0	1186	6	653	17	54	14	5	0.956	0.467	39	118	28	41	0.072	0.166
Ot	4	4	6.9	1189	7	560	19	60	13	4	1.099	0.480	22	108	31	42	0.096	0.161
Ot	6	1	7.0	1279	12	726	20	69	23	3	1.099	0.465			33	48	0.083	0.184
Ot	6	2	7.1	1336	9	796	21	61	17	4	1.147	0.482	38	113	36	44	0.111	0.106
Ot	6	3	7.0	1207	8	775	21	52	16	2	1.015	0.474	53	106	33	46	0.092	0.194
Ot	6	4	7.0	1181	13	658	21	53	20	4	1.125	0.496	45	95	34	43	0.114	0.129
HH	1	1	7.2	1153	2	828	18	73	10	5	0.640	0.349	35	105	22	36	0.094	0.140
HH	1	2	7.2	1038	3	707	15	55	9	5	0.685	0.375	24	77	19	32	0.066	0.181
HH	1	3	7.2	1050	3	705	15	64	9	5	0.645	0.329	28	104	22	36	0.092	0.136
HH	1	4	7.1	1058	2	744	17	64	10	4	0.601	0.324	28	114	22	32	0.102	0.121
HH	4	1	7.2	1073	3	702	17	67	10	6	0.596	0.311	34	96	24	35	0.105	0.143
HH	4	2	7.2	1043	3	803	17	57	11	6	0.660	0.376	30	78	19	32	0.078	0.149
HH	4	3	7.2	1038	3	681	17	61	10	6	0.701	0.373	33	93	22	33	0.095	0.131
HH	4	4	7.1	1225	4	926	23	74	14	8	0.708	0.367	45	121	28	38	0.098	0.144

Appendix 2.2. (cont.)

Site	treat	block	pH	total N	Min N	HCl	PB	HW	$CaCl_2$-t	$CaCl_2$-o	UV 205	UV 260	>200	200-63	14 days	84 days	14-84	rate
				N – mg kg^{-1}							absorbance		N – mg kg^{-1}				d^{-1}	mg $kg^{-1}d^{-1}$
R	1	1	7.1	1416	10	1016	24	80	22	9	0.951	0.451	77	122	42	77	0.043	0.510
R	1	2	7.2	1331	9	915	24	72	17	7	0.895	0.423	74	116	44	71	0.066	0.359
R	1	3	7.1	1347	10	902	27	67	17	4	0.864	0.413	65	100	37	60	0.054	0.361
R	1	4	7.1	1382	10	965	24	78	20	6	0.900	0.419	71	123	41	68	0.053	0.411
R	2	1	7.2	1198	12	833	20	62	16	4	0.820	0.380	42	85	27	51	0.04	0.347
R	2	2	6.9	1179	12	798	24	56	15	5	0.857	0.399	31	78	27	48	0.053	0.281
R	2	3	7.0	1266	11	849	23	59	17	6	0.883	0.401	31	81	28	50	0.046	0.320
R	2	4	7.1	1222	11	848	20	57	18	5	0.887	0.408	26	79	32	49	0.058	0.281
R	3	1	7.2	1131	13	770	20	56	17	0	0.808	0.345	22	66	26	47	0.05	0.303
R	3	2	7.1	1131	10	797	22	57	16	6	0.889	0.386	25	60	23	42	0.048	0.293
R	3	3	7.0	1188	11	812	21	51	14	4	0.796	0.382	24	67	26	45	0.043	0.300
R	3	4	7.2	1197	9	816	21	62	18	6	0.835	0.386	31	75	25	45	0.058	0.269

Appendix 3.1. Mineral N content as NH4+-N (mg kg-1) in a soil amended with wheat straw (0.8; 2.4 and 4.8 g kg-1 rates), without and with N fertilization (80 mg kg-1 rate). For each sampling means with the same letter are not significantly different (P<0.05). Letters are used only when the analysis of variance showed significant differences

	Days of incubation											
	2	7	14	21	28	35	49	63	79	91	106	120
N0-WS0	5.6 b	5.6 d	2.9 b	3.6	3.2	1.4	4.6	1.5	1.8	0.9	0.4	1.5
N0-WS0.8	4.8 b	5.1 d	3.5 b	2.1	2.1	1.5	1.7	1.5	1.9	0.9	0.9	1.2
N0-WS2.4	5.3 b	4.8 d	2.8 b	2.6	2.4	1.6	1.9	1.1	2.6	1.1	0.5	2.5
N0-WS4.8	4.9 b	5.0 d	3.0 b	1.8	2.7	1.8	1.8	1.6	2.4	0.8	0.3	0.9
N80-WS0	51.4 a	22.9 a	3.9 a	2.5	3.1	1.2	1.6	1.3	2.3	0.9	0.7	4.3
N80-WS0.8	53.6 a	18.8 b	3.7 a	2.3	2.1	1.3	1.9	1.3	2.4	1.8	0.7	0.8
N80-WS2.4	54.4 a	10.6 c	3.0 b	1.6	3.4	0.9	2.1	1.4	2.3	2.6	0.4	1.3
N80-WS4.8	45.1 a	5.6 d	3.0 b	2.8	2.2	1.1	2.2	1.2	2.2	1.7	0.6	1.1

Appendix 3.2. Mineral N content as NO_3^--N (mg kg^{-1}) in a soil amended with wheat straw (0.8; 2.4 and 4.8 g kg^{-1} rates), without and with N fertilization (80 mg kg^{-1} rate). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation											
	2	7	14	21	28	35	49	63	79	91	106	120
N0-WS0	17 b	21 c	25 d	27 d	31 d	36 d	42 d	53 d	62 d	73 d	79 c	90 c
N0-WS0.8	16 b	15 d	17 e	18 e	23 e	27 e	34 e	46 e	54 e	63 e	70 cd	84 c
N0-WS2.4	16 b	1 e	2 f	5 f	7 f	13 f	21 f	34 f	46 ef	56 ef	66 cd	78 c
N0-WS4.8	13 c	1 e	1 f	3 f	3 f	4 g	8 g	26 g	41 f	54 f	65 d	83 c
N80-WS0	32 a	65 a	83 a	83 a	94 a	96 a	101 a	113 a	124 a	144 a	152 a	163 a
N80-WS0.8	28 a	62 a	81 a	83 a	90 a	95 a	97 a	111 a	125 a	140 a	144 a	158 a
N80-WS2.4	29 a	58 ab	69 b	70 b	74 b	82 b	88 b	96 b	111 b	123 b	133 a	142 b
N80-WS4.8	32 a	54 b	58 c	57 c	61 c	68 c	69 c	83 c	97 c	106 c	118 b	134 b

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Appendix 3.3. Mineral N content (mg kg^{-1}) in a soil amended with wheat straw (0.8; 2.4 and 4.8 g kg^{-1} rates), without and with N fertilization (80 mg kg^{-1} rate). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation											
	2	7	14	21	28	35	49	63	79	91	106	120
N0-WS0	23 b	27 e	28 d	31 d	34 d	37 d	46 d	54 d	64 d	74 d	80 c	92 c
N0-WS0.8	21 b	20 f	20 e	20 e	23 e	28 e	36 e	47 e	56 de	64 de	71 cd	85 cd
N0-WS2.4	21 b	6 g	5 f	7 f	9 f	14 f	22 f	35 f	48 de	58 e	67 d	80 d
N0-WS4.8	18 b	6 g	4 f	4 f	6 f	5 g	9 g	27 g	43 e	55 e	65 d	84 cd
N80-WS0	83 a	88 a	87 a	85 a	97 a	97 a	103 a	115 a	127 a	145 a	153 a	167 a
N80-WS0.8	82 a	81 b	85 a	85 a	95 a	97 a	99 a	112 a	128 a	142 b	145 a	159 a
N80-WS2.4	83 a	69 c	72 b	72 b	77 b	83 b	90 b	97 b	113 b	126 c	133 a	143 b
N80-WS4.8	77 a	60 d	61 c	59 c	63 c	69 c	71 c	85 c	100 c	108 d	119 b	135 b

Appendix 3.4. Respiration rates (CO_2-C mg $kg^{-1}d^{-1}$) in a soil amended with wheat straw (0.8; 2.4 and 4.8 g kg^{-1} rates), without and with N fertilization (80 mg kg^{-1} rate). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation									
	7	14	28	35	49	63	79	91	106	120
N0-WS0	15.3 d	14.3 c	16.4 b	14.2 cd	14.8 c	14.7 bc	15.2 c	13.6 c	10.7 b	10.5 b
N0-WS0.8	21.5 c	21.2 b	18.3 b	17.5 c	16.6 c	16.5 b	17.3 b	15.2 b	11.3 b	12.2 b
N0-WS2.4	29.7 b	25.7 a	19.6 b	20.7 b	20.5 b	18.2 b	17.6 b	13.9 c	12.6 b	13.6 b
N0-WS4.8	30.8 b	29.7 a	27.0 a	27.4 a	25.0 a	24.5 a	21.0 a	17.2 a	12.6 b	14.3 b
N80-WS0	12.8 d	14.2 c	14.7 c	12.8 d	12.8 d	13.1 c	14.8 c	12.1 c	10.7 b	12.2 b
N80-WS0.8	16.2 d	23.5 b	20.6 b	13.2 d	12.8 d	13.8 c	13.3 c	12.7 c	11.2 b	12.7 b
N80-WS2.4	25.0 c	24.0 b	18.8 b	16.2 c	14.8 c	13.7 c	14.3 c	12.6 c	13.8 b	11.8 b
N80-WS4.8	38.7 a	29.3 a	19.4 b	20.9 b	16.0 c	16.6 b	18.3 b	15.8 b	15.6 a	14.3 a

Appendix 3.5. Mineral N content as NH_4^+-N (mg kg^{-1}) in a soil amended with wheat straw (WS at 0 and 2.4 g kg^{-1} rates). Different N fertilizers were added (100 mg kg^{-1} rate). N fertilizers: urea (U); Ammonium sulphate (AS) and Potassium sulphate (PN). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation					
	2	5	11	18	25	32
WS-N0	2 b	5 c	4 c	2 c	3 b	3 b
WS-U	74 a	42 b	9 c	3 c	2 b	3 b
WS-AS	99 a	53 a	19 b	6 c	5 b	3 b
WS-PN	4 b	4 c	3 c	2 c	1 b	2 b
N0	2 b	3 c	3 c	2 c	1 b	3 b
U	95 a	51 a	32 a	14 b	7 b	4 b
AS	86 a	70 a	51 a	33 a	28 a	22 a
PN	3 b	5 c	2 c	4 c	1 b	3 b

Appendix 3.6. Mineral N content as NO_3^--N (mg kg^{-1}) in a soil amended with wheat straw (WS at 0 and 2.4 g kg^{-1} rates). Different N fertilizers were added (100 mg kg^{-1} rate). N fertilizers: urea (U); Ammonium sulphate (AS) and Potassium sulphate (PN). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation					
	2	5	11	18	25	32
WS-N0	11 c	3 d	3 e	1 e	1 d	1 e
WS-U	16 b	37 c	72 c	67 d	77 b	70 c
WS-AS	16 b	37 c	56 d	69 d	76 b	72 c
WS-PN	113 a	109 b	90 b	92 b	90 a	90 b
N0	8 c	7 d	8 e	10 e	13 c	15 d
U	14 b	39 c	65 c	86 c	104 a	108 b
AS	13 b	35 c	55 d	74 d	93 a	95 b
PN	110 a	113 a	109 a	116 a	124 a	129 a

Appendix 3.7. Mineral N content as NO_3^--N (mg kg^{-1}) in a soil amended with wheat straw (WS at 0 and 2.4 g kg^{-1} rates). Different N fertilizers were added (100 mg kg^{-1} rate). N fertilizers: urea (U); Ammonium sulphate (AS) and Potassium sulphate (PN). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation					
	2	5	11	18	25	32
WS-N0	13 b	7 c	7 d	3 d	3 c	3 e
WS-U	90 a	80 b	80 c	69 c	72 b	73 c
WS-AS	115 a	90 ab	75 c	74 c	80 b	75 c
WS-PN	116 a	113 a	93 b	94 b	91 b	92 b
N0	9 b	10 c	11 d	12 d	15 c	18 d
U	109 a	90 ab	97 b	100 b	112 a	112 b
AS	99 a	105 a	106 a	107 b	121 a	117 b
PN	113 a	118 a	111 a	120 a	126 a	131 a

Appendix 3.8. Respiration rates (CO_2-C mg kg^{-1} d^{-1}) in a soil amended with wheat straw (WS at 0 and 2.4 g kg^{-1} rates). Different N fertilizers were added (100 mg kg^{-1} rate). N fertilizers: urea (U); Ammonium sulphate (AS) and Potassium sulphate (PN). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation										
	1	2	5	9	13	15	18	21	25	29	32
WS-N0	19.6 c	18.6 a	17.6 b	13.9 b	12.1 d	9.2 c	8.6 a	9.2 a	9.4 a	9.0 a	8.7 a
WS-U	38.5 a	20.5 a	20.8 a	19.0 a	15.3 a	11.8 a	8.9 a	8.4 ab	6.8 b	5.9 b	4.9 b
WS-AS	17.8 c	16.7 b	16.8 b	17.3 a	14.4 b	11.5 a	8.9 a	7.7 ab	6.7 b	6.3 b	4.7 b
WS-PN	19.9 c	17.7 ab	16.6 b	18.3 a	13.6 c	10.6 b	9.1 a	8.0 b	6.5 b	5.9 b	4.5 b
N0	13.8 d	10.8 c	7.4 c	5.3 c	5.8 e	5.4 d	4.4 b	4.8 c	4.1 c	4.6 c	3.2 c
U	26.9 b	8.8 d	5.8 cd	4.4 cd	4.8 e	3.9 e	3.7 b	3.2 d	2.9 d	2.1 d	1.5 d
AS	12.4 d	9.0 d	8.6 c	4.5 c	2.9 f	2.5 e	2.9 c	2.3 d	2.5 d	2.3 d	2.1 d
PN	10.4 d	7.9 d	3.5 d	3.1 d	2.7 f	3.5 e	3.0 c	3.4 d	2.2 d	1.9 d	1.8 d

Appendix 3.9. pH ($CaCl_2$) of a soil amended with wheat straw (WS at 0 and 2.4 g kg^{-1} rates). Different N fertilizers were added (100 mg kg^{-1} rate). N fertilizers: urea (U); Ammonium sulphate (AS) and Potassium sulphate (PN). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation								
	1	2	5	9	13	15	18	25	32
WS-N0	5.0 b	5.0 a	5.0 a	4.9 a	4.8 b	4.9 a	4.9 a	5.0 a	5.1 a
WS-U	5.3 a	5.2 a	5.0 a	4.7 c	4.6 d	4.6 c	4.7 b	4.7 b	4.9 c
WS-AS	4.9 bc	4.8 c	4.7 c	4.5 d	4.4 e	4.4 e	4.5 c	4.5 d	4.6 e
WS-PN	5.0 b	4.9 b	5.0 a	4.9 a	4.9 a	4.9 a	4.9 a	5.0 a	5.1 a
N0	4.9 bc	4.9 b	4.9 b	4.8 b	4.8 b	4.8 b	4.8 a	4.9 a	5.0 b
U	5.2 a	5.3 a	5.0 a	4.8 d	4.6 d	4.6 c	4.6 b	4.6 c	4.7 d
AS	4.8 c	4.8 c	4.7 c	4.4 e	4.4 e	4.5 d	4.5 c	4.5 d	4.6 e
PN	4.9 bc	4.9 b	4.9 b	4.7 c	4.7 c	4.8 b	4.8 a	4.9 a	5.0 b

Appendix 3.10. Regression of cumulative evolved CO_2-C (mg kg^{-1}) on immobilized N (mg kg^{-1}) in a soil amended with wheat straw (0.8; 2.4 and 4.8 g kg^{-1} rates), without and with N fertilization (80 mg kg^{-1} rate). After day 63 not significant relationships were found (P<0.05).

	Days of incubation					
	7	14	28	35	49	63
Intercept	83.04	224.1	475.5	627.4	754.21	997.1
Slope	6.22	9.28	10.71	11.8	15.75	19.8
R^2	0.86	0.9	0.92	0.93	0.99	0.74

Appendix 4.1. Respiration rates (CO_2-C mg kg^{-1} d^{-1}) in a soil amended with wheat straw, sunflower and maize residues (WS, Sun, M at 2.4 g kg^{-1} rates). without and with N fertilization (80 mg kg^{-1} rate). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation					
	6	13	33	61	82	131
N0-R0	4.5 b	4.3 c	2.5 b	4.2 b	2.4 a	3.6 a
N0-WS	10.1 a	9.2 ab	6.7 a	6.5 a	2.7 a	2.8 a
N0-Sun	10.2 a	9.6 a	5.9 a	6.7 a	4.1 a	3.7 a
N0-M	10.3 a	10.7 a	6.3 a	8.6 a	3.6 a	3.7 a
N80-R0	3.5 b	3.9 c	2.6 b	3.8 b	2.2 a	2.9 a
N80-WS	8.1 a	7.2 b	3.5 b	5.1 ab	2.6 a	3.5 a
N80-Sun	9.1 a	5.7 bc	2.6 b	2.9 b	3.2 a	3.5 a
N80-M	10.4 a	8.7 a	5.4 ab	6.7 a	2.4 a	4.3 a

Appendix 4.2. Mineral N content as NH_4^+-N (mg kg^{-1}) in a soil amended with wheat straw, sunflower and maize residues (WS, Sun, M at 2.4 g kg^{-1} rates). without and with N fertilization (80 mg kg^{-1} rate). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation								
	5	12	19	33	47	61	82	103	131
N0-R0	4.2 c	2.1 e	0.1 e	0.1 c	2.2 c	2.4 d	2.1 c	0.8 d	1.6 e
N0-WS	3.7 c	3.0 e	0.1 e	0.1 c	1.6 c	3.1 d	2.3 c	0.9 d	1.4 e
N0-Sun	5.1 c	2.1 e	0.1 e	0.4 c	2.0 c	2.4 d	2.5 c	0.9 d	1.3 e
N0-M	4.0 c	2.8 e	0.1 e	0.1 c	1.7 c	2.7 d	2.4 c	1.1 d	1.8 e
N80R0	47.2 a	33.6 a	20.2 a	8.1 a	7.1 a	12.1 a	10.8 a	7.8 a	8.1 a
N80-WS	43.0 a	23.3 b	13.1 b	2.2 b	5.8 b	7.8 b	7.8 a	6.0 b	7.1 b
N80-Sun	35.0 b	15.5 d	3.2 d	0.8 bc	1.8 c	3.4 d	3.8 bc	1.3 d	2.8 d
N80-M	35.1 b	20.6 c	8.8 c	3.4 b	2.4 c	5.2 c	4.3 b	2.0 c	4.4 c

Appendix 4.3. Mineral N content as NO_3^--N (mg kg^{-1}) in a soil amended with wheat straw, sunflower and maize residues (WS, Sun, M at 2.4 g kg^{-1} rates). without and with N fertilization (80 mg kg^{-1} rate). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation								
	5	12	19	33	47	61	82	103	131
N0-R0	13.8 b	14.6 b	17.0 d	23.8 c	30.7 d	35.6 c	47.3 c	56.1 c	69.1 d
N0-WS	2.0 c	0.9 c	0.1 e	2.5 d	9.5 e	19.6 d	34.0 d	44.3 d	54.1 e
N0-Sun	0.3 c	1.1 c	0.1 e	0.2 d	5.3 ef	15.2 d	31.7 de	40.6 de	52.4 e
N0-M	0.1 c	1.0 c	0.1 e	0.1 d	2.4 f	12.9 de	28.0 e	38.9 e	48.6 e
N80R0	44.5 a	66.8 a	84.1 b	105.2 a	111.8 a	115.7 a	127.3 a	144.4 a	150.7 a
N80-WS	47.9 a	66.3 a	80.0 c	100.2 b	109.3 a	110.5 b	124.8 a	129.3 b	142.2 ab
N80-Sun	46.6 a	67.8 a	88.5 a	102.2 ab	106.3 b	108.3 b	124.8 a	129.3 b	143.9 b
N80-M	47.3 a	62.3 a	78.8 c	92.1 b	98.0 c	106.2 b	115.1 b	129.1 b	126.2 c

Appendix 4.4. Mineral N content in a soil amended with wheat straw, sunflower and maize residues (WS, Sun, M at 2.4 g kg^{-1} rates). without and with N fertilization (80 mg kg^{-1} rate). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation								
	5	12	19	33	47	61	82	103	131
N0-R0	18.0 b	16.6 d	17.0 d	23.9 d	32.9 d	38.0 d	49.4 c	56.8 d	70.7 d
N0-WS	5.7 c	3.9 e	0.2 e	2.6 e	11.1 e	22.7 e	36.3 d	45.2 e	55.5 e
N0-Sun	5.4 c	3.2 e	0.2 e	0.7 e	7.4 ef	17.7 f	34.2 d	41.5 f	53.7 e
N0-M	4.0 c	3.8 e	0.2 e	0.2 e	4.1 f	15.6 f	30.5 d	40.1 f	50.3 e
N80R0	91.8 a	100.4 a	104.4 a	113.3 a	118.9 a	127.8 a	138.1 a	152.2 a	158.8 a
N80-WS	90.9 a	89.6 b	93.1 b	102.4 b	115.1 a	118.3 b	132.6 a	135.2 b	149.3 b
N80-Sun	81.6 a	83.3 c	91.7 b	103.0 b	108.1 b	111.7 c	128.6 ab	130.6 bc	146.7 b
N80-M	82.5 a	83.0 c	87.6 c	95.5 c	100.5 c	111.5 c	119.4 b	131.1 c	130.5 c

Appendix 4.5. Respiration rates (CO_2-C mg kg^{-1} d^{-1}) in a soil amended with egyptian clover young shoots (C-y); egyptian clover mature shoots (C-m) egyptian clover roots (C-r) sunflower leaves and stems (Sun-L and Sun-S); Soybean leaves and stems (Soy-L and Soy-S); and maize residues (M) at 2.4 g kg^{-1} rates. For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation										
	1	2	4	8	11	15	18	22	25	30	32
C-y	52.3 bc	48.5 c	25.8 b	12.8 bc	7.7 ab	4.4 b	3.8 d	3.8 b	3.5 bc	4.6 ab	4.0 cd
C-m	57.4 b	42.9 c	22.6 b	13.4 b	7.7 ab	5.3 b	3.9 d	3.7 b	2.4 cd	4.3 b	6.0 a
C-r	47.3 c	56.2 b	33.2 a	15.0 a	8.9 a	5.1 b	4.7 c	3.7 b	3.9 ab	3.6 bc	4.8 bc
Sun-L	25.6 d	28.5 d	22.5 b	11.3 d	7.5 ab	4.8 b	5.5 bc	3.8 b	3.2 bc	3.9 b	4.8 bc
Sun-S	66.7 a	65.3 a	25.5 b	11.4 cd	6.8 b	4.8 b	4.2 cd	4.1 b	3.2 bc	3.4 bc	4.1 cd
Soy-L	27.9 d	19.7 e	16.2 c	10.9 e	7.6 ab	4.9 b	4.5 c	3.7 b	2.9 c	3.8 b	4.8 bc
Soy-S	30.8 d	19.2 e	17.6 c	12.5 bc	7.8 ab	5.9 a	5.3 ab	5.3 a	3.2 bc	5.0 a	5.6 ab
M	12.4 e	11.5 f	15.4 c	13.7 ab	8.7 a	6.6 a	6.4 a	5.9 a	4.8 a	4.6 ab	5.8 ab
Control	3.4 f	2.3 g	1.4 d	2.6 f	2.3 c	1.7 c	2.0 e	1.6 c	1.9 d	2.0 d	3.1 d

	Days of incubation (cont.)									
	39	46	53	60	67	77	81	88	95	102
C-y	3.2 cd	3.2 bc	2.9 ab	2.8 bc	2.8 a	2.8 ab	3.9 a	1.4 a	1.4 a	1.6 a
C-m	3.4 b	3.1 c	2.7 ab	2.9 bc	2.5 ab	2.2 b	3.6 a	1.8 a	1.1 a	0.9 a
C-r	3.2 cd	3.4 b	3.3 a	3.2 ab	3.3 a	3.2 ab	4.0 a	2.2 a	1.5 a	1.2 a
Sun-L	2.6 d	2.2 d	2.2 b	2.6 bc	2.9 a	2.4 b	3.4 a	1.8 a	1.7 a	0.8 a
Sun-S	2.6 d	3.1 c	2.7 ab	3.2 ab	2.9 a	2.8 ab	4.2 a	2.1 a	1.4 a	1.6 a
Soy-L	3.4 b	4.0 a	3.2 a	2.4 bc	2.8 a	2.8 ab	3.7 a	1.9 a	1.6 a	1.0 a
Soy-S	4.5 a	3.6 a	3.4 a	3.7 a	3.5 a	3.4 a	4.4 a	1.8 a	1.1 a	1.2 a
M	3.6 b	3.5 a	3.1 a	4.0 a	3.1 a	2.8 ab	3.9 a	2.1 a	1.5 a	0.8 a
Control	1.4 e	1.3 d	1.2 c	1.8 c	1.5 b	2.1 b	3.7 a	1.7 a	1.0 a	1.1 a

Appendix 4.6. Mineral N content (mg kg^{-1}) in a soil amended with egyptian clover young shoots (C-y); egyptian clover mature shoots (C-m) egyptian clover roots (C-r) sunflower leaves and stems (Sun-L and Sun-S); Soybean leaves and stems (Soy-L and Soy-S); and maize residues (M) at 2.4 g kg^{-1} rates. For each sampling means with the same letter are not significantly different ($P<0.05$).

	Days of incubation							
	1	8	16	23	30	44	58	79
C-y	64.2 c	78.7 a	92.4 a	96.0 a	106.2 a	107.3 a	128.3 a	146.1 a
C-m	66.7 cb	70.1 b	84.5 b	87.1 b	92.9 b	100.3 b	116.5 b	122.8 c
C-r	68.9 ab	71.5 b	78.4 c	82.1 b	90.9 b	93.4 c	112.2 b	129.9 b
Sun-L	66.0 cb	57.5 c	62.2 e	61.3 d	66.5 d	72.1 e	78.3 e	82.5 f
Sun-S	43.4 d	46.1 d	48.4 f	50.2 e	53.4 e	60.1 f	62.6 g	72.2 g
Soy-L	66.4 cb	58.8 c	69.2 d	70.7 c	81.2 c	78.6 d	88.5 d	89.6 e
Soy-S	66.0 cb	56.2 c	61.5 e	60.9 d	61.3 d	70.0 e	72.1 f	75.8 g
M	71.0 a	57.1 c	56.9 e	55.4 de	61.2 d	61.7 f	69.9 f	73.7 g
Control	71.3 a	73.9 b	79.1 c	81.9 b	87.2 bc	89.1 c	96.4 c	98.0 d

Appendix 5.1. Respiration rates (CO_2-C mg $kg^{-1}d^{-1}$) in a clay soil incubated at different temperatures (5; 12; 21; 31 and 40^{0}C). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation																
Temp	1	3	6	8	10	13	15	17	20	22	27	30	32	35	38	41	43
40^{0}C	57.0 a	16.4 a	18.1 a	14.8 a	12.7 a	14.1 a	13.3 a	13.0 a	13.9 a	12.5 a	12.9 a	12.0 a	11.1 a	11.4 a	12.3 a	14.0 a	13.0 a
31^{0}C	21.2 b	12.2 b	12.6 b	8.6 b	7.1 b	8.0 b	6.7 b	6.8 b	7.0 b	5.2 b	5.5 b	5.3 b	4.0 b	3.9 b	5.9 b	4.9 b	4.8 b
21^{0}C	5.7 c		5.3 c	6.1 c		3.4 c	4.3 c		3.7 c	3.5 b	3.3 b	3.0 c		3.1 b	4.2 b	3.4 b	2.5 c
12^{0}C	3.6 d			2.1 d			1.6 d			1.3 c		1.0 d		1.7 c			1.1 d
5^{0}C	1.9 d			1.0 e			0.9 e			0.8 c		0.3 e		0.5 d			0.7 e

	Days of incubation (cont.)																
Temp	45	48	50	52	55	58	62	66	69	73	76	80	83	87	90	94	97
40^{0}C	10.9 a	10.7a	10.5 a	11.0 a	10.4 a	10.5 a	9.9 a	9.7 a	11.8 a	11.1 a	9.6 a	10.1 a	11.4 a	11.8 a	10.6 a	11.2 a	11.5 a
31^{0}C	4.7 b	5.3 b	4.7 b	4.0 b	3.9 b	4.8 b	4.1 b	3.6 b	3.9 b	3.6 b	3.3 b	3.8 b	3.5 b	4.3 b	4.1 b	3.7 b	4.3 b
21^{0}C		3.1 b	2.6 c		2.9 b	2.4 c	3.3 b	2.9 b	1.6 b	2.0 c		2.7 b		2.3 c		2.8 b	
12^{0}C			1.1 d			1.1 d		1.3 c		1.2 d		1.1 c		0.9 d		1.2 c	
5^{0}C			0.7 e			0.7 d		0.7 c		0.5 d		0.7 c		0.7 d		0.8 c	

	Days of incubation (cont.)																
Temp	101	104	108	111	115	122	129	136	143	150	157	164	171	178	185	192	199
40^{0}C		10.5 a	10.9 a	13.3 a	12.9 a	11.9 a	12.5 a	12.2 a	12.3 a	12.4	13.8 a	13.5 a	14.1 a	13.4 a	14.1 a	13.4 a	14.8 a
31^{0}C	4.3 a	3.9 b	4.1 b	3.8 b	4.0 b	3.5 b	3.5 b	3.6 b	3.2 b	2.9	3.0 b	2.7 b	2.5 b	2.4 b	2.4 b	2.0 b	3.0 b
21^{0}C	2.8 b		2.2 c		2.2 c	2.0 b	2.4 b	1.9 c	2.0 c	1.5	2.5 b	1.4 c	2.1 b				0.8 c
12^{0}C	0.7 c		1.4 c		0.6 d		0.8 c		0.5 d		0.8 c		0.9 bc		0.8 c		0.3 d
5^{0}C	0.6 c		1.0 d		0.5 d		0.6 d		0.4 d		0.6 c		0.3 c		0.5 d		

	Days of incubation (cont.)													
Temp	206	213	220	227	234	241	248	255	267	282	297	317	338	366
40^{0}C	14.2 a	12.5 a	12.4 a	11.8 a	11.6 a	12.8 a	13.7 a	12.3 a						
31^{0}C	2.6 b	2.7 b	2.4 b	2.7 b	2.3 b	2.7 b	2.3 b	2.3 b						
12^{0}C		0.7 c		0.6 c		0.4 c		0.6 c	0.8 a	0.4 a	0.6 a	0.5 a	0.3 a	0.3 a
5^{0}C		0.5 c		0.2 d		0.3 c		0.4 c	0.3 b	0.2 b	0.3 a	0.2 b	0.1 a	0.2 a

Appendix 5.2. Respiration rates (CO_2-C mg $kg^{-1}d^{-1}$) in a sandy soil incubated at different temperatures (5; 12; 21; 31 and 40^0C). For each sampling means with the same letter are not significantly different ($P<0.05$).

	Days of incubation																
Temp	2	4	8	12	16	19	23	26	30	33	37	40	44	47	51	54	58
40^0C	13.8 a	12.1 a	10.3 a	8.8 a	7.9 a	7.3 a	7.2 a	7.4 a	8.3 a	8.0a	7.0 a	5.7 a	6.2 a	6.4 a		6.1 a	5.0 a
31^0C	9.7 a	6.5 b	5.3 b	5.2 b	4.7 b	4.8 b	3.9 b	5.1 b	4.4 b	3.4b	4.2 b	4.5 b	4.5 b	5.3 a	4.9 a	4.0 b	3.6 ab
21^0C		6.2 b	2.4 c	2.1 c	2.8 c	1.9 c	1.7 c		2.9 c		2.2 c		3.1 b		2.1 b		2.6 b
12^0C			1.6 d		1.2 d		0.6 d		1.2 d		1.0 d		1.2 c		0.9 c		1.1 c
5^0C			1.1 e		0.3 e		0.3 d		0.4 e		0.2 e		0.6 c		0.7 c		0.6 c

	Days of incubation (cont.)																
Temp	61	65	72	79	86	93	100	104	107	114	121	128	135	142	149	156	163
40^0C	6.7 a	4.9 a	5.9 a	4.9 a	4.4 a	4.4 a	3.5 a		4.5 a	3.3 a	2.8 a	3.1 a	3.2 a	2.9 a	3.5 a	3.8 a	3.2 a
31^0C	3.4 a	3.5 b	3.3 b	3.7 b	3.5 b	3.1 b	3.4 a		2.9 b	2.8 a	2.2 a	2.3 a	2.8 a	2.7 a	3.1 b	2.8 a	3.1 a
21^0C		1.9 c	1.8 c	3.1 b	2.4 c	2.4 c	2.0 b		2.4 b	1.9 b	2.2 a						
12^0C		0.5 d		0.8 c		0.5 d		0.8 a			0.8 b		0.8 b		0.7 c		0.5 b
5^0C		0.3 d		0.5 c		0.3 d		0.5 b			0.4 b		0.4 b		0.2 d		0.5 b

	Days of incubation (cont.)												
Temp	170	176	183	190	197	204	211	223	238	253	274	283	311
40^0C	3.4 a		3.3 a	3.3 a	3.7 a	3.8 a	3.5 a						
31^0C	3.5 a		2.8 a	2.7 a	3.2 a	3.2 a	3.1 a						
12^0C		0.4 a			0.5 b		0.6 b	0.9 a	0.4 a	0.5 a	0.4 a	0.3 a	0.3 a
5^0C		0.2 a			0.5 b		0.3 b	0.2 b	0.2 a	0.3 a	0.2 b	0.2 a	0.1 a

Appendix 5.3. Mineral N content as NH_4^+-N (mg kg^{-1}) in a clay soil incubated at different temperatures (5; 12; 21; 31 and 40^0C). For each sampling means with the same letter are not significantly different (P<0.05). Letters are used only when the analysis of variance showed significant differences.

	Days of incubation													
Temp	7	14	21	28	35	42	49	63	77	91	112	126	147	175
40^0C	2.7	1.1	0.7	1.0	0.1	0.1	0.4	3.5	2.9	8.2 a	20.3 a	22.6 a	46.5 a	53.0 a
31^0C	1.6	0.8	0.1	0.1	0.1	0.5	0.1	1.1	0.5	1.1 b	6.1 b	3.8 b	6.4 b	3.0 b
21^0C	2.3	1.7	0.2	0.7	0.3	0.4	0.1	1.0	0.8	1.1 b	7.4 b	1.9 b	1.7 b	1.5 b
12^0C	1.7	1.7	1.3	1.4	0.1		0.3	2.0	1.1	1.3 b	6.8 b	2.5 b	1.9 b	1.8 b
5^0C	3.8	2.7	0.8		0.1		0.1	2.0	2.0	0.7 b	8.3 b	2.2 b	1.9 b	1.7 b

Appendix 5.4. Mineral N content as NO_3^--N (mg kg^{-1}) in a clay soil incubated at different temperatures (5; 12; 21; 31 and 40^0C). For each sampling means with the same letter are not significantly different (P<0.05). Letters are used only when the analysis of variance showed significant differences.

	Days of incubation													
Temp	7	14	21	28	35	42	49	63	77	91	112	126	147	175
40^0C	40.0 a	54.5 a	71.7 a	82.5 a	95.4 a	106.8 a	121.0 a	161.3 a	177.6 a	203.7 a	195.6 a	214.9 a	207.3 a	238.4a
31^0C	26.9 b	33.6 b	42.6 b	53.3 b	56.3 b	64.4 b	69.3 b	80.3 b	100.6 b	126.0 b	128.9 b	140.5 b	144.2 b	187.4 b
21^0C	21.8 bc	24.7 c	27.9 c	29.8 c	31.1 c	33.6 c	42.7 c	38.4 c	46.6 c	56.2 c	58.9 c	68.7 c	76.5 c	92.3 c
12^0C	20.7 c	23.6 c	25.9 d	28.1 c	27.2 c		30.4 d	32.9 cd	34.7 d	38.7 d	40.3 d	46.3 d	44.8 d	53.3 d
5^0C	18.0 c	22.4 c	24.8 d		25.8 c		28.6 d	29.1 d	32.0 d	35.0 e	36.9 d	37.7ed	40.1 d	45.2 e

Appendix 5.5. Mineral N content (mg kg^{-1}) in a clay soil incubated at different temperatures (5; 12; 21; 31 and 40^0C). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation													
Temp	7	14	21	28	35	42	49	63	77	91	112	126	147	175
40^0C	42.7 a	55.6 a	72.3 a	83.5 a	95.4 a	107.0 a	121.4 a	158.6 a	174.8 a	211.8 a	216.0 a	237.5 a	253.8 a	291.4 a
31^0C	28.4 b	34.5 b	42.6 b	53.3 b	56.5 b	64.9 b	69.4 b	81.4 b	102.8 b	127.0 b	135.0 b	144.3 b	150.7 b	190.4 b
21^0C	24.1 bc	26.4 c	28.2 c	30.6 c	31.4 c	34.0 c	42.8 c	39.5 c	47.5 c	57.2 c	66.3 c	70.5 c	78.2 c	93.8 c
12^0C	22.5 c	25.4 c	26.9 d	29.5 c	27.2 c		30.6 d	34.9 cd	35.7 d	40.1 d	47.1 d	48.8 d	46.7 d	55.1 d
5^0C	21.8 c	25.1 c	25.6 d		26.0 c		28.7 d	31.1 d	34.0 d	35.7 e	45.2 d	39.9 e	42.0 d	46.9 e

Appendix 5.6. Mineral N content as NH_4^+-N (mg kg^{-1}) in a sandy soil incubated at different temperatures (5; 12; 21; 31 and 40^0C). For each sampling means with the same letter are not significantly different (P<0.05). Letters are used only when the analysis of variance showed significant differences

	Days of incubation							
Temp	6	13	27	41	62	76	97	125
40^0C	2.6	3.2	2.0 a	3.8 a	17.0 a	27.9 a	28.2 a	
31^0C	0.3	2.3	0.6 b	1.1 b	4.7 b	3.9 b	3.7 b	
21^0C	0.5	2.4	0.2 b	0.2 b	3.3 b		1.7 b	0.8
12^0C	0.8	2.3	0.2 b	0.8 b	5.2 b		2.3 b	1.7
5^0C	0.6	0.7	0.1 b	0.8 b	3.0 b		2.0 b	1.3

Appendix 5.7. Mineral N content as NO_3^--N (mg kg^{-1}) in a sandy soil incubated at different temperatures (5; 12; 21; 31 and 40^0C). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation							
Temp	6	13	27	41	62	76	97	125
40^0C	28.1 a	44.5 a	65.3 a	79.9 a	64.4 a	97.4 a	99.6 b	
31^0C	19.3 b	30.3 b	48.6 b	65.3 b	64.6 a	92.0 a	109.2 a	
21^0C	15.1 c	20.3 c	28.5 c	36.4 c	38.7 b		60.7 c	71.6 a
12^0C	13.4 d	16.2 d	21.5 d	25.2 d	22.9 c		34.2 d	39.2 b
5^0C	13.8 d	16.8 d	20.0 d	21.6 e	23.4 c		29.5 e	31.7 b

Appendix 5.8. Mineral N content mg kg^{-1}) in a sandy soil incubated at different temperatures (5; 12; 21; 31 and 40^0C). For each sampling means with the same letter are not significantly different (P<0.05).

	Days of incubation							
	6	13	27	41	62	76	97	125
40^0C	30.7 a	47.7 a	67.2 a	83.6 a	81.3 a	125.3 a	127.8 a	
31^0C	19.6 b	32.7 b	49.2 b	66.4 b	69.3 b	95.9 b	112.9 b	
21^0C	15.7 c	22.7 c	28.7 c	36.7 c	42.0 c		62.4 c	72.4 a
12^0C	14.2 c	18.5 d	21.7 d	26.0 d	28.0 d		36.4 d	40.9 b
5^0C	14.4 c	17.5 e	20.1 d	22.4 e	26.4 d		31.5 e	33.0 b

Appendix 5.9. Respiration rates (CO_2-C mg $kg^{-1}d^{-1}$) in a clay soil incubated at different soil water potentials (-0.01; -0.10; -0.14; -0.90; -1.00 and –2.00 MPa). For each sampling means with the same letter are not significantly different (P<0.05).

Water level		Days of incubation											
Grav.	W. Pot.	1	5	9	12	16	23	30	37	44	51	58	63
0.37	-0.01	26.3 a	18.4 a	8.2 a	6.7 a	7.6 a	5.2 a	4.5 a	5.4 a	4.4 a	4.0 a	3.3 a	2.8 a
0.28	-0.10	21.6 ab	17.5 ab	8.0 a	5.4 b	6.2 b	4.1 b	3.0 b	3.5 b	2.9 b	3.0 b	2.3 b	2.4 ab
0.26	-0.14	21.6 ab	16.2 b	7.1 b	5.3 b	5.7 b	3.9 b	2.9 b	3.5 b	2.6 b	2.7 bc	2.2 bc	2.0 bc
0.19	-0.90	19.1 bc	11.9 c	6.1 c	4.2 c	4.9 c	3.2 c	2.5 bc	3.1 c	2.5 b	2.4 c	2.0 bc	2.0 bc
0.18	-1.00	17.8 cd	9.3 d	5.1 d	3.7 cd	4.7 c	3.1 c	2.2 c	2.8 cd	2.2 c	2.3 c	1.8 c	1.8 cd
0.16	-2.00	17.0 d	8.5 d	4.9 d	3.2 d	4.2 c	2.5 d	2.1 c	2.5 d	2.2 c	1.8 d	1.8 c	1.6 d

Appendix 5.10. Respiration rates (CO_2-C mg $kg^{-1}d^{-1}$) in a sandy soil incubated at different soil water potentials (-0.01; -0.03; -0.08; -0.28; and –0.67 MPa). For each sampling means with the same letter are not significantly different (P<0.05).

Water level		Days of incubation													
Grav.	W. Pot.	2	6	9	13	16	20	23	29	36	43	50	57	64	71
0.18	-0.01	3.4 a	3.3 a	5.4 a	3.8 a	3.4 a	3.5 a	3.0 a	2.1 a	2.0 a	3.1 a	2.1 a	2.1 a	2.9 a	1.9 a
0.16	-0.03	2.8 ab	2.9 b	4.9 a	3.5 a	3.5 a	3.1 a	2.7 a	1.6 ab	1.8 ab	2.4 b	1.8 a	1.8 a	2.7 a	1.5 ab
0.14	-0.08	2.7 ab	3.1 b	4.5 ab	3.6 a	2.8 b	2.6 b	2.2 ab	1.5 ab	1.5 bc	2.8 ab	2.0 a	1.1 b	2.4 a	1.2 b
0.12	-0.28	1.2 c	2.4 c	4.3 b	3.3 ab	2.2 c	2.1 c	1.8 bc	1.2 b	1.0 c	1.5 c	1.1 b	0.9 b	1.4 b	0.9 b
0.10	-0.67	1.5 c	2.4 c	3.2 b	2.8 b	2.0 c	2.1 c	0.6 c	1.0 b	1.0 c	1.4 c	1.2 b	0.8 b	1.1 b	0.8 b

Appendix 5.11. Net mineralized N (mg $kg^{-1}d^{-1}$) in a clay soil incubated at different soil water potentials (-0.03; -0.12; -0.28; and –1.81 MPa). For each sampling means with the same letter are not significantly different (P<0.05).

Water level		Days of incubation			
Grav.	W. Pot.	7	21	35	49
0.32	-0.03	8.4 a	34.0 a	47.4 a	50.7 a
0.27	-0.12	5.1 ab	25.4 ab	36.3 b	41.7 b
0.23	-0.28	5.1 ab	23.2 bc	31.0 b	32.4 c
0.17	-1.81	3.5 b	16.8 c	23.4 c	23.1 d

Appendix 5.12. Mineral N content (mg kg^{-1}) in a sandy soil incubated at different potentials (-0.01; -0.03; -0.08; -0.28; and –0.67 MPa). For each sampling means with the same letter are not significantly different (P<0.05).

Water level		Days of incubation								
Grav.	W. Pot.	1	7	14	21	28	35	49	63	72
0.18	-0.01	22.7 a	26.8 a	31.9 a	33.4 a	35.6 a	37.6 a	42.9 a	48.8 a	51.2 a
0.16	-0.03	23.0 a	29.2 a	30.7 a	32.4 a	34.9 a	37.6 a	41.0 a	44.3 b	49.3 a
0.14	-0.08	23.9 a	27.3 a	28.4 b	30.2 b	31.8 b	34.0 b	39.1 b	41.7 bc	45.2 b
0.12	-0.28	23.3 a	27.9 a	28.2 b	30.3 b	31.4 b	33.1 bc	35.5 c	39.7 c	40.2 c
0.10	-0.67	21.4 a	26.1 a	25.8 b	27.4 b	28.6 b	28.6 c	31.9 c	34.3 d	34.3 d

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