Optimisation of N Release

Influence of plant material chemical composition on C and N mineralisation

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2003

Acta Universitatis Agriculturae Sueciae
Agraria 381

ISSN 1401-6249
ISBN 91-576-6422-6

Tryck: SLU Service/Repro, Uppsala 2003

Abstract

Gunnarsson, S. 2003. *Optimisation of N release - influence of plant material chemical composition on C and N mineralisation*. Doctor's dissertation.
ISSN 1401-6249, ISBN 91-576-6422-6

Nitrogen mineralisation from green manure needs to be managed to meet crop N demand and minimise N losses. One way of achieving this can be to use differences in chemical composition between plant materials as a means to steer the processes either towards immobilisation or mineralisation during specific periods. The overall objective of this thesis was to improve the understanding of N mineralisation processes during decomposition of temperate-zone grasses and legumes, especially in relation to concentrations of specific carbohydrates and N compounds. Possible ways of using differences in composition as a means to steer the N mineralisation pattern were also investigated.
Mineralisation of C and N during decomposition of pure plant components and materials were studied under controlled conditions. The most abundant plant components could be subdivided into three groups based on when they had their main influence on C and N mineralisation. This subdivision can be used when choosing plant material to achieve a desired effect on the pattern of N mineralisation. To enhance immobilisation of N during the initial days of decomposition plant materials rich in free sugars and fructans may be used. To prolong the initial immobilisation phase for a period of up to two weeks plant materials rich in starch, pectin, or with a hemicellulose characterised by a high arabinose-to-xylose ratio may be used. To reduce N mineralisation during later stages of decomposition plant materials dominated by slowly decomposable structural carbohydrates, such as xylan and cellulose, may be used. Under Swedish field conditions these periods will be approximately two to three times longer than this depending on the time of incorporation due to lower temperature and suboptimal moisture conditions.
In order to obtain the necessary information about concentrations of pectin, arabinose and xylose detailed plant analysis, rather than the common proximate neutral/acid-detergent analysis, is required. More knowledge is also required concerning the significance of the effect of chemical composition on N mineralisation in a field situation. New tailor-made plant materials with a greater range of chemical properties, compared to the present plant materials, may be needed to achieve the intended effects in a field situation.

Keywords: green manure, decomposition, free sugars, fructans, pectic substances, hemicellulose, arabinose, xylose, cellulose, protein

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Nu har jag kommit fram till
Att man aldrig kommer fram till
Någonting
Man blir aldrig fääääääärdig!

Så där ja, - nu e jag klar!

-Robert Broberg

Contents

Appendix

Papers I-IV

The present thesis is based on the following papers, which are referred to by their Roman numerals:

I. Gunnarsson, S. & Marstorp, H. 2002. Carbohydrate composition of plant materials determines N mineralisation. *Nutrient cycling in agroecosystems 62*, 175-183.

II. Gunnarsson, S. Marstorp, H. & Witter, E. Influence of non-cellulose structural carbohydrate composition on plant material decomposition in soil. (Submitted)

III. Gunnarsson, S. Influence of plant carbohydrate composition on net N immobilisation and mineralisation. (Manuscript).

IV. Sindhøj, E. Andrén, O. Kätterer, T. & Gunnarsson, S. Projections of 30-year soil carbon balances for a semi-natural grassland under elevated CO_2 based on measured root decomposability. (Submitted).

Introduction

N mineralisation and environmental goals

Nitrogen (N) is a plant nutrient essential for cropping, but N also constitutes a considerable environmental threat causing eutrophication and damage to valuable water resources. Approximately 50% of the total N loading to inland waters in Sweden has its origin in Swedish agriculture (SEPA, 1997; Johnsson and Hoffman, 1998). Depending on region, leaching of N from Swedish agricultural soils to inland waters has been estimated to range between 6 and 47 kg N ha^{-1} $year^{-1}$, with a total average loss of around 22 kg N ha^{-1} $year^{-1}$ (Johnsson and Mårtensson, 2003). Depending on soil type and land use a substantial portion of European groundwater is affected by excess input of N. In areas with intensive agriculture high concentrations of NO_3^- in the surface water are also related to increased concentrations in groundwater reservoirs. The Swedish government has therefore stated that prior to 2010 water-carried N losses caused by human activity should be reduced by 30% or more, to 38 500 tonnes, compared to the losses during 1995 (SEPA, 2000).

The increased leaching of NO_3^- from arable land is linked to the extensive use of N fertilisers in conventional farming, but also to the use of organic manure in biological farming. Kirchmann and Bergström (2001) showed that leaching losses expressed in relation to unit of yield will be as large in organic as in conventional farming systems. Incorporation of an N-rich plant material increases the amount of organic N in the soil leading to greater N mineralisation and nitrification and thus an increased risk of N losses. The use of biological N_2-fixation, rather than fertilizer N, as a source of N input in biological farming may lead to an increase in N leaching losses (Bergström, 1987; Torstenson, 1998, 2003, Kirchmann and Bergström, 2001). Nevertheless, it is a goal stipulated by the Swedish government to increase biological farming to encompass 20% of the cultivated land in Sweden. To minimise the risks of large leaching losses from N_2-fixing green manure crops the pattern of N mineralisation from green manure needs to be optimised in relation to crop N demand. The ability of the growing crop to utilise the N mineralised from the incorporated green manure must be maximised, while the losses of N must be minimised. This is accomplished by making sure that the N is available just prior to or synchronised with crop N uptake, while N mineralisation during time periods of low or no crop uptake is minimised. Optimisation of N release from decomposing N-rich plant materials and plant N uptake is a central issue and goal in applied research concerning N in agricultural systems based on high inputs of organic N, and also in this thesis.

Plant materials as a N source - The state of the art

Problems…

The N applied to soil through plant materials is mainly organically bound. Prior to crop uptake it needs to be transformed, mineralised, into plant-available mineral N, even if it has been shown that plants to some extent may utilise small organic N-containing molecules, such as amino acids (Hodge *et al.,* 2000). Mineralisation is performed by soil microorganisms, and depend on several factors, such as temperature, moisture, properties of the incorporated plant material (Swift, 1979), and may be extended over time.

However, it is very difficult to optimise N delivery from decomposing plant materials to meet crop N demand. Nitrogen in organic materials can be mineralised at times when little or no crop uptake is taking place leading to increased leaching of mineral N (Breland, 1994a; Myers *et al*., 1994; Jensen, 1997). Conversely, N deficiency will occur if N uptake exceeds the N mineralisation rate. In addition, crops take up around 5 – 50% of the green manure N during the subsequent growing season (Ladd and Amato, 1986; Ta and Faris, 1990; Myers *et al*., 1994; Wivstad, 1997), while the rest is either lost through volatilisation or leaching, or is immobilised in soil organic matter, from where some of it is stabilised into humic substances.

Green manure may give rise to gaseous losses of N through denitrification or ammonia emission. Most N denitrifies to N_2 gas, and only a smaller proportion to nitrogen oxides constituting a pollution problem, of which N_2O is especially noxious because it also is a powerful greenhouse gas. During decomposition of an incorporated N-rich plant residue emissions of N_2O are higher, 1-4 kg ha^{-1} $year^{-1}$, compared to a N-poor residue or bare soil, ca 0.5 kg ha^{-1} $year^{-1}$ (Ghosh *et al*., 2002). Losses of N_2O through denitrification are, however, often small in proportion to accumulated amounts of mineralised N or to other types of losses, such as leaching. Quemada and Cabrera (1997) reported that the loss of N_2O through denitrification was less than 4.5% of apparent net mineralised N. Total loss of N through denitrification may however be much higher. In a budget covering the main sources of inputs, *e.g*. fertilisers, biological N-fixation, deposition etc., and outputs, *e.g*. denitrification, gaseous emissions, transport to sea *etc*., of N in Europe, losses of N through denitrification equalled 40% of total N input (van Egmond *et al*., 2002). However, denitrification did not solely take place in the agricultural fields but represents the sum of all denitrification occurring during storage, application, leaching *etc*. In general, denitrification increases with increasing soil temperature and water content (Granli and Bøckman, 1994).

Prior to incorporation of green manure there is a large risk of ammonia volatilisation (NH_3). Substantial losses of NH_3, sometimes as high as 50% of added N, have been documented from NH_4^+-containing fertilisers and animal manure applied to the soil surface (Terman, 1979). In general, approximately 10-

20% of applied plant material N has been estimated to volatilise (Janzen and McGinn, 1991; Granli and Bøckman, 1994). In mulching systems, where the green manure is left on the soil surface, volatilisation of ammonia occurs mainly during the first month after application with the highest emissions from wet material (Larson *et al*., 1998). Quemada and Cabrera (1997) reported that as much as 21% of the apparent net mineralised N was lost through ammonia volatilisation when the green manure was placed on the surface. Ammonia volatilisation from fresh plant material is however low during the first days after cutting (Marstorp, 1995), which is why incorporation in soil within a few days will almost eliminate the losses (Janzen and McGinn, 1991).

…and possibilities

N_2-fixation has always been the natural pathway for N to enter the soil. The N_2 present in the atmosphere can be fixed by groups of free-living N_2-fixing bacteria, *i.e*. *Azospirillum* spp, as well as by leguminous plants through symbioses with *Rhizobium* bacteria in root nodules (Trevors and van Elsas, 1997). The contribution of this interaction to N_2-fixation has been estimated to 150-300 kg N ha^{-1} $year^{-1}$ depending on habitat and climatic conditions (Stevenson, 1986; Claesson *et al*., 1991; Trevors and van Elsas, 1997). The roots and stubble of a grass-legume crop may contain 60 to 156 kg N per hectare (Laidlaw *et al*., 1996; Hauggaard-Nielsen *et al*., 1998; Eriksen and Jensen, 2001) potentially available for the succeeding crop even if most of the above-ground material is harvested and removed from the field. The accumulation of N in a legume crop is accompanied by a rhizo-deposition of N-rich exudates, such as amino acids, readily available for plant uptake (Jensen, 1997). The rhizospheric deposition results in an active root zone supporting not only microbial growth and N mineralisation, but also microbial assimilation of N in the rhizosphere (Recous *et al*., 1990).

Incorporation of a plant material increases the amount of soil organic matter, which has several beneficial effects such as improved soil structure, water-infiltration capacity and water-holding capacity while reducing the risk of erosion (Tisdall and Oades, 1979; Swift *et al*., 1979; Piccolo, 1996). A green manure crop stimulates the activity of soil microflora and fauna, which may affect soil aggregation and stabilisation (Broersma *et al*., 1997; Nilson *et al*., 2000).
Undersown green manure crops function as catch crops after harvest. Nitrogen that otherwise could have been lost through leaching is taken up by the roots and stored in the plant material. Several studies have shown that grass species, such as *Lolium perenne* and *L. multiflorum*, used as catch crops effectively reduce N leaching (Gladwin and Beckwith, 1992; Shepherd, 1999; Aronsson, 2000). Catch crops have been found to reduce N leaching by, on average 60% (Torstensson and Aronsson, 2000). Also legumes and grass/legume mixtures have been shown to reduce the risk of N leaching during the winter period (Båth, 2000).

Management practices as a means to optimise N release

The timing of incorporation of the green manure in soil influences the N mineralisation pattern, due to variations in temperature and soil moisture.

Delaying or excluding autumn tillage has been shown to considerably reduce the risk of N leaching during this period (Møller Hansen and Djurhuus, 1997; Stenberg, 1998; Känkänen *et al*., 1998; Torstensson, 1998; Shepherd *et al*., 2001; Korsaeth *et al*., 2002). Soil cultivation and incorporation of the plant material stimulates the mineralisation as new aggregate and particle surfaces are exposed to microbial attack. In contrast, leaving the soil undisturbed leads to decreased mineralisation of organic N. The beneficial effect of delaying the incorporation of a green manure crop is enhanced by a prolonged N uptake by the green manure itself during the autumn. If the incorporation is delayed until spring, immediately prior to sowing of the subsequent crop, there is however a risk that net N mineralisation will occur too late for an optimal utilisation of N by the subsequent crop (Thorup-Kristensen, 1996; Aronsson, 2000).

Pretreating of the plant material by cutting it into small pieces prior to incorporation also makes it possible to manipulate the decomposition and mineralisation (Jensen, 1994, 2000; Tarafdar *et al.,* 2001). The decomposition rate is positively correlated to a decreasing particle size of the plant material (Wilson, 1993; Jensen, 2000). Small particles offer a relatively larger surface, increasing the possibilities for microbial attack and activity. Moreover, small particle sizes also result in higher levels of soluble organic C and N. The increased availability of soluble components will lead to a more extensive microbial assimilation of both C and N (Rovira and Vallejo, 1997; Ambus and Jensen, 1997), which also can favour the temporary stabilisation of N added through the plant material. As an example, the reduction of N leaching by incorporation of straw in the autumn was improved when the straw material had a smaller particle size (Ambus *et al.,* 2001). In contrast, a large particle size will further delay the decomposition of recalcitrant plant components.

Also the spatial distribution determines the availability of the incorporated plant materials to the decomposers. By combining a fine particle size with a thorough incorporation of a plant material, such as straw, to soil the rate of N immobilisation will be maximised. If the plant material is not evenly distributed in the soil “cold and hot decomposition spots” may occur in which the rate of N immobilisation may differ considerably, mainly depending on the level of available N at each spot (Jensen, 2000; Hesselsøe *et al.,* 2001). In addition, the distance between hot spots and cold spots also effects the competition between plant roots and microorganisms for mineralised N. Wang and Bakken (1997) found that if the distance between incorporated N-rich and N-poor plant residues exceeds 6 mm, plant roots outcompete the microorganisms more or less completely.

In soil, oxygen level, temperature and moisture may be significantly different at different depth. Surface horizons, at a depth of 5 cm, are relatively more exposed and affected by water deficits and drying-wetting and freezing-thawing cycles than deeper horizons (Rovira and Vallejo, 1997). If the plant material is incorporated deeper, both the moisture and temperature conditions in the soil are more even and the decomposition may proceed more evenly than on the surface. Application of plant material on the soil surface, however, moderates soil

temperature and moisture fluctuations in the soil and also protects the soil against erosion (Collins *et al.,* 1990). Applications of N-rich residues on the surface, provided dry conditions, may be put into practice to delay microbial degradation as well as N mineralisation (Breland, 1994a; Myers *et al.,* 1994), but with the risk of considerable losses through NH_3 volatilisation, as previously discussed.

Several of the management options suggested above may be difficult to realise and the benefits may be of only small practical significance because of all the uncertainties involved, particularly concerning the weather. Additional management options are therefore needed to achieve the desired level of N mineralisation. Control of plant chemical composition through choice of plant species as a means to improve optimisation of N release in relation to crop demand may be a promising option.

Plant material composition as a means to optimise N release

The plant cell is mainly composed of different polysaccharides, polypeptides and lignin, with varying rates of decomposition after litter formation. Oades (1988) demonstrated that plant material composition, amount of plant material input, and proportions between plant parts and plant tissues are decisive for the formation of humus in soils. Plant material chemical composition determines the availability of plant C to the soil decomposers, and will therefore have a crucial influence on the dynamics of N mineralisation during decomposition. The importance of specific plant components in relation to C and N mineralisation changes depending on factors such as plant species, and age (Kühbauch and Kleeberger, 1975; Haynes, 1986; Parton *et al.,* 1987; Bending *et al.,* 1998; Bernard-Reversat, 1998; Henriksen and Breland, 1999; de Neergaard *et al.,* 2002; Kögel-Knabner, 2002).

Plant material composition

The anatomical structure of a plant cell can be divided into two main regions (Galston *et al.,* 1980; Raven *et al.,* 1992). Firstly, the protoplast that consists of a cell nucleus and cytoplasm (Figure 1). The cytoplasm includes all cell organelles and systems of membranes and non-membranous entities. The cytoplasm is rich in salts and sugars and dissolved organic N compounds. Secondly, the cell wall that is made up of a relatively rigid, highly structured and chemically heterogeneous network of substances.

Components found in the protoplast

Storage compounds such as free sugars, *e.g.* sucrose, fructose and glucose, and in grasses also fructans, are found in the cell protoplast (Smith, 1973; Kühbauch and Kleeberger, 1975; Kögel-Knabner, 2002). Fructose and glucose are both monomers while sucrose is a dimer, composed of a glucose molecule attached to a fructose molecule. Sugars and fructans are essential for osmoregulation and freezing point depression in plant cells (Turner and Pollok, 1998; Kögel-Knabner, 2002). Depending on plant species, plant part, age, temperature *etc*., the concentration of these compounds varies considerably.

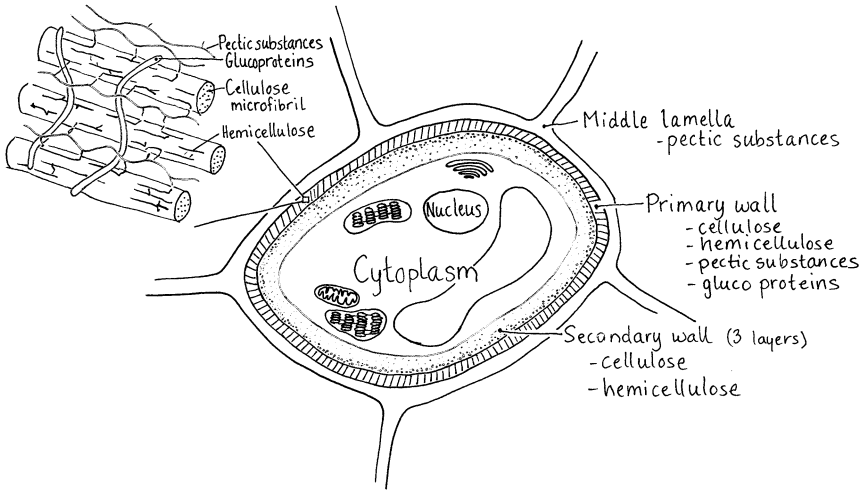

Figure 1. A schematic picture of a plant cell composed of a protoplast surrounded by a cell wall. The protoplast contains different organelles as well as the nucleus and the cytoplast, which is rich in salts and sugars and dissolved organic N compounds. The cell wall is divided in layers starting with the middle lamella, composed mainly of pectic substances, functioning as an interlayer between plant cells. The primary wall consists of cellulose surrounded by hemicellulose, pectic substances and glucoproteins, as illustrated in the top left corner. The secondary wall mainly consists of cellulose but is to some extent also surrounded by hemicellulose.

Starch is another important storage carbohydrate that quickly may convert into free sugars supporting winter survival in legumes (Turner and Pollock, 1998). Starch is a polymeric chain made of α-linked glucose units. The amount of starch in grasses is generally low. In legumes the concentration can be much larger, especially in stolons of *Trifolium repens* which can contain up to 30% of plant material dry weight (dw) during the cold season (Turner and Pollock, 1998).

Proteins are the major organic N constituent of the protoplast and consist of polypeptides built of long chains of various amino acids. They have metabolic functions as well as structural functions associated with carbohydrates as a part of the cell wall (Raven *et al.,* 1992; Galston *et al.,* 1980). Legumes, particularly their leaves, generally have higher protein concentrations than many non-fixing plant species. Free amino acids are also found in the protoplast (Galston *et al.,* 1980).

Components found within the cell wall

The structure and composition of plant cells have been highlighted in the following reviews: Carpita and Gibeaut (1993); Wilson (1993); Heredia *et al.,* (1995); Chesson (1997) and Kögel-Knabner (2002), which have been used as source material in the following text. The plant cell wall is heterogeneous in thickness and in chemical composition, depending partly on the role the cells play in the structure of the plant and partly on the age of the individual cell. The plant

cell wall mainly consists of carbohydrates, *i.e.* pectic substances, hemicellulose and cellulose, as well as proteins and lignin organised in several layers with partly different properties concerning chemical composition and function (Figure 1).

Pectic substances are a group of structural acidic polysaccharides consisting mainly of (1,4)-α-D-galacturonic and glucuronic acid residues linked together as a core, which may be interspersed with residues of rhamnose and have neutral side chains of galactose and arabinose attached (Jarvis *et al.,* 1988; Carpita and Gibeaut, 1993; Heredia *et al.,* 1995; Kögel-Knabner, 2002). The approximate degree of polymerisation, *e.g.* the number of linked monosaccharide units, of the pectic polysaccharide is somewhat larger than 100 (Chambat and Joseleau, 1980). The structure of pectin can be highly variable with sections of un-branched backbone and sections with a strongly branched structure. Pectins form the binding substance between the cells in the middle lamella and they also occur in the primary cell wall (Figure 1). Legumes can consist of up to 30 % pectic substances on dw basis while only a few percent are found in grasses (Jarvis *et al.,* 1988; Hatfield, 1992; Åman, 1993).

Hemicelluloses are a group of structural carbohydrates surrounding the cellulose fibrils and cementing them together (Figure 1). They consist of more or less branched chains of cellulose-like sugar units bound together but with a lower degree of polymerisation than cellulose. The most abundant non-cellulose polysaccharides in the majority of angiosperms are the arabinoxylans (Figure 2). They consists of short side chains of (1-3)-α-L-arabinose and (1-2)-α-D-glucuronic acid residues attached to a long linear backbone chain of (1-4)-β-D-xylose residues linked to each other (Wilkie, 1979; Aspinall, 1980; McNeil *et al.,* 1984; Åman, 1993; Gruppen *et al.,* 1993; Vinkx *et al.,* 1995a, 1995b). Besides the arabinose and glucuronic acid side chains, or branches, small amounts of mannose and galactose residues are also found on the xylan chain (Cotta and Zeltwangen, 1995). The number of branches on the linear xylan backbone varies markedly from nearly all xylose units being branched to those where only 10% or less of the xylose units bear side groups. The degree of branching affects the structure of the hemicellulose. The less the branching, the more likely the linear xylan chain is to form hydrogen bonds either to cellulose or to each other, stabilising the linear structure even more. In contrast, if the xylan chain is heavily branched the branches constitute a hindrance to the formation of hydrogen bonds, making the structure more amorphous (Carpita and Gibeaut, 1993). In general a growing plant cell contains higher concentrations of branched arabinoxylans, while more un-branched arabinoxylans are accumulated after cell growth.

The total concentration of hemicelluloses can vary between approximately 15 – 35% in both legumes and grasses depending on age, species, and the morphological part of the plant (Wilkie, 1979; Åman, 1993; Nelson and Moser, 1994). Leaves however often contain concentrations of hemicellulose lower than 15% (Nordkvist *et al.,* 1987; Müller *et al.,* 1988; Åman, 1993; Henriksen and Breland, 2002). The proportions between arabinose and xylose (A/X ratio) vary both between and within species. In *Lolium multiflorum*, the composition has been

found to vary greatly, with A/X ratios varying from 0.1 to 1.1 (Chesson *et al.,* 1985).

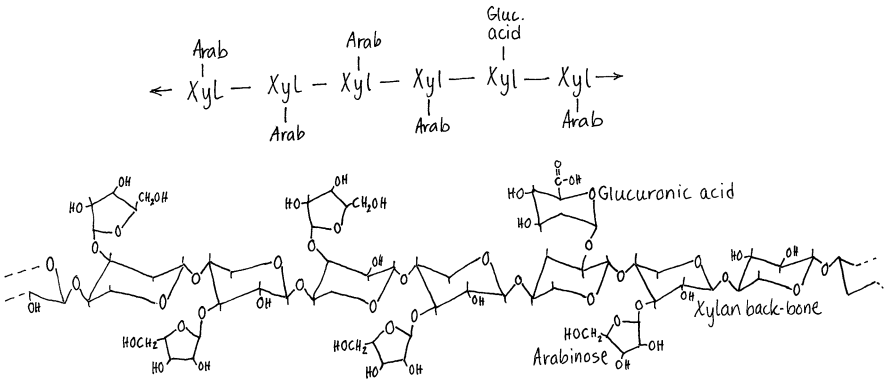

Figure 2. Chemical structure of one of the most abundant hemicelluloses, arabinoxylan, consisting of short side chains of (1-3)-α-L-arabinose and (1-2)-α-D-glucuronic acid residues attached to a long linear backbone chain of (1-4)-β-D-xylose residues.

Cellulose is composed of (1,4)-β-D-glucose, comprising the major structural component of the plant cell wall with a much higher degree of polymerisation (>10 000) compared to pectic substances and hemicellulose (Aspinall, 1980; Bacic *et al.,* 1988; Kögel-Knabner, 2002). Several dozen of the linear long chains are regularly linked together forming microfibrils with partly crystalline properties. The microfibrils wind together forming macrofibrils (Figure 1). The structure and aggregation of cellulose is partly regulated by the presence of hemicellulose. Hemicellulose and cellulose are co-aggregated, as hemicellulose is included within and between the cellulose structure (Atalla *et al.,* 1993). Grass generally contains higher concentrations of cellulose than legumes (Chesson *et al.,* 1985).

Lignin is a large molecule consisting of phenolic groups composed of aromatic rings with three carbon side chains. Lignin is found in the primary and secondary cell wall as well as interwoven in the middle lamella bonded to parts of the cellulose and hemicelluloses forming a so-called ligno-polysaccharide complex (Bacic, 1988; Hatfield *et al.,* 1999). In grasses, lignification is initiated through cross-linking of lignin to arabinose (Hatfield *et al.,* 1999). The degree of lignification is generally known to reduce the decomposition of structural carbohydrates as it physically protects the cell wall against microbial attack (Swift *et al.,* 1979; Berg *et al.,* 1982; Wilson, 1993; Heredia *et al.,* 1995; Chesson, 1997). Lignification of cell walls is generally considered as the premier impediment to decomposition of forage crops in ruminants (Deetz *et al.,* 1993).

The lignified cell walls of legumes contrast with those of grasses because they appear to be partly less digested than grass cell walls in the rumen environment (Wilson *et al.,* 1993). The lignin in legumes probably is concentrated to only one

tissue type, the xylem, rather than spread between several different tissue types, as in the grasses (Wilson, 1993). The lignin concentration in the xylem cells, especially in stems can be very high, preventing decomposition. In terms of overall plant material decomposability this is worth considering as mainly one tissue type in the legumes seems to be affected by lignification.

Lignin content in plant materials varies widely, increasing with maturation and senescence. The concentration of lignin in fresh leaves ranges from 5 to 20% while that of senescent litter ranges from 10 to 40% (Palm and Rowland, 1997). Cereal and legume straw and litter from annual crops usually contain less than 10-15% lignin (Heal *et al.,* 1997). It has been suggested that if the concentration of lignin exceeds 15% of plant material dw decomposition is reduced (Chesson, 1997).

Other plant cell components

Other phenolic substances than lignin include a range of compounds, such as tannins. Phenols can serve as a C substrate (Martin and Haider, 1980) but many of them, especially tannins, can also hinder the growth or function of the decomposer organisms by binding to enzymes or make N unavailable by chemically binding to protein (Palm and Rawland, 1997). Phenols are common in tropical forage but are also found in relatively large amounts (ca 2% of plant material dw) in temperate legume forage, *e.g. Lotus* spp (Min *et al.,* 2000; Hedqvist *et al.,* 2000).

Lipids are a heterogeneous group of fats and fatlike substances that occur in plants whereof cutin and suberin are insoluble lipid polymers that are structural components of many plant cell walls (Harwood, 1980). In general the concentrations of these components, *e.g.* phenols, lipids, cutin and suberin, are rather small in proportion to the other more abundant components, *e.g.* carbohydrates and proteins (Haynes, 1986; Harwood, 1980).

Plant material decomposition

Decomposition is the result of a three-component process (Swift *et al.,* 1979; Heal *et al.,* 1997). Firstly catabolism, a biochemical term which describes energy-yielding enzymatic reactions usually involving the transformation of complex organic compounds to smaller and simpler molecules. Secondly comminution, which is the physical process of reducing the particle size of organic material by the feeding activity of decomposer animals. Finally leaching which influences the access of substrates to microbial decomposition or even remove them from the system.

Decomposer community

The majority of the decomposers is heterotrophic bacteria and fungi which obtain carbon (C) and energy from organic material (Swift, 1979; Benbi and Richter, 2002). Generally, the initial decomposition of a plant material is performed by so-called opportunistic bacteria as well as sugar fungi that are specialised in exploiting readily metabolised non-polymeric resources such as simple sugars, and amino acids, starch and to some extent also pectic substances. These organisms die

as their nutrient resource is finished and the decomposition is taken over by species of bacteria and fungi with a slower growth rate, which are specialised in decomposition of more refractory structural carbohydrates which are enzymatically broken down to smaller molecules which then can be absorbed and utilised. Two important attributes of the decomposers are to be able to penetrate the protective surface of a substrate and to invade it at a cellular and molecular level (Swift, 1979; Heal *et al.,* 1997). Many fungi accomplish this by producing specific hyphae, which mechanically penetrate the cuticularised surface of plants. The penetration is followed by exploitation of available substrates found within and between the cells which can be enzymatically attacked. An alternative course of action used by both bacteria and fungi is that of enzymatic attack on cutin and cell wall polymers by extracellular enzymes (Heal and Dighton, 1986; Sjökvist, 1995; Hammel, 1997). The water-soluble parts inside the plant cell may leach out from the cell if they come in contact with soil water and are decomposed (Collins *et al.,* 1990).

Soil animals, such as earthworms, collembola, mites, and nematodes, also affect decomposition of plant residues and soil organic matter, *e.g.* by comminution and mixing (Verhoef and Brussaard, 1990; Wolters, 2000). The proportion of degradation originating from soil animals is however small. The degradation of, for example, clover only increased by approximately 5% in the presence of earthworms (Uvarov, 1982).

Decomposition of plant components

Plant proteins can be decomposed by a multitude of organisms (Kögel-Knabner, 2002). In general, proteins outside the cell wall, being non-structural, are more susceptible to degradation than structural proteins embedded in the cell walls. Approximately 5% of the cell wall is made up of proteins such as glucoproteins and extensin (Hatfield *et al.,* 1999) linked to the cellulose (Figure 1). However, most of the plant material N compounds have high turnover rates (Martin and Haider, 1986; Marstorp, 1996).

The decomposition rate of pectic substances exceeds that of xylan and cellulose, both in the rumen (Nordkvist *et al.,* 1987; Hespell and Cotta, 1995; Jung *et al.,* 1998; Hatfield *et al.,* 1999;), and in soil (Swift *et al.,* 1979). Moreover, it seems likely that pectic substances surrounding the hemicellulose and the cellulose may function as an entrance for microbial attack of the cell wall (Jarvis *et al.,* 1988). Consequently, the microbial attack of the cell wall may be somewhat delayed if the concentration of pectic substances is low in the cell wall and the wall might stay intact for a longer time after incorporation in soil.

In several studies the degradation and utilisation of hemicelluloses present in plant materials and feedstuffs have been investigated. In an early study by Dehority (1967), hemicellulose was isolated from *Linum usitatissimum*, *Zea mayze* and a *Festuca* spp and the results showed that the overall hemicellulose degradation varied with the species and was related to differences in the inherent

hemicellulose composition. Consequently, the hemicelluloses can be more or less rapidly decomposed depending on differences in their chemical composition.

Both in vitro studies and studies in vivo in the rumen have shown that the degradability of hemicellulose increases with the degree of branching (Albrecht *et al.,* 1987; Vinkx *et al.,* 1995b). The presence of the side groups affects the susceptibility to enzymatic attack. A low arabinose-to-xylose ratio indicates a linear hemicellulose with few bonds to other monosaccharides forming side groups and branches. A high ratio between arabinose and xylose on the other hand indicates a chain with a large amount of branches and side groups. Whether this ratio is related to decomposition of hemicellulose in soils had not previously been investigated, which is why this became one of the main aims of this work. Hemicelluloses, as well as pectic substances, are decomposed more rapidly than cellulose by many aerobic and anaerobic bacteria and fungi (Swift *et al.,* 1979; Palm and Rawland, 1997).

External enzymes, produced by a few organisms, are required to break down the cellulose polymer. Fungi constitute the major organisms able to decompose cellulose. In general the decomposition of cellulose takes place during aerobic conditions and is a slow process (Martin and Haider, 1986).

Lignin is relatively resistant against microbial decomposition and only few organisms are known to produce the enzymes necessary for degradation. White-rot fungi are the most abundant degraders of wood and are able to decompose lignin completely (Hammel, 1997). Other fungi such as soft-rot and brown-rot fungi may cause structural changes by attacking the side chains or by cleavage of certain bonds to expose the more easily decomposable cellulose (Martin and Haider, 1980; Hammel, 1997; Chesson, 1997). These species however are not able to complete degradation. A consortium of decomposer organisms most likely degrades lignin in soil (Kögel-Knabner, 2002). The degradation of lignin is an oxidative process and therefore no degradation takes place under anaerobic conditions (Kirk and Farrel, 1987).

C and N mineralisation

Carbon and N cycles in soil are strongly linked due to the simultaneous uptake, *i.e.* assimilation, of C and N by the decomposing microflora. The fraction of consumed C that is converted to microbial biomass C varies widely depending on differences in substrate quality, *e.g.* ratio between C and N (C/N), molecular complexity, and availability of inorganic nutrients, especially N (Bloem *et al.,* 1997). The C flow and the C/N ratio of the decomposers determine the requirements for microbial assimilation of N (Mary *et al.,* 1996).

A simplified chart of the C and N flows between plant residues, soil organic and inorganic pools, growing plants, and air during decomposition is presented in Figure 3. Mineralisation occurs when inorganic forms of an element are released during catabolism of organic resources, *e.g.* CO_2 from carbohydrates, and NH_4^+ from organic N components. The consequence of catabolism is the release of

energy for anabolic activity that also involves the uptake and use of nutrients, *i.e.* assimilation. The NH_4^+ ions are subsequently converted through oxidation to NO_3^-. The process whereby NH_4^+ is oxidised to NO_3^-, referred to as nitrification, is mediated by autotrophic nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter* (Stevenson, 1986; Benbi and Richter, 2002). N is then transformed back into organic N through microbial assimilation or plant uptake. Through these processes N is immobilised into a form that is temporarily unavailable to other organisms. Immobilisation and mineralisation always accompany each other operating in the reverse directions. Immobilised N is sooner or later re-mineralised due to the turnover of plant material and the decomposer community. The amount of mineral N resulting from the mineralisation and immobilisation processes corresponds to the net N mineralisation, equalling the extent to which mineralisation exceeds immobilisation.

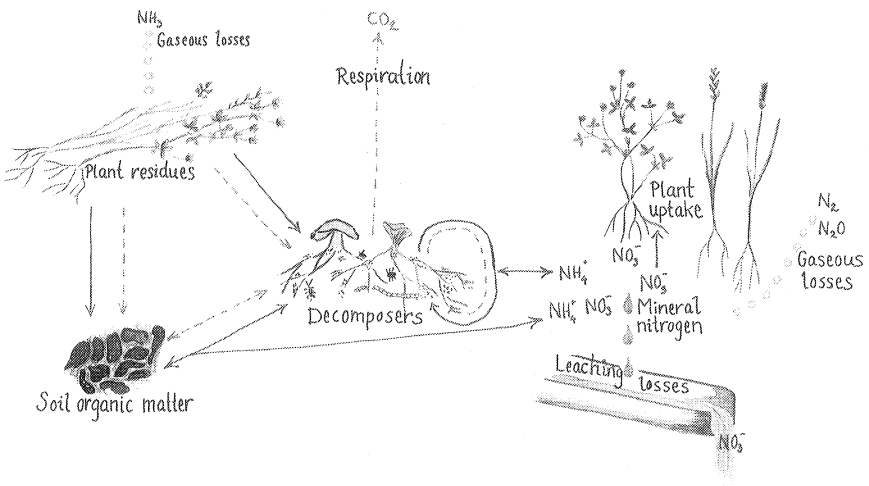

Figure 3. Flow chart of C and N transformations during decomposition of plant residues in soil. Dotted lines: C fluxes; continuous line: N fluxes.

Variables used to characterise plant materials

The C/N ratio and the concentrations of N and lignin have been, and still are looked upon as important characteristics governing the rate of decomposition and N mineralisation. The theoretical optimum C/N ratio of the substrate for microbial growth should not exceed 25 (Swift, 1979) but both fungi and bacteria can decompose plant materials with much higher C/N ratios. In a medium-term perspective, such as a vegetation season, residues with a C/N ratio greater than 30 in general result in a lowering of mineral N because of net immobilisation (Stevenson, 1986); while residues with a C/N ratio below 20 lead to an increase in the mineral N level through net N mineralisation. Quemada and Cabrera (1995) however showed that net N mineralisation may occur at higher C/N ratios than 20. Net immobilisation at C/N ratios much lower than 20 is also common. Looking at the initial few weeks of decomposition, plant materials with low C/N ratios can

cause net N immobilisation initially (Jensen, 1994; Marstorp, 1996; Trinsoutrot *et al.,* 2000). The total C/N ratio is consequently not the only variable to use when predicting N mineralisation. One explanation for this is that the total C content in itself is not closely related to decomposition while the presence of carbohydrates with differing decomposition rates is of greater importance.

Nitrogen mineralisation requires the presence of an organic N source. de Neergaard *et al.,* (2002) found a strong and linear relationship between plant material N content and N mineralisation ($R^2 = 0.92$). Initial immobilisation of N is also related to the initial plant N content, but with a negative relationship ($R^2 = 0.98$) (Kuo and Sainju, 1998). However, the point when N immobilisation turns to N mineralisation does not occur at a specific concentration of N. As an example, a grass sward was expected to release less N than a grass/clover sward, as the presence of clover increases the N concentration. But Davies *et al.,* (2001) found the opposite result. The release of N from the grass exceeded the clover/grass mix by 205 kg N ha^{-1} after incorporation, which was assumed to be related to compositional differences between the materials determining the rate of C and N mineralisation. Likewise, decomposition of different legumes with more or less similar N concentrations resulted either in an initial net immobilisation or a net mineralisation of N (Frankenberger and Abdelmagid, 1985), which was probably related to differences in concentrations of more or less easily decomposable carbohydrates, which will be discussed later on.

The ratio of lignin to N (lignin/N) is sometimes used as a plant material characteristic. The decomposition of needle litter is strongly related to its lignin and N concentration (Berg *et al.,* 1982; Palm and Rawland, 1997). When the concentration of lignin increases or the concentration of N decreases, both the rate of decomposition and net N mineralisation decrease (Müller *et al.,* 1988). The lignin/N ratio may serve as a good indicator of C availability to microbial degradation when the C/N ratio of the plant material exceeds 75. A high C/N ratio is often accompanied by a high lignin concentration modifying the decomposability of the plant material (Heal *et al.,* 1997; Palm and Rawland, 1997). However, many of the plant materials used as green manures, catch crops or crop residues incorporated in soil are relatively low in lignin and more or less high in N, which is why these characteristics do not facilitate understanding or help in estimating their decomposition pattern.

Several studies have shown that soluble phenols slow down the rate of N mineralisation during decomposition but not necessary the mineralisation of C (Bending *et al.,* 1998; Bernard-Reversat, 1998; Palm and Sanchez, 1991; Constantinides and Fownes, 1994; Handayanto *et al.,* 1994; Handayanto *et al.,* 1997). In addition phenols are believed to increase the formation of recalcitrant soil N and organic matter formation in both the long and short term. Therefore it has been suggested that the concentration of soluble phenols or the phenol-to-N ratio may be an important indicator for describing plant material composition (Heal *et al.,* 1997; Palm and Rowland, 1997). However, the cited studies were conducted under tropical conditions with tropical plant species containing in general higher concentrations of phenols than temperate legumes and grasses.

Nevertheless, it has been shown that the presence of phenols in the temperate legume *Lotus corniculatus* reduced the plant protein degradation rate in the rumen (Min *et al*., 2000), which is why specific consideration of phenols may be of importance, at least for some species.

The release of N is largely determined by the temporal pattern of decomposition of plant components containing C and N such as different carbohydrates and proteins. Therefore I suggest that the concentration and composition of different carbohydrates, such as free sugars, pectic substances, hemicellulose, and cellulose should be considered to understand the mechanisms regulating processes of decomposition of and N release from temperate plant materials. In addition, the concentration of organic N as well as plant modifiers, such as lignin, is also significant (Palm and Rowland, 1997).

Short- or long-term decomposition

The importance of different components on decomposition and nutrient release changes over time. Berg and Staaf (1980) showed that during early decomposition of needles from *Pinus sylvestris* the availability of N was the most important factor while lignin was shown to be the main controlling factor during later stages. Concerning N release from decomposing legumes in tropical agroforestry systems, Palm (1995) concluded that the polyphenol/N ratio was well related to short-term N release while the lignin + polyphenol/N ratio was well related to long-term release of N. Also in temperate grasses and legumes different components are of varying importance during the process of decomposition. When looking at long-term changes in C and N mineralisation, it may be sufficient to consider just a few important factors, such as C/N ratio and the concentration of lignin. But if short-term decomposition is considered, *e.g.* decomposition during one vegetative season, additional components, such as free sugars, fructan, protein, hemicellulose and cellulose, need to be included.

Objectives

The comprehensive aim of the experiments carried out and presented in this thesis was to improve understanding of the decomposition and N mineralisation processes specifically in relation to chemical composition of temperate-zone grasses and legumes. The specific objectives were:

- To investigate if timing between decomposition of plant carbohydrates and proteins is crucial for the course of net N mineralisation, and if this relationship can be used to determine and modify N mineralisation from plant materials in a predictable way. Specific hypotheses are formulated in Paper I.

- To investigate whether a more detailed plant analysis than that offered by traditional proximate analysis (NDF/ADF) would give a better explanation of the pattern of C mineralisation during decomposition of a range of plant materials common in temperate agricultural systems. Special emphasis was placed on investigating whether determinations of starch, pectin, and the

proportions between arabinose and xylose in the hemicellulose fraction could help explain differences in C mineralisation patterns. Specific hypotheses are formulated in Paper II.

- To investigate if the pattern and amount of N mineralisation could be predicted from the plants' chemical composition, with special emphasis on the composition of the hemicellulose fraction. A further aim was to test if hourly measurements of C mineralisation in combination with plant N concentration could be used to predict the pattern of net N mineralisation. Specific hypotheses are formulated in Paper III.

- To investigate if roots grown in a semi-natural grassland in central Sweden under elevated CO_2 levels decompose more slowly than roots grown under ambient CO_2 levels. Moreover, to make a 30-year soil C balance projection for the different treatments, based on differences in root composition, quantity and microclimate.

Materials and Methods

An overview of the experiments presented in the papers

Paper I

C mineralisation was measured during decomposition of single pure plant components to find out the time when specific plant components are decomposed. The pure plant components were divided based on their decomposition rate. By combining pure carbohydrates with different decomposition rates with protein, the effect of the C source on the fate of protein-N during decomposition was studied. With the aim of modifying the pattern of N mineralisation originating from a decomposing N-rich plant material, represented by *Medicago sativa*, a second plant material was added. Changes in both C and N mineralisation during decomposition were studied.

Paper II

C mineralisation during decomposition of ten plant materials, grasses and legumes, was measured for approximately four months. The aim was to establish the level of detail required of the chemical composition of plant materials, in order to successfully relate compositional differences between the plant materials to the patterns of C mineralisation. Special focus was placed on investigating if the arabinose-to-xylose ratio could work as a measure of hemicellulose composition, rather than total concentration of hemicellulose, in relation to decomposition rate. Principal component analysis (PCA) based on two different chemical determination methods was used to investigate the ability of each method to separate the plant materials from each other. Multiple regression was used to calculate the relationship between C mineralisation and plant material composition during decomposition.

Paper III

Net N mineralisation during decomposition of combined pure plant components and ten plant materials was measured for approximately one and four months, respectively. The aim was to set the pattern of N mineralisation in relation to the chemical composition and concentration of different carbon sources during different stages of decomposition. Once again special emphasis was placed on investigating the influence of the arabinose-to-xylose ratio on net N mineralisation. Multiple regression was used to calculate the relationship between N mineralisation and plant material composition during decomposition.

Paper IV

C mineralisation during decomposition of roots grown at ambient or elevated CO_2 level was measured and used as input in the Introductory Carbon Balance Model (ICBM) to predict the long-term (30-year) treatment effects on soil C-balances in a semi-natural grassland. The aim was to measure possible long-term effects of treatment-induced differences in C-input quality and quantity.

Chemical analysis of plant materials

Total C and N in all plant materials was measured on an elemental analyser (Paper I-IV). Total N concentration was separated into water extractable and non-water extractable organic N (Paper II-III). Total protein concentration was calculated by assuming a protein C/N ratio of 3.1 (Paper I-III). Concentrations of free sugars, fructan and starch were measured with enzymatic methods (Larsson and Bengtsson, 1982) (Paper I-II). Structural carbohydrates were determined sequentially as neutral- and acid-detergent fibres (NDF/ADF) according to Goering and van Soest (1970) (Paper I). Or alternatively, after extraction as described by Asp *et al.,* (1983) with minor modifications, by specific determination of pectic substances, hemicellulose monosaccarides and cellulose according to Theander *et al.,* (1995) (Paper II). Klason lignin was determined in all plant materials according to Theander *et al.,* (1995) (Paper I-IV). A schematic flow chart of the carbohydrate determination procedures is presented in Appendices A and B.

The performance of the incubation experiments

The rate of C mineralisation during decomposition of pure plant components and plant materials was measured (Paper I-IV) on a 1-hour basis for two to five weeks with an automatic respirometer described by Marstorp, (1996), and thereafter by titration according to Stotzky (1965). In all incubation experiments an equivalent of 40 g dw soil was used. The soil moisture was kept at 45% of water holding capacity during the incubation, which was carried out at 20 °C. A nutrient solution containing N, P and K was added to the soil to avoid nutrient limiting conditions during decomposition.

Inorganic soil N was determined during decomposition of pure plant components and plant materials (Paper I and III) by KCl-extraction with a higher frequency during the initial phase of decomposition. Concentrations of ammonium and nitrate were measured colorimetrically on a TRAACS 800 (Krom, 1980; Keeney and Nelson, 1982). The net effect of substrate addition on N mineralisation was calculated as (NH_4^+ + NO_3^- in treatment) – (NH_4^+ + NO_3^- in control).

Results and Discussion

Plant material components that determine the pattern of C and N mineralisation

In an incubation study I measured the patterns of N and C mineralisation during decomposition at 20 ºC of *Medicago sativa* and *Trifolium pratense*, with equal concentrations of N and C (unpublished). The final accumulation of mineral N in the soil at the end of incubation was more or less the same. But, the patterns of N mineralisation were completely different (Figure 4a). The C mineralisation also revealed two separate patterns, which were assumed to be related to compositional differences between the materials that determined the rate of C and N mineralisation (Figure 4b).

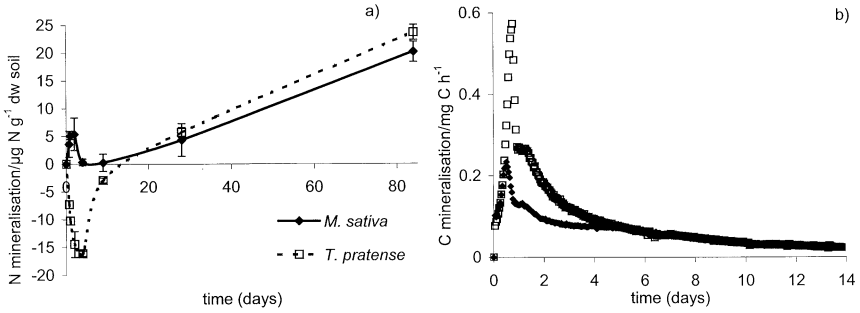

Figure 4. The pattern of a) N mineralisation (µg N g^{-1} dw soil) during a period of 2.5 months and b) C mineralisation (mg C h^{-1}) during the first two weeks of decomposition of *M. sativa* and *T. pratense* with equal concentrations of N and C (C/N=16). The different patterns of N and C mineralisation from the two plant materials were assumed to be related to compositional differences between the materials influencing the rate of C and N mineralisation.

In Paper II principal component analysis (PCA) based on a detailed chemical analysis of plant material, including determination of free sugars, fructans, starch, pectic substances, individual hemicellulose and cellulose monomers, lignin and soluble and non-soluble N clearly separated legumes from grasses. A similar difference was observed using a PCA based on the C mineralisation pattern from

each decomposing plant material. This suggested that the differences in the decomposition pattern of the two groups of plant materials were due to differences in their chemical composition. The question is – Which plant components do we need to explain the dynamics of C and N mineralisation during different time periods of decomposition?

Components influencing C and *N mineralisation during the initial days*

The experiments in which decomposition of pure plant components were studied showed that free sugars, such as sucrose and fructose, and fructans were the most rapidly decomposed plant components with approximately 40% of the added C respired within the first two days (Papers I and III). The concentration of free sugars in the grass materials used in Papers I to III ranged from 2 to 12% of plant material dw. In the legume materials concentrations of 3 to 10% of dw were found. The concentration of fructans ranged from below the detection limit in the legumes to 12% in *Phleum pratense* (Paper I).

As expected the plant materials with the highest concentration of these easily decomposable components caused the highest and most rapid increase of C mineralisation during the initial day of decomposition (Papers I and II). The amount of C mineralised during the first day of plant material decomposition (Paper II) was well related to the amounts of C in free sugars and fructans combined in a multiple regression model and explained 77% of the variation in C mineralisation between the ten plant materials.

No determination of free amino acids was carried out but the concentration of water-soluble organic N was measured to find the most easily decomposable organic N components to which free amino acids belong. In general, relatively small amounts of the total plant material dw consisted of soluble organic N (0.1 – 0.4%) equalling 15-18% of plant material total N. The legumes contained a higher proportion of soluble organic N than the grasses (Paper III). Adding C in soluble organic N compounds to the amounts of free sugars and fructans improved the degree of explanation to 83%, indicating that compounds containing N may also have been mineralised during the first day. Net N mineralisation due to decomposition of pure protein was initiated already during the first days of decomposition and was possibly related to decomposition of small amounts of amino acid residues or short peptide chains present in the pure protein (Papers I and III). Amendments of the abundant plant protein rubisco to soil resulted in an increase in C mineralisation already during first two days of decomposition (Papers I and III) equalling a loss of 11% of added C.

The amount of N mineralisation during the first two days of plant material decomposition (Paper III) was also well related to the amounts of soluble organic N and C in free sugars and fructans, and explained 70% of the variation in net N mineralisation. The soluble organic N, the N source, was positively related to initial net N mineralisation, while an increasing amount of free sugars and fructans reduced net N mineralisation, as indicated by the negative relationship.

As decomposition proceeded during days 2 to 5, the relationship between the amount of mineralised C and C in the most easily decomposable components weakened, $R^2 = 0.51$, according to a regression model not shown in Paper II. The result confirmed that their main impact on C mineralisation took place during the very first days of decomposition. The degradation of these components had probably more or less finished after two days of decomposition at 20 ºC, whereas decomposition of other types of plant components had just been initiated.

Components influencing C and N mineralisation during the following two weeks

Several studies have shown (Martin and Haider, 1986; Nordkvist *et al.,* 1987; Hespell and Cotta, 1995) that mainly non-cellulose structural carbohydrates and proteins are decomposed after soluble sugars but mainly before cellulose starts to decompose. This corroborates the results from Paper I, which show that the decomposition of proteins, starch, pectic substances and a range of different hemicelluloses, such as arabinoxylan, mainly took place between day two and nine at 20 °C; a time period during which a range of 17 to 46% of the added C from the different carbohydrates was respired.

The hemicellulose arabinoxylan used in Papers I and III showed a consistently higher decomposition rate than the hemicellulose xylan, thus it contributed less to C mineralisation during the first nine days. The chemical composition of arabinoxylan and xylan differed extensively. The almost equal proportion of arabinose and xylose monomers (43 and 56% respectively) in arabinoxylan indicated that arabinoxylan was rich in branches of arabinose. While xylan was almost entirely composed of xylose (93%) indicating that this hemicellulose formed long linear xylan chains with only a few branches, in this case of 7% glucuronic acid substituents. Therefore, the treatments containing arabinoxylan in the incubation experiment with mixed pure plant components in Paper III were expected to result in more N being immobilised and consequently less net N mineralisation at an earlier period compared to the treatments containing xylan. This was confirmed by the addition of arabinoxylan, which reduced net N mineralisation to a greater extent than the addition of xylan between days one and nine (Paper III).

Total concentrations of hemicellulose in the plant materials ranged from approximately 7 to 21% of plant material dw (Paper II). A specific determination of the concentration of pectic substances, *e.g.* uronic acids, was done for the plant materials used in Papers II and III. The grasses had a lower range of pectic substances (2 – 4% of plant material dw) than the legumes (7 – 12%).

Total N concentration found in the plant materials ranged from 0.7% to 5.5%. Recalculated to protein the protein concentration ranged from 4 to 34% of plant material dw. The availability of plant proteins can however vary as parts of the plant proteins are structurally bound, thus delaying their time of microbial decomposition. Breland (1997) found that approximately half of the N bound to the structural part of the plant material resulted in an efficient estimation of N

mineralisation during decomposition of *Trifolium repens*. Nevertheless, in Papers II and III the amount of non-soluble organic N was used to roughly estimate the proportion of N compounds assumed to be mainly decomposed during this intermediate period (Papers II-III), structural proteins being a part of this fraction.

However, a regression model with the amounts of C in starch, pectic substances, protein and hemicellulose only showed a weak relation with the amounts of C mineralised between days one and nine ($R^2 = 0.47$; Paper II). This result indicated the need for a higher level of detail concerning differences in the hemicellulose composition between the plant materials. The A/X ratio in the plant materials (Paper II) ranged from 0.21 to 1.25 and was generally lower in the grasses than in the legumes. When hemicellulose was exchanged in the model for the variables arabinose and xylose the relationship was greatly improved ($R^2 = 0.87$). This result suggested that the composition of the hemicellulose fraction as described by these two components may be more important for predicting C mineralisation during this period of decomposition than total concentration of hemicellulose.

A simplified regression model containing non-soluble organic N, pectin and arabinose explained as much as 89% of the variation in net N mineralisation from the decomposing plant materials between days 2 to 14 (Paper III). The net N mineralisation will decrease with increasing amounts of pectic substances and arabinose and increase with an increased amount of non-soluble organic N.

Components influencing C and N mineralisation after more than two weeks

CO_2 evolution from xylan-amended soil showed a relatively high rate equalling a loss of 11% of added C between days 9 and 17, even if the decomposition of xylan started at an earlier stage (Paper I). C mineralisation from soil amended with cellulose both started and continued at a lower rate compared to all other pure components tested. 25% of C added through cellulose was lost between days 9 and 17. The concentration of xylan in the plant materials used in Papers II and III was generally higher in the grass materials, ranging from 10 to 16% of plant material dw, compared to the legume materials, ranging from 1.5 to 8% of plant material dw (Paper II). The concentrations of cellulose, determined as glucose, ranged from 8 to 29% of plant material dw. The concentration of lignin, modifying the decomposability of the structural carbohydrates in the cell wall, ranged from 8 to 18% with the lowest concentrations in stems and leaves from *Trifolium repens* and the highest in *Festuca rubra*.

The amount of C mineralised from the plant materials during the interval 9 to 34 days was explained by a regression model that included not only xylan and cellulose, but also lignin as a decomposition-rate-modifying component ($R^2 = 0.72$; Paper II). The result confirmed that cellulose and the more recalcitrant fraction of the hemicellulose were the main contributors to C mineralisation during this later time interval. Lignin was negatively related to the change in mineralised C indicating its resistant and protective properties.

Amendments with slowly decomposable substrates, such as straw or cellulose, will induce a slower rate of late N mineralisation (Vinten *et al.,* 2002). In agreement with this the amount of net N mineralised later than day 14 was negatively related to the amounts xylan and cellulose but positively related to the amount of non-soluble N, explaining 84% of the variation between the ten plant materials (Paper III).

Long-term effects of plant composition on C and N mineralisation

No relationship between the amount of C mineralised and the slowly decomposable plant components, *i.e.* xylan and cellulose, in combination with lignin was found when C mineralisation after more than one month was considered (Paper II). At this late time of decomposition, roughly corresponding to a period of two months after spring incorporation in temperate climatic regions, the plant materials have been modified, due to microbial degradation, to such an extent that the components originally present no longer determine the pattern of decomposition. Carbon mineralisation originating from decomposition of earlier-produced decomposition products that have become a part of the soil organic matter pool starts to set in and becomes increasingly important.

An increase in soil C input through decomposition of plant material leads to an accumulation of C in the soil organic matter pool, *i.e.* a changed soil C balance in the long term. In paper IV a projection of a 30-year C balance for a semi-natural grassland based on short-term root decomposability was made to investigate the effect of differences in soil C input. One of the treatments involved changes in C input during periods of elevated CO_2 levels. An elevated CO_2 level in the atmosphere is believed to increase C input to soil through an increased plant material C/N ratio. A common hypothesis is that elevated CO_2 levels will result in a greater transport of easily decomposable carbohydrates to the roots (Gorissen *et al.,* 1995). This is believed by some to result in a reduced root decomposition rate (Cotrufo and Ineson, 1995; Gorissen *et al.,* 1995; van Ginkel *et al.,* 2000) but not by others (Dukes and Field, 2000; Randlett *et al.,* 1996). Therefore, one of the aims in Paper IV was to investigate if the decomposition rate of roots grown under elevated CO_2 level differed from those grown under ambient CO_2 level. Another was to make a 30-year soil C balance for the different treatments to investigate possible effects on long-term storage of C in the soil organic matter. The C mineralisation pattern during decomposition of the roots indicated that the roots grown at elevated CO_2 level contained slightly lower concentrations of soluble carbohydrates compared to roots grown under ambient CO_2 levels. However, even if differences in C input and root decomposability were seen on short-term basis, almost no differences were found when extrapolating to a 30-year perspective. Initially, the incubation seemed to support the findings that decomposition of plant materials grown under elevated levels of CO_2 will be somewhat reduced as the elevated treatment had the lowest initial respiration peak (Paper IV). However, after three weeks the rate of CO_2 evolution from elevated roots increased and remained 25% higher than the other treatments. But despite differences in the

quantity and quality of C input as well as the environmental microclimate, only minimal differences were found when extrapolating to a 30-year soil C perspective. The soil C balance was found to be in decline and the amount of C lost from the elevated and ambient treatments was estimated to be equal to 1.9 and 2.5% respectively, of the initial 3.6 kg C m^{-2} soil C.

As a comparison, a simulated change of land management from grazed grassland to ungrazed green fallow resulted, on the other had, in an increase in C input to soil equalling 5.3 kg C m^{-2} over 30 years (Paper IV). Yet only 0.42 kg C m^{-2} was sequestered in the soil, if compared to the control conditions. The rest was respired back to the atmosphere. Moreover, half of the sequestered C was incorporated as labile C and would be decomposed within a few years if the original management system were resumed.

A C pool built up by frequent use of green manure will probably also decrease relatively quickly with changes in management practices. Such a change in management regime can be compared with the break up of a pasture. Large amounts of both C and N are accumulated and preserved in the soil during the years of pasture. But once the pasture is broken up both the C and N are rapidly sent into circulation, with large risks of N losses as a consequence.

A short summary of the results presented so far

The decomposition and the influence of many of the plant components presented above overlap with one another making it rather difficult to differentiate precisely the time of importance of several of the components. Nevertheless, a suggestion of how to divide the plant components based on when their main influence on the pattern of both C and N mineralisation during decomposition occurs is as follows:

I) During approximately the initial two days of decomposition at 20 ºC free sugars, fructans and soluble organic N components constitute the main C and N substrates thus influencing C and N mineralisation.

II) During the following two weeks of decomposition at 20 ºC starch, pectic substances, the most readily decomposable part of the hemicellulose fraction and non-soluble organic N compounds constitute the main C and N substrates, thus influencing C and N mineralisation. The concentration of arabinose and xylose showed a stronger relationship to C mineralisation during the intermediate time period of decomposition compared to the total concentration of hemicellulose. Consequently, arabinose and xylose could be used to characterise the composition, and thus the influence of the hemicellulose fraction on C and N mineralisation.

III) After more than two weeks of decomposition at 20 ºC the recalcitrant parts of the hemicellulose, such as xylan and cellulose, constitute the main C source for soil organisms, with lignin more or less modifying the rate of decomposition, influencing C and N mineralisation.

Methods required to determine chemical composition - a greater degree of detail increases the explanation of C and N mineralisation

In Paper I the chemical composition of the structural carbohydrates was determined by the neutral/acid-detergent extraction method (NDF/ADF; Appendix A) (Goering and van Soest, 1970). This method is a proximate method analysing groups of plant compounds, rather than detailed analysis of individual compounds, and has been used to characterise plant materials in several investigations (Quemada and Cabrera, 1995; Henriksen and Breland, 1999; Seguin *et al.,* 2002). However, a disadvantage with using the NDF/ADF extraction to determine plant material chemical composition is that the three fractions obtained may be very heterogeneous depending on the type of plant material. As a consequence the size of the fractions determined by NDF/ADF, and in the next step their influence on decomposition, may be miscalculated. In Paper II it was concluded that a more detailed plant analysis than that offered by traditional proximate analysis gave a better explanation of the pattern of C mineralisation during decomposition of a range of plant materials.

The PCA in Paper II, based on the grouped fractions resembling NDF/ADF determination, did not manage to separate the grasses from the legumes. There were differences in the chemical composition of the plant materials that could not be detected by the NDF/ADF procedure, such as the ratio between arabinose and xylose and the concentration of pectic substances. But, as mentioned earlier, if a more detailed method, *i.e.* the "Uppsala method" (Theander *et al.,* 1995), was used (Appendix B) sufficient chemical characteristics to achieve a separation of the plant materials was obtained (Paper II).

Free sugars, fructan and soluble N rather than total soluble components

The total concentration of C in soluble plant material components as obtained by ND extraction only explained 60% of the variation in C mineralisation during the first day of decomposition (Paper II). This was probably related to the fact that the influence of proteins and starch, which largely ended up in this fraction, was overestimated. The concentration of C in free sugars, fructans and water-soluble organic N components, on the other hand, markedly improved the degree of explanation (87%) as presented earlier.

Pectic substances, arabinose and xylose rather than total non-cellulose structural carbohydrates

In Paper II it was concluded that a more detailed chemical analysis of the non-cellulose structural carbohydrates was required. From the proximate NDF/ADF determination used in Paper I the concentration of the non-cellulose structural carbohydrates of stems from *L. perenne* and *T. pratense* was shown to be more or less the same. At the same time relatively large differences in C mineralisation during the intermediate period of decomposition of the two materials was observed. The relationship between C mineralisation from the plant materials used in Paper II and their concentration of non-cellulose structural carbohydrates,

representing the intermediate fraction from the NFD/ADF procedure, only explained 18% of the variation in C mineralisation (Paper II). This provided further evidence that this method of determination is too crude.

As discussed earlier the relative amounts of arabinose and xylose as well as pectic substances were shown to be of great importance in improving predictions of C and N mineralisation. It has previously been suggested by Palm and Rawland (1997) that a standardised method to determine plant material chemical composition should include soluble C, soluble phenols, cellulose, lignin and total N. However, my suggestion is that a minimum analysis set for temperate legumes and grasses also should include free sugars, starch, pectic substances, arabinose, and xylan.

Main disadvantages of chemical determination methods

The determination methods used in this work are based on chemical treatments of the plant materials sometimes performed in several steps with the purpose of removing different plant material fractions. The treatments are relatively rough, which is why the original composition of the plant material may not be accurately reflected. The concentrations of pectic substances and hemicellulose in the plant material (Papers II and III) were for instance slightly underestimated as part of this fraction was probably lost during the stepwise enzymatic treatment performed at high temperature that preceded the determination. In addition, if the pretreatment of the plant materials differs, *i. e*. if their particle size differs prior to chemical determination, it may severely influence the results (Vanlauwe *et al.,* 1997).

Alternative or complementary methods

C mineralisation measurements instead of chemical determination

An alternative to the time consuming and expensive chemical determinations is to characterise plant materials by their specific CO_2 evolution dynamics during decomposition, measured on an hourly basis with an automatic respirometer (Papers III and IV). The measured C mineralisation, if calibrated against reliable and detailed chemical analysis, works as a direct mirror of the availability of a plant material if the decomposition is allowed to take place under non-nutrient limiting conditions. The idea is that if properly managed, the pattern of C mineralisation could work as a fingerprint of the C quality, being highly specific for each single plant material. One possible field of application could be to connect the C measurements to a predictive model calculating N mineralisation or to use them as a tool to improve the parameterisation and calibration of decomposition models, as in Paper IV. In Paper IV, differences in C mineralisation during short-term decomposition of roots grown under ambient or elevated CO_2 levels was used to extrapolate treatment-induced compositional differences of the roots on long-term soil C pools.

In Paper III C mineralisation measurements were successfully used to characterise plant material quality in order to explain variations in N mineralisation during the first two weeks of decomposition, divided in an initial

and intermediate time period. The result showed relatively strong relationships to N mineralisation explaining 69 and 90% of the variation in net N mineralisation respectively. As expected, net mineralisation of N was positively influenced by an increasing concentration of N but negatively influenced by increasing amounts of C mineralisation.

However, it cannot always be taken for granted that an increase in C mineralisation will generate a decrease in net N mineralisation. Between days 14 and 123 the C mineralisation showed a positive relationship to N mineralisation (R^2 = 0.67; Paper III). During later stages of decomposition, when the easily decomposable carbohydrates are already decomposed, the opposite will occur if a N-rich substrate is present, such as a protein, leaves from legumes or decomposer biomass. In addition, N mineralisation during later stages may also be a result of remineralisation of earlier immobilised N (Vinten *et al.,* 2002; Paper III). This ambiguity in the relationship between C and N mineralisation complicates the use of C mineralisation as a predictor of N mineralisation.

Microscopic and spectroscopic complements

Several alternative methods to determine plant material composition exist. They can work either as complements or alternatives to the more traditional chemical determinations to improve predictions of C and N mineralisation. Different microscopic techniques, such as electron microscopy and polarised infrared spectroscopy, may be used to observe the orientation of structural polysaccharides in isolated cell walls and intact tissues (Carpita and Gibeaut, 1993). Paying attention to the three-dimensional orientation of structural carbohydrates inside the cell wall and plant tissues both prior to as well as during decomposition may add to the understanding of plant structural factors influencing the possibilities of microbial attack and thus the pattern of C and N mineralisation.

By using nuclear magnetic resonance (NMR) spectroscopy it is possible to define differences in chemical composition between plant materials prior to decomposition. It is also possible to follow the transformation of the plant materials during decomposition without the complications of extracting organic matter from the soil (Carpita and Gibeaut, 1993; Hopkins and Chudek, 1997; Kögel-Knabner, 2002). Although specific compounds are not identified, this technique can reveal the approximate chemical composition of plant materials (Kögel-Knabner, 2002). Also near-infrared reflectance spectroscopy (NIR), if calibrated against detailed chemical composition, may be used to identify groups of compounds and determine concentrations of chemical constituents of plant materials in a rapid and economical way (Palm and Rowland, 1997).

A standardised method of analysing green manure plant material not only with respect to chemical extraction and determination methods and which chemical components that are of most relevance to determine, but also with respect to possible complementary methods as mentioned above would be worth aiming for.

Is it possible to optimise N release on basis of plant material chemical composition?

The pattern of plant N uptake differs between crops. When fertilising with organic materials to different crops, the pattern of crop N uptake is as important as the total N uptake. As an example, Pang and Letey (2000) illustrated the difficulty of synchronising N release from organic manure to the N-uptake pattern of *Zea mays* while the uptake pattern of *Triticum aestivum*, grown under the same climatic conditions, was easier to match. The uptake pattern of *T. aestivum* was more extended and slow compared to the pattern of *Z. mays*, the latter's initial high N demands was impossible to meet through the addition of organic manure. But even if the N uptake of *T. aestivum* was easier to match at the beginning, N mineralisation exceeded N uptake towards the end of the vegetative period. If *T. aestivum* is established during autumn, the N demand in the spring is higher and earlier compared to spring-sown crops. Optimisation of N release to crop N demand thus may be more or less difficult depending on the succeeding crop.

Several studies have shown that by combining plant materials of different chemical composition, modifications of the pattern of N mineralisation can be effected (Myers *et al.,* 1994; Quemada and Cabrera, 1995; Ranells and Wagger, 1996; Handayanto *et al.,* 1997; Kuo and Sainju, 1998; Båth, 2000). In Paper I a model experiment was conducted in which combined pure plant components differing in decomposition rates were added to soil with the aim of modifying the pattern of N mineralisation originating from a protein. By modifying an N-rich substrate by adding a second N-poor substrate it is possible to increase the stabilisation of N into soil organic matter through increased N immobilisation, which at least temporarily decreases the risk of N losses through leaching. The aim was either to bind as much N as possible to soil organic matter or to release as much N as possible to match plant N uptake and to minimise N losses. By varying the quality and the quantity of the carbohydrates, *e.g.* sucrose, xylan and cellulose, but keeping the same C/N ratio it was possible to change the course of net N mineralisation into either a rapid initial net immobilisation or a rapid net mineralisation.

Extrapolation of a laboratory experiment to a field situation

In the next step, the aim was to accomplish the same modification result using real plant materials. Based on chemical composition (Paper I) leaves from *M. sativa* were selected to represent an N-rich plant material. A second plant material was added with the aim of modifying the N mineralisation from *M. sativa* in different directions. Stem materials from *P. pratense*, *L. perenne* and *T. pratense* were chosen to represent plant materials rich in free sugars and fructan, hemicellulose and cellulose, and pectic substances, respectively (Paper I). The total addition of C and N through the mixed plant materials was kept almost equal with C/N of 12-13. The result obtained was, as presented here, theoretically adjusted to field conditions to visualise the effect of modification of N mineralisation in different field situations. However, there was no verification with field data, which is why the temperature adjustment must be seen simply as a hypothetical calculation.

Further, no adjustment to field moisture was made. The temperature adjustment was merely done to illustrate hypothetical field scenarios. By using mean soil temperatures based on measurements over a 10-year period at 0.2-m depth in Uppsala, Sweden (59º50'N. 17º40'E), published in Kirchmann and Marstorp (1991), the results obtained at 20 ºC were recalculated, using a straight temperature sum, as if they were obtained under field temperatures. For example, two days of incubation at 20 ºC with a temperature sum of 40 ºC, corresponds to 13 days in November with a mean temperature of 3.1 ºC, but only 2.5 days in July with a mean temperature of 15.9 ºC. Two scenarios were calculated. First, autumn incorporation of plant materials on the 15th October and second, spring incorporation on the 1st of May.

Autumn incorporation

According to the N mineralisation patterns obtained after incorporation of the different plant material combinations, modification of *M. sativa* leaves with *P. pratense*, with its high concentration of free sugars and fructans causing initial immobilisation of N, would suit a situation with incorporation on the 15th of October (Figure 5a). Favourable conditions for rapid microbial assimilation and stabilisation of N were produced reducing the mineral N content in the soil and consequently also the risk of leaching. Kuo and Sainju (1998) suggested that to achieve an increase in microbial immobilisation of N, the proportion of an N-rich legume in a legume-grass mixture should not exceed 40%, provided that the grass material contains adequate amounts of easily decomposed carbohydrates resulting in microbial immobilisation. However, in this experiment the proportion of legume leaves to grass material was 50/50 and an initial period of immobilisation was still obtained. The effect of net immobilisation upon incorporation depends not only on the composition of the plant carbohydrates, but also on the amount of incorporated plant material as well as on the N conditions (Kuo and Sainju, 1998; Jensen, 2000). The length of an initial period of immobilisation increases with an increasing proportion of soluble carbohydrates and decreased proportion of N in a legume and grass mixture.

Net N mineralisation was kept low during the entire autumn and winter, but started to increase with increasing soil temperature during spring. The increase in net N mineralisation took place around the end of May and beginning of June (Figure 5a), which may be too late if, for example, autumn-established *T. aestivum* is the succeeding crop. This scenario is however usually more severe during spring incorporation (Thorup-Kristensen, 1996; Aronsson, 2000; Båth, 2000). The somewhat slow release of N during the early vegetative season could be compensated for by a small addition of fertiliser or by slightly delaying the sowing of the succeeding crop to ensure the presence of plant-available N at the establishment of the crop. Maximum nutrient uptake in cereal crops usually occurs during the vegetative stage, *e.g.* stem elongation, followed by declining uptake rate during generative growth. This corresponds approximately to the period between the end of May until the end of June in central Sweden (Hansson *et al.,* 1990). Hauggaard-Nielsen *et al.,* (1998) found that incorporated plant material giving high concentrations of N in the soil during early decomposition, such as

green manure with high proportions of N-rich legumes, generated a higher early N uptake in barley during the early vegetative stage following incorporation. However, at this stage the crop is only in the very early vegetative growth stage, which is before actual maximum crop N uptake occurs. But the early soil N availability and resultant high plant N concentration have been seen to affect the barley crop physiology, *e.g.* by promoting strong tillering which gives these plants an advantage compared to those with low early soil N availability (Hauggaard-Nielsen, 1998). N mineralised after the period of maximum crop N uptake may be of limited benefit for increasing the crop grain yield through enhanced N uptake.

Due to variations in topography, hydrology, and type of soil, fields within a region or even different areas of the same field do not contribute evenly to mineralisation and losses of N. It is therefore important to identify areas of a field or within a region where losses are likely to be largest to be able to apply N site specifically. This is suggested as an important means to reduce N leaching when artificial N fertiliser is applied (Kirchmann *et al.,* 2002). However, this is not so easily done when the N supply is based on organic N originating from incorporated plant materials. Therefore, greater precautions are generally needed when using green manure in order to decrease the risk of NO_3^- leaching.

Spring incorporation

For spring incorporation, *M. sativa* leaves as a single plant material, without any modifier, created the best conditions for fast and extensive net N mineralisation to meet crop N demand (Figure 5b). However, legume leaves alone are not sufficient as a green manure, which is why the pattern of N mineralisation from the treatment also including stems from *T. pratense* would be more realistic in practice. Korseath *et al.,* (2002) found that the expected benefit of spring incorporation of *T. repens* was counteracted by a large loss (36%) of the above ground plant material due to freeze/thaw damage and subsequent runoff during the winter. However they concluded that such winter loss is likely to be a rare phenomenon. In general large amounts of clover N are mineralised within a month of incorporation (Frankenberger and Abelmagid, 1985; Breland, 1994b; Franzluebbers *et al.,* 1994; Wivstad, 1997). But the presence of the more quickly decomposed structural pectic substances and hemicellulose in the clover stem material reduced the N mineralisation during an intermediate period of decomposition limiting the availability of mineral N (Figure 5b). However, during the spring the growing crop is a good competitor with the microorganisms for the available N, being able to also partly utilise low molecular organic N (Hodge *et al.,* 2000).

According to the results the theoretical combination of *M. sativa* leaves with *L. perenne* could also be suitable as a spring-incorporated green manure. The low levels of easily decomposable carbohydrates in *L. perenne* do not cause initial net N immobilisation. But the high concentration of slowly decomposable structural carbohydrates reduces the amounts of mineral N in the soil in July when crop N demand starts to decrease, thus reducing the risk of N losses (Figure 5b). Korsaeth *et al.,* (2002) obtained the same result but after incorporating *T. repens*, high in N,

together with straw, high in slowly decomposed structural carbohydrates. At the beginning mineral N originating from the *T. repens* accumulated in the soil. Later on the microbial demand of N during the decomposition of straw gave net N immobilisation of most of the *T. repens* N. They also concluded that this pattern would be suitable during spring rather than autumn.

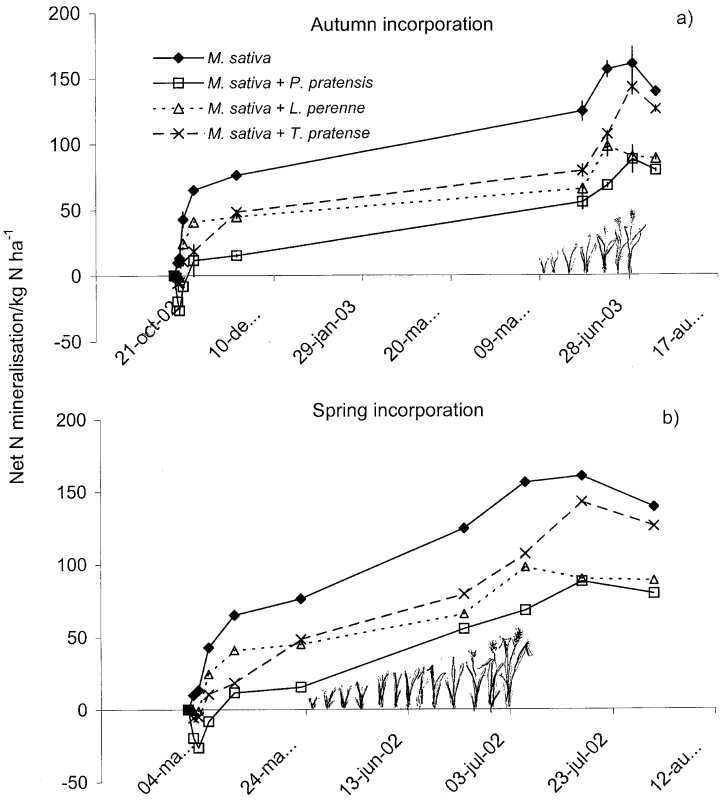

Figure 5. Net N mineralisation (kg N ha^{-1}) during decomposition of leaves from *M. sativa* combined with either *P. pratense*, *L. perenne* or *T. pratense*, which are rich in free sugars and fructan, hemicellulose and cellulose, and pectic substances, respectively, in order to optimise N mineralisation. Two scenarios were calculated: a) autumn incorporation and b) spring incorporation. Results obtained in the laboratory were extrapolated to a field situation by using a straight temperature sum, as if they were obtained under field temperatures in central Sweden.

As discussed earlier different crops have different N-uptake patterns. A crop with a very high maximum N-uptake rate would be difficult to fertilise with only

organic N. It would be impossible to meet initial peak demands without excessive N in the soil before and after crop growth. For the same reason crops with high maximum N demand during a short period are poorly suited to organic systems. In systems based on organic N supplied through plant materials, crops with a more extended N-uptake pattern immobilising available N over a longer period are more suitable. Such a scenario facilitates synchronisation. Depending on the N-uptake pattern of the crop, it might be worth considering a combination of green manure and fertiliser to keep the addition of organic N down while meeting peak N demand with artificial N.

In general however, I believe that when spring incorporation is practised the main focus does not necessarily have to be on exactly synchronising N release and plant uptake. It is more important to make sure that the N really is present in the soil profile in the springtime and then the plant will utilise it when it is needed. On the other hand, for soils with a high risk of leaching there is a greater need to actually try to manipulate the pattern of N mineralisation, or maybe even to avoid the use of green manure. As an example, the losses of N through leakage from a farming system based on N-fixation in south-west of Sweden were found to represent 60% of the total amount of removed N (Torstensson, 2003). More N was lost through leakage (44 N kg ha^{-1} $year^{-1}$) than through the harvested crop (32 kg N ha^{-1} $year^{-1}$). Why in this case a system based on N_2-fixing crops were evaluated as most inappropriate both from a productive and an environmental point of view (Torstensson, 2003).

Complicating factors in the field

Interactions between plant materials

The combination of two or more plant materials that are different in composition may impair the prediction of C and N mineralisation: the combined materials may interact with each other changing the pattern of decomposition. Collins *et al.,* (1990) found that by mixing plant parts the mineralisation of C increased by up to 25% above that predicted by summing cumulative C mineralisation evolved from each individual plant part. Similar effects on the mineralisation of N have also been discovered. It has been concluded that strong interactions are likely to be found during decomposition of a combination of plant materials when large amounts of soluble C are present in one of the residues (Handayanto *et al.,* 1997). Quemada and Cabrera (1995) found that the amount of N mineralised from a soil amended with a mixture of leaves and stems from a monocotyledon was lower than that predicted based on mineralisation measurements from each single plant fraction. The difference in N concentration between plant materials or plant parts influences the pattern of mineralisation (Hesselsøe *et al.,* 2001; Jensen, 2000) creating zones of net N mineralisation and net N immobilisation respectively. In such situations decomposition performed by fungi may be of great importance as fungi, in contrast to bacteria, have the possibility to extend across substrates by their hyphae. The hyphal extension from plant materials high in nutrients permits utilisation of plant materials low in nutrients, transporting necessary nutrients from the “hot-spot” to the “cold-spot”, which will further stimulate decomposition of combined plant materials.

In general there is a lack of knowledge about the interaction between plant materials of different composition during decomposition. It has been observed that the proportion of respired C increases with an increasing addition of C (Marstorp and Witter, 1999). This is one possible explanation of why measured C mineralisation can exceed the predicted value. The pattern of microbial colonisation may depend on the chemical composition of plant materials. Plant materials with a high content of easily available substrates will give the decomposer organisms an opportunity for early growth, resulting in a subsequent more rapid rate of decomposition of the more slowly degradable plant components. Understanding of these effects however needs to be improved as interactions between plant materials may have great impact on the pattern of N mineralisation. For example, if an initial immobilisation of N from two plant materials deliberately combined in order to stabilise N during the autumn does not result in the predicted proportions, N losses may increase.

Changes of the soil organic N pool

Repeated additions of plant material will increase soil organic matter. Organic material that is not completely mineralised during the first year contributes to improved long-term N availability through carryover of un-mineralised material (Pang and Letey, 2000). Mineralisation of the native organically bound N on a yearly basis accounts for 1 to 2% of the total organic N present in soil (Johnston, 1986), equalling 80 to 160 kg N ha^{-1} in a Swedish soil with a N content of ca 8 ton ha^{-1} (Paustian *et al.,* 1990). Accumulated un-mineralised material from previous years contributes to an increased yield and N leaching in successive years. By using a model describing organic C turnover Kirchmann *et al.,* (2002) estimated that regular incorporation of straw during a 10-year period would result in 24 kg more N ha^{-1} $year^{-1}$ being mineralised. Frequent addition of organic matter will in the long run change the level of soil organic matter and hence N mineralisation potential. Therefore Pang and Letey (2000) recommended that altered amounts of organic N should be applied from one year to another. With such management the pool of organic N originating from a year with high amendments of green manure will be utilised the following year. Thus, yields can be kept high with a relatively small risk for increased leaching.

Moisture and Temperature

As all the work included in this thesis was done under controlled conditions no effects of changes in water and temperature regimes were considered. However, as discussed earlier, moisture and temperature affect the rate of mineralisation (Swift, 1979; Haynes, 1986). The optimum moisture level for mineralisation of N is ranging approximately between 45 to 60% water-filled porosity (WFP) (Beauchamp and Hume, 1997; Linn and Doran, 1984). At about 60% WFP there is a sharp transition to O_2-limiting conditions and denitrification begins to increase (Beauchamp and Hume, 1997). If incorporation of an N-rich plant material coincides with a heavy rainfall saturating the soil, there may be a great risk of substantial losses of N through denitrification.

In the temperature recalculations (Figure 8), the N mineralisation activity was set as absent at 0 °C, even if Lomander *et al.,* (1998) suggested that –5 ºC was the minimum temperature for decomposition activity. The low microbial activity at soil temperatures of 0 °C or less affects both the C and N mineralisation process. Andersen and Jensen (2001) and Nicolardot *et al.,* (1994) discovered that decomposition of recalcitrant substances, such as structural carbohydrates, was more limited at low temperatures than that of the more easily degradable substances. Consequently, immobilisation of N due to decomposition of easily available C might be expected even at relatively low temperatures. However, the process of N immobilisation has been observed to be more sensitive to low temperatures than the process of N mineralisation.

But even if low temperatures slow down microbial processes, suggesting low N mineralisation and low risks of leaching during wintertime, mineralisation of N is not negligible. High release of N from incorporated plant material during periods with soil temperatures below zero or close to zero has been found (Breland, 1994b; Magid *et al.,* 2001). A positive net N mineralisation was obtained during decomposition of *L. multiflorum* at 3 ºC while net N immobilisation was obtained during decomposition at 9 ºand 15 ºC from the same plant material (Andersen and Jensen, 2001). Also periods of freezing and thawing, which can occur in late winter and early spring, showed a significant increase in N mineralisation as N flushes (Herrmann and Witter, 2002). Based on this information the initial period of N immobilisation due to modification by *P. pratense* might not have been so extensive in a more realistic situation.

O_2 levels

The process of immobilisation is enhanced under aerobic conditions and impaired under less aerobic conditions. When large amounts of organic material are added conditions leading to anaerobiosis may develop. Anaerobic conditions affect the opportunities for soil microorganisms to assimilate C and N, with seriously impaired N immobilisation as a result (Paul and Beauchamp, 1989; Vinten *et al.,* 2002). Vinten *et al.,* (2002), moreover, found that low O_2 levels apparently reduced decomposition of easily decomposed carbohydrates, such as sucrose and glucose, more than slowly decomposed cellulose. If this is so, low O_2 levels in combination with low temperatures, a combination that may very well occur during incorporation of plant material during late autumn, could have an extensive impact on the process of N immobilisation and mineralisation. Special care must therefore be taken to make sure that the soil is carefully cultivated during autumn incorporation to allow aeration of the soil.

The right plant material for the right effect

Present plant materials

Assuming that plant material components control the dynamics of C and N mineralisation and consequently may be used to optimise N release will improve the possibility of finding the right plant material for the right effect. Cereal straw has so far often been used as an ‘immobiliser’. But according to the results in

Paper III which are in agreement with others (Vinten *et al*., 2002), straw is not the ultimate plant material to use to obtain an efficient and quick net immobilisation of N. There are other plant materials more suitable. Several grasses, more commonly used as forage crops, have been developed to provide high energy and nutritionally efficient food for ruminants. As the exchange of C as well as N must be as high as possible, several forage grasses contain large concentrations of easily decomposed storage compounds. As a further improvement, 'super sweet' forage grass species, containing up to 35% of easily available low molecular carbohydrates on plant material dw basis, have been developed (Radojevic *et al.,* 1994; Smith *et al.,* 1998). An alternative plant material not yet so commonly utilised for this purpose is *Cichorium intybus* that is rich in fructan (Koch *et al.,* 1999). It is also possible to achieve a relatively high concentration of easily decomposable carbohydrates in more common grass species. Among the plant materials used in this thesis, the highest concentration of easily decomposable sugars was found in *P. pratense*, which had a concentration of as much as 24% of plant dw. However, using a grass species or an alternative plant species specially selected to accumulate high concentrations of simple sugars is probably more reliable.

Significant differences in rate and amount of N mineralised during decomposition of different legume species have been found (Frankenberger and Abdelmagid, 1985; Marstorp and Kirchmann, 1991). The compositional properties of *M. sativa* stems used in Papers II and III may be worth taking a closer look at. The low arabinose-to-xylose ratio in *M. sativa* stems, more comparable to the grasses than to the other legumes, indicated that the hemicellulose fraction was dominated by slowly decomposable linear xylan. The stem material caused even less C mineralisation than the grasses (Paper II). The effect on N mineralisation was only a small initial N immobilisation induced by the stem material, and compared to the other two legume stem materials the net N mineralisation started at an earlier stage (Paper III). A combination of *M. sativa* stems with its N-rich leaves, such as the whole plant material, would probably not induce initial immobilisation. On the contrary, it probably would give favourable conditions for initial net N mineralisation if compared to the other legume stems used in Papers II and III that have higher proportions of easily decomposable C, leading to initial immobilisation instead. The slowly decomposable structural carbohydrates in *M. sativa* stems might on the other hand provide a reduction of the N mineralisation during a later vegetative stage when the N demand of the crop has decreased.

Future plant materials

Even if it has been shown to be possible to change and steer the pattern of net N mineralisation by taking advantage of plant material chemical composition, I would like to address the question of whether the present plant materials really offer an adequate variability in composition. Perhaps more specifically adjusted, tailor-made green manure crops are required? What properties should they have? As an example, to make sure that the release of N from a spring-incorporated plant material is sufficient both in amount and in time it might be necessary to work on further modifying and adjusting the chemical composition to achieve this. Today

the stem fraction from legumes such as *T. pratense* and *T. repens* often contains high concentrations of pectic substances and hemicellulose that have a relatively high decomposition rate. This creates favourable conditions for large amounts of N being immobilised at early stages that otherwise could have been net mineralised. In addition, the compositional differences between plant materials expected to generate a specific pattern of N mineralisation during decomposition may be too small to induce significantly different results, especially under field conditions where several additional factors may influence and mask the effect of plant material chemical composition.

During the 1960s serious efforts to genetically improve the nutritional value of forage crops by selection were initiated. Since then successful increases in digestibility have been documented in new cultivars of several forage crops, including legumes and grasses (Casler and Vogel, 1999). Similarly, the compositional properties of plant materials may be changed by different breeding methods with the purpose of finding the best possible green manure materials suitable for specific management practices. Special emphasis should be placed on developing plant materials with even higher or lower capacities to immobilise or release N at an early stage of decomposition than already existing plant materials; as well as plant materials with even higher or lower capacities to reduce net N mineralisation during a late stage of decomposition than it is possible to find today.

There are at least two compositional properties that are possible to modify to either improve or impair the digestibility of the cell wall. First, the total level of pectic substances in both stem and leaf of *T. pratense* and *M. sativa* was found to be quite variable (Hatfield, 1992). Through selective breeding legumes high in pectic substances could be created. This would offer a plant material with the properties to immobilise more N to soil microbial biomass during a prolonged initial decomposition period, a situation that would be preferable in a situation with autumn incorporation. In contrast, legumes as low as possible in pectic substances could also be created. In combination with a high N content these materials would facilitate initial and intermediate net N mineralisation improving synchronisation with crop N demand in the spring. Similarly, it is probably also possible to modify the composition of the hemicellulose towards a high or low arabinose-to-xylose ratio through selective breading.

The second compositional property that could modify the digestibility of the cell wall is the lignification. Through more detailed knowledge about the biosynthesis of lignin in a plant cell, more possibilities to modify the biosynthesis occur (Wilson, 1993; Bavage *et al.,* 1997; Hatfield *et al.,* 1999; Hopkins *et al.,* 2001). Natural or induced mutations were earlier identified as leading to abnormal structures and altered amounts of lignin in cell walls (Bacic, 1988). Jung and Engels (2002), suggest that the degradability in the rumen, and presumably also in soil (my comment), of legumes and grasses could be improved if the lignified secondary tissues in legumes and grasses are prevented from being deposited. A reduction of lignification is already being effected today through development of gene modification programs through down regulation of enzymes in the lignin

pathway (Schuch *et al.,* 1990; Hopkins *et al.,* 2001). By this procedure it is possible to maintain the decomposability of the structural carbohydrate and consequently increase the microbial assimilation of N. By manipulation of the lignin biosynthesis it could also be possible to create a plant material that is extensively lignified, thus ensuring a slow decomposition rate of the structural carbohydrates through physical protection (Berg *et al*., 1982; Chesson, 1997), while maximising the potential net mineralisation of N. Such properties may be achieved with a legume with a degree of lignification exceeding 15% of plant material dry weight, which is the minimum level for reduced decomposition by lignification (Chesson, 1997).

An even more sophisticated strategy would be to combine the two proposed ways of modifying plant material composition. Either by creating a plant material low in pectic substances and with a low arabinose-to-xylose ratio, but with a high degree of association between lignin and structural polysaccharides to impair C availability. Or alternatively, by creating a plant material high in pectic substances and with a high arabinose-to-xylose ratio but low in lignin association to improve C availability. The result would be two different plant materials with properties to either release or immobilise as much N as possible.

Conclusions

- The timing between decomposition of plant carbohydrates and N components is crucial for the course of both C and N mineralisation. Based on the time interval of decomposition, and thus the time of influence on C and N mineralisation, the most important plant components in temperate-zone grasses and legumes determining the pattern of C and N mineralisation can schematically be sub-divided in three groups.

  1. Free sugars, fructans and soluble N components, being the most easily decomposable plant components, have the main influence on C and N mineralisation during the first few days of decomposition at 20 ºC.

  2. Starch, non-cellulose structural carbohydrates, *e.g*. pectin and the most easily decomposable parts of the hemicellulose fraction, and non-soluble N components, being mainly decomposed later than free sugars but prior to cellulose, have their main influence on C and N mineralisation during this intermediate interval, taking place after approximately two days but before two weeks of decomposition at 20 ºC.

  3. Xylane and cellulose, being the most slowly decomposable structural carbohydrates, and to some extent modified by lignin, have the main influence on C and N mineralisation during decomposition after approximately two weeks at 20 ºC.

- The relative amounts of arabinose and xylose in the hemicellulose fraction proved to be useful as a measure of the inherent hemicellulose composition

with obvious effects on C and N mineralisation after approximately two days but before two weeks. It is therefore suggested that the concentration of arabinose and xylose rather than the total concentration of hemicellulose should be used when predicting C and N mineralisation from plant chemical composition.

- N mineralisation from an N-rich plant material can be modified by addition of a second plant material. Plant materials rich in free sugars, fructans, starch, pectin, and with a hemicellulose characterised by a high arabinose-to-xylose ratio may be used to enhance early immobilisation of N. Plant materials dominated by slowly decomposable structural carbohydrates, such as xylan and cellulose, may be used to reduce N mineralisation during later stages of decomposition.

- The utilisation of green manure has great potential for improvement in terms of optimising N mineralisation to meet crop N demand and to minimise N losses. But, more knowledge is required concerning the significance of the effect of chemical composition on N mineralisation in a field situation. New tailor-made plant materials with a greater range in chemical properties, compared to the present plant materials, may be needed to achieve the intended effects in a field situation.

- Plant material chemical composition may offer a valuable complement to other management options, such as soil cultivation and timing of incorporation, that aim to optimise N mineralisation in relation to crop N demand and to minimise N losses.

- To further improve optimisation of N mineralisation an alternative could be to reduce the N input from a carefully chosen green manure to minimise the risk of N losses, and replace this with a small addition of fertilizer to meet crop peak N demand. All the beneficial soil properties, such as improved microbial activity, aggregate stabilisation *etc*. obtained through the added plant material would be maintained, but crop N demand also satisfied.

- There were no major effects on root composition leading to reduced decomposition rate in roots grown at elevated CO_2 levels compared to roots grown at ambient CO_2 levels. Despite treatment-induced differences found on root composition, input and microclimate there were only small differences in soil C dynamics on a 30-year timescale, compared to the change in soil C dynamics caused by a change in land use.

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Acknowledgements

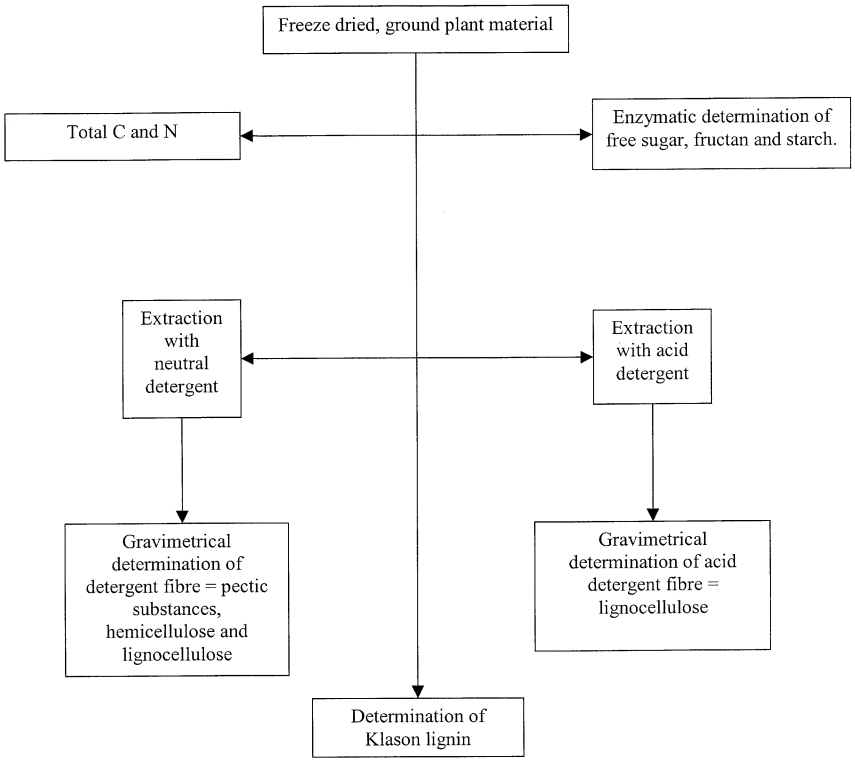
I would like to thank all of you, colleagues, friends, students and dogs, that in one way or another have been involved in making my time as a Ph.D. student so valuable and memorable.

I would like to dedicate special thanks to…
Food 21, financed through MISTRA, offering me the possibility to become a Ph.D. student within the programme.
Håkan Marstorp, my supervisor, for guiding me and bringing light into the dark and complex world of soil science as well as for being available no matter what. Thanks also for trying your best to teach me everything from how to mix substrate and soil thoroughly, to perform necessary and unnecessary stuff on the computer, not to speculate so much or to explain my results, to dig under each stone before someone else does, and how to express myself briefly and concisely without hiding my own results. I still have much to learn! But there is one thing you will never convince me of – and that is how on earth it would be possible to have a closer and more dedicated relationship to a tractor than to a dog, even if the dog has bad breath.
Ernst Witter, my assisting supervisor, for all discussions about hypotheses, methods, results, manuscripts, *etc*., and for helping me urgently with the respirometer at times of flooding. For inviting us to your lovely home, filled with delicious food, to discuss ^{15}N-techniques or, even better, just for enjoying ourselves as well as for supplying my parents with a half of a 'happy', but unfortunately dead, lamb now and then.
Inger Juremalm for making the environment in the lab so friendly and pleasant and for always being available when I needed a helping hand. The lovely handmade rag carpets you made will decorate my floors for many years. Rose-Marie Ericsson for analysing all those hundreds of extracts, even if I sometimes forgot to replace the screw corks with rubber corks. Pär Hillström for contributing with great expertise when I was cultivating my grasses and legumes.
Sigrun Dahlin for valuable comments on my thesis and for supplying me with chocolate perfectly synchronised with my demands!
Jan Persson for supporting and re-filling me with enthusiasm at exactly the right moment.
Shelagh Green for revising my English and for preparing all that delicious food we ate in your and Ernst's home.
Anke, Anna, Barbro, Erik, Erika, Gudrun, Gunilla, Helena, Ingela, Katarina, Lena, Magnus, Petra, Pål, and Stefan - my Ph.D. colleagues. To be honest, the best thing during the years at the department was meeting you all.

Katarina Börling, I suppose this is where our study relationship ends. After being used to having you as good support by my side for almost 12 years, it is going to feel strange without you. But you will not lose me as a personal friend for many years to come!
Anna Hedlund, you are one of the most colourful persons I have met, both in spirit and in looks and you inspire me a lot. I have really enjoyed our small projects on the side and I'm looking forward to future projects and performances, skating and skiing tours, cosy evenings at Skogshyddan, or why not another trip to Stockholm?
Barbro Beck-Friis, you and your lovely family, Johan, Lars and Caroline, opened up your house to me and made it more or less my second home. In my heart you will always be like the big sister I never had.
Erik and Lotta Sindhøj– thank you for your warm and generous friendship and for making my own life in my small world full of experiences through you. I am so happy that you are back home and I wait impatiently to meet the third member of the Sindhøj family.
Jim Greatorex with whom Erik, Barbro and I have shared so many good laughs and moments that I will always treasure. For example, the unforgettable evenings composing what should by now have become the worldwide hit and top of the blouse lists – 'Erik Sindhøj blues'. It's about time we all met for another semla bun.
Leticia Pizzul, Elga Monaci and Kristin Steger - you beautiful and ambiguous girls at the Department of Soil Microbiology - what would Sweden be without you? You'd better hurry up finishing your theses so that we can open that Argentinean/Italian/German/Swedish restaurant. Elga, whatever you do and wherever you are remember that you are forever adopted by the 'Gunnarsson family'.
All my friends outside SLU, who enrich my life with other things than work. Special thanks to all my friends in Uppsala Vokalensemble with whom I share so many musical experiences while re-charging my batteries.
Rikard, my big brother, Anna and Elias Owenius, you are so dear to me. Despite living so far away during recent years, you have always been close. Having you and especially Elias, my beloved nephew, in my thoughts always makes me feel like the happiest and most fortunate aunt in the world. I have counted the days until your return back home – and now the time has arrived. Lucky me!
Mother, father and Pilli – you are my absolute favourites. You have always been and will always be the best!

Appendix A

A schematic flow chart of the procedure used for proximate chemical analysis of the plant materials used in Paper I.



Appendix B

A schematic flow chart of the procedure used for the more detailed chemical analysis of the plant materials used in Papers II and III.