Lignin Degradation

Long-term effects of nitrogen addition on decomposition of forest soil organic matter

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2003

Acta Universitatis Agriculturae Sueciae
Agraria 419

ISSN 1401-6249
ISBN 91-576-6449-8

Tryck: SLU Service/Repro, Uppsala 2003

Abstract

Sjöberg, G. 2003. Lignin Degradation – Long-term effects of nitrogen addition on decomposition of forest soil organic matter.

Doctoral dissertation.

ISSN 1401-6249, ISBN 91-576-6449-8

Long-term addition of nitrogen (N) reduces the decomposition rate of humified soil organic matter (SOM) in forest ecosystems causing an increase in SOM storage. This could be an effect of (1) suppressed enzyme activity among white-rot fungi degrading lignin (2) formation of recalcitrant phenolic compounds or (3) altered microbial composition. The aims were to study the long-term effects of N addition on CO_2 evolution, production of dissolved organic matter (DOM), lignin degradation and structural changes of the C and N composition. CuO oxidation, solid-state and dipolar-dephasing CPMAS ^{13}C and ^{15}N NMR, Klason lignin and acid detergent lignin (ADL) were used as methods.

Fresh and decomposed needle litter, Oi, Oe and Oa layers, and fresh and decomposed root litter were investigated. The needle litter was decomposed under laboratory conditions, whereas the root litter was decomposed in litterbags under field conditions. To estimate CO_2 evolution and DOM production, Oe and Oa layers were laboratory incubated in columns. The samples originated from the long-term N fertilization experiment Skogaby in southwestern Sweden (plots denoted NS receiving 100 kg N ha^{-1} yr^{-1} as $(NH_4)_2SO_4$). The long-term N fertilization experiment Stråsan in south-central Sweden (plots denoted N1, N2 and N3 receiving 35, 73 and 108 kg ha^{-1} yr^{-1} as NH_4NO_3) was also used in the column incubation.

The unfertilized and the N-fertilized plots at both sites were compared with each other for each layer or decomposition period. The CO_2 evolution rate was significantly lower in the NS Oe layer than in the control Oe layer at Skogaby. The N3 Oe layer at Stråsan showed a similar trend. No significant treatments effects on the degree of lignin degradation (expressed as $Ac/Al)_V$) could be found except for 853-day decomposed root litter. The lignin degradation products (VSC) obtained by CuO oxidation did not show any significant treatment effects. No heterocyclic N compounds could be detected by ^{15}N NMR in the forest floor at Skogaby. The dipolar-dephasing technique showed that tannin was present. Tannin might have caused the slight increase in phenolic C with depth. In conclusion, no major qualitative change in the SOM due to N was found and no heterocyclic N compounds were observed. Thus, the common explanations for decreased decomposition due to N additions did not seem to be valid.

Key words: Norway spruce, Litter, Mor humus, Lignin, CuO oxidation, Klason lignin, Acid detergent lignin, ^{13}C NMR, ^{15}N NMR

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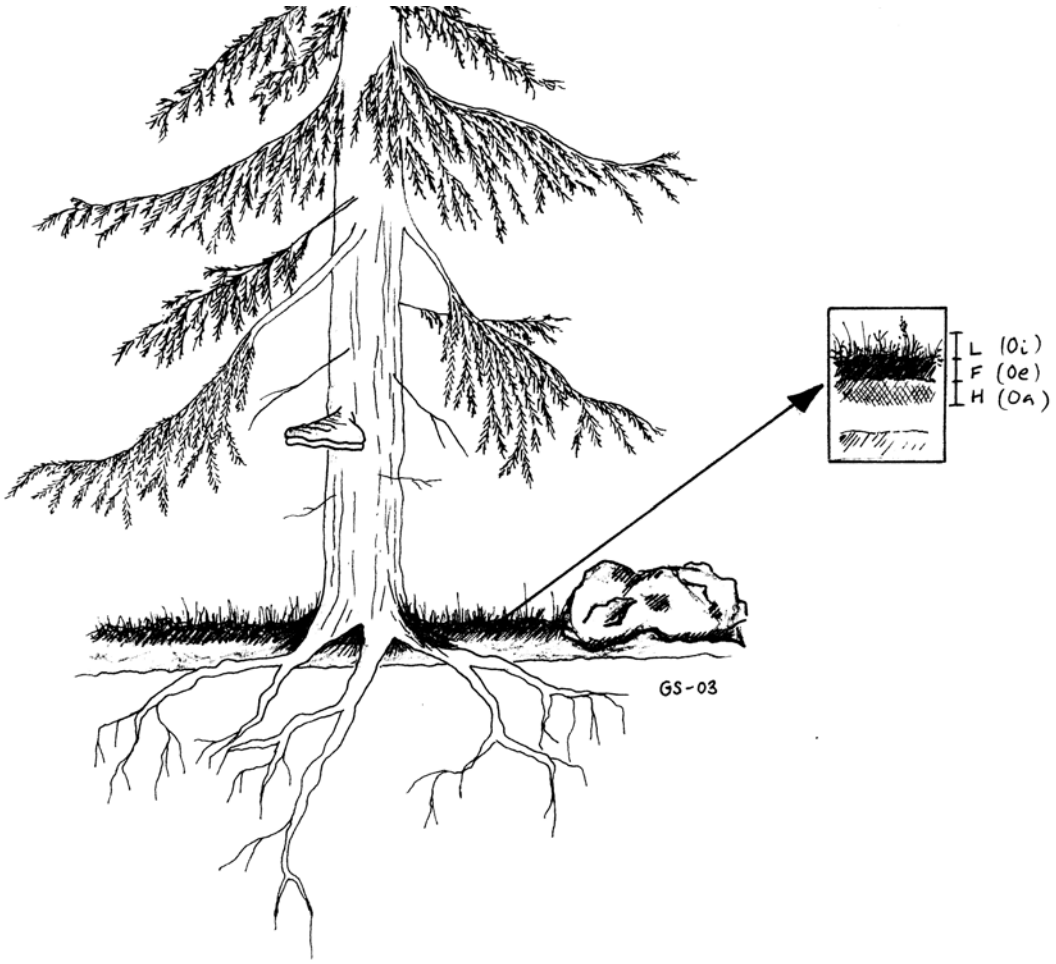
L (Oi)
F (Oe)
H (Oa)
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Contents

Appendix

Papers I-III

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

I Sjöberg, G., Bergkvist, B., Berggren, D., Nilsson, S.I. 2003. Long-term N addition effects on the C mineralization and DOC production in mor humus under spruce. Soil Biology & Biochemistry (In press).

II Sjöberg, G., Knicker, H., Nilsson, S.I., Berggren, D. Impact of long-term N fertilization on the structural composition of spruce litter and mor humus. Soil Biology & Biochemistry (In revision).

III Sjöberg, G., Nilsson, S.I., Persson, T., Karlsson, P. Degradation of cellulose and lignin in decomposing spruce needle litter in relation to N. Soil Biology & Biochemistry (Submitted).

Introduction

Nitrogen (N) is released to the atmosphere as oxides through combustion of fossil fuels, as ammonia from animal wastes and fertilizers and as gases formed by natural soil processes (such as denitrification). These N compounds may thereafter be transported and deposited. Atmospheric deposition of N is especially high in central parts of Europe. In south-western Sweden, atmospheric deposition of inorganic N may reach levels of 30 kg N ha^{-1} yr^{-1} whereas in northern Sweden it is reaching 6 kg N ha^{-1} yr^{-1} (Bernes, 1993; Lövblad *et al*., 1995; Lövblad, 2000). At the end of the 1960s, the deposition of S and N compounds in Europe were debated because of their potential effects on forest ecosystems in Sweden (Odén, 1968), mainly through acidification. In the 1980s, Bernes (1981), Hallbäcken and Tamm (1986) and Nilsson (1993) among others claimed that lakes and forest ecosystems in Sweden were severely affected. Since the 1990s, there has been a considerable decrease in S deposition, but similar declining trends have not been observed for N deposition (Galloway, 2001). An additional effect of the high N deposition may be an increase in soil C. More recently this effect has come into focus due to its connection to the climate change issue. Increased addition of N to forest ecosystems may cause the following effects:

- Increasing amount of litterfall due to enhanced tree growth and litter production (Tamm, 1991).

- Decreasing decomposition rate of humified soil organic matter (SOM) on a long-term basis (Bååth *et al*., 1981; Berg *et al*., 1982; Söderström *et al*., 1983; Nohrstedt *et al*., 1989; Arnebrant *et al*., 1996; Sjöberg, 2000; Persson *et al*., 2001; Michel and Matzner, 2002). However, addition of N has a positive effect on SOM decomposition during early stages. For example, Berg *et al*. (1982) showed an initial stimulation of litter decomposition by raised N concentrations, whereas high N concentrations resulted in a declining decomposition rate when the litter became more humified.

Increasing input of N-rich litter to the forest floor as well as a reduced decomposition rate on a long-term basis may explain why storage of SOM in the upper soil horizons can increase with time. An increased storage of SOM may have an impact on the carbon dioxide (CO_2) level in the atmosphere since less CO_2 will be released from the ecosystem. It is therefore important to know why forest soils have a reduced decomposition of SOM after long-term additions of N. At present, little is known about the mechanisms that are responsible for a decreased C mineralization. One hypothesis is that the lignolytic activity among white-rot fungi is reduced in the presence of high inorganic N levels (Keyser *et al*., 1978; Reid, 1991). Another hypothesis is that N stabilizes phenols by forming recalcitrant compounds (Nömmik and Vahtras, 1982). It has also been hypothesized that reduced decomposition is a result of a change in the decomposer composition (Ågren *et al*., 2001).

Aims

In this thesis I have focused on studying the long-term effects of N on:

1. Heterotrophic respiration and production of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in Oe and Oa layers (Paper I)
2. Structural composition of organic C and N compounds in Oi, Oe and Oa layers with an emphasis on lignin degradation (Paper II)
3. Decomposition rate and composition of organic compounds in fresh needle litter with an emphasis on lignin degradation (Paper III)
4. Lignin degradation in root litter with emphasis on why long-term root litter decomposition may be enhanced by additions of N (Complementary Study)

Papers I and III were carried out as laboratory experiments whereas Paper II was based on characterization of sampled forest floor material. The Complementary Study was based on root litter decomposition in the field. All samples (Papers I-III and the Complementary Study) were collected from the long-term N fertilization experiment at Skogaby. The experimental site Stråsan was included in Paper I.

Background

Forms of organic C in soils

SOM contains plant and animal residues, microbial products and humified substances. The proportions of cellulose, hemicellulose, lignin, proteins and other compounds can vary. For instance, plant remains may contain 15-30% lignin, 15-50% cellulose and 15-20% hemicellulose and other compounds such as proteins, lipids, tannins, suberin and cutin (Swift *et al*., 1979; Kögel-Knabner, 2002). Johansson (1995) found that needles that were shaken down from spruce trees contained on average 32% lignin, 29% cellulose and 21% hemicellulose. During litter decomposition, the energy-rich large molecules of carbohydrates and proteins are depolymerized by microorganisms (Mellilo *et al.*, 1989) and converted into smaller molecules such as carboxylic acids, amino acids and CO_2. The complex polymer lignin accumulates with time.

Cellulose and hemicellulose

Cellulose and hemicellulose are polysaccharides. Cellulose consists of glucose units in long polymeric chains $(C_6H_{10}O_5)_n$, whereas hemicellulose is composed of branched chains of cellulose-like sugar units. Hemicellulose is less polymerized than cellulose and therefore decomposes faster (Swift *et al*., 1979). Decomposition of cellulose occurs in the presence of enzymes (cellulases) produced by bacteria and fungi. During decomposition of hemicellulose, the pectinase enzymes are active. Hemicellulose may either occur free or covalently associated with lignin

through ether and ester bonds in the cell walls. Together they surround cellulose, forming a ligno-cellulose complex (Kirk and Farrell, 1987; Melillo *et al*., 1989).

Lignin and tannin

Lignin is a component in cell walls of vascular plants (Kögel-Knabner, 2002). The highest concentration of lignin is found in the middle lamella and in corner regions, causing these areas to be delignified last (Kirk and Farrell, 1987; Blanchette, 1991). Lignin can be degraded by wood-rot fungi but is only completely degraded by white-rot fungi. Some bacteria species can also degrade lignin (Crawford *et al*., 1983; Kirk and Farrell, 1987). Lignin is a polymer with an aromatic structure (Fig. 1) built up by phenylpropane units (aromatically bound $CH_3CH_2CH_3$). The proportions of these units within the lignin structure vary between different life forms of plants. Gymnosperms (softwoods, such as coniferous trees), angiosperms (hardwoods, such as deciduous trees) and grasses have therefore varying lignin structures. Gymnosperm lignin is mainly built up of phenylpropane units called vanillyl phenols, *i.e.* coniferyl alcohol-derived units (Hedges and Mann, 1979). Other groups in gymnosperm lignin are sinapyl-type phenols (*i.e.* syringyl phenols) and coumaryl-type phenols (*i.e.* cinnamyl phenols). Adler (1977) showed the relative proportion of these three phenolic units (coniferyl:sinapyl:coumaryl) in spruce lignin to be 94:5:1. The content of sinapyl-type phenols is higher in angiosperm lignin whereas coumaryl-type phenols can be found in larger amount in lignin from non-woody tissues. Angiosperm lignin has a relative proportion of 56:40:4 between coniferyl:sinapyl:coumaryl, whereas grass lignin is built up of equal amounts of these three phenolic units (Crawford, 1981).

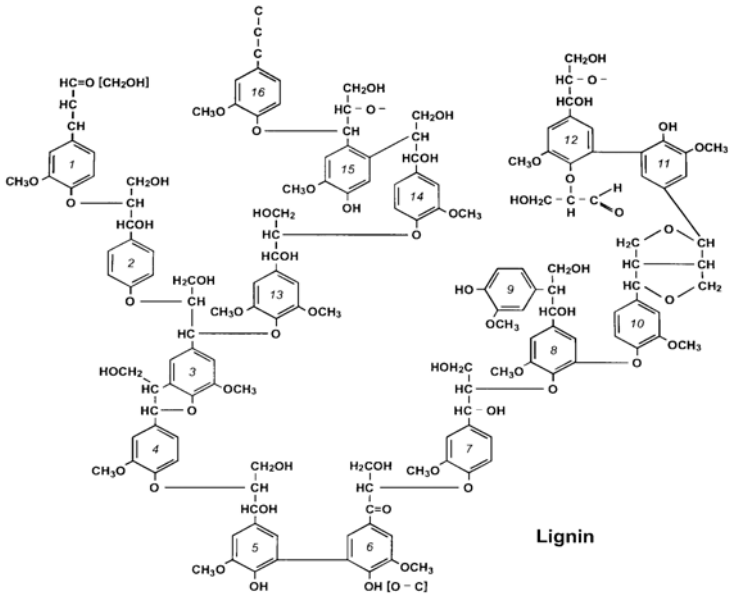

Figure 1. The chemical C structure of spruce lignin (Kögel-Knabner, 2002).

Tannin is a polyphenolic macromolecule found in leaves, needles, bark and roots. Spruce litter in particular contains a high amount of tannin (Behrens *et al*., 2003). Tannins can be divided into condensed tannin (CT, proanthocyanidin) and hydrolyzable tannin (HT, gallic acid). Flavanol monomer units (polyhydroxy-flavan-3-ol) that are linked together by C-C bonds (C4-C8 or C4-C6) form condensed tannins. Condensed tannins are more insoluble and decompose more slowly than the hydrolyzable ones. After being released into the soil, some of the tannins can bind with proteins present, forming water insoluble protein-tannin complexes (Northup *et al*., 1995; Hagerman *et al*., 1998). Tannins can therefore affect the availability of N in soils. However, many questions concerning the stability and function of tannins in soils remain unanswered. This is mainly due to the fact that common extraction and identification techniques are not suitable for isolating tannins in humus (Lorenz *et al*., 2000; Lorenz and Preston, 2002) whereas they have been successfully applied to fresh needle and leaf litters (Behrens *et al*., 2003; Maie *et al*., 2003).

Forms of organic N in soils

Scientists have discussed the possibility that SOM may become recalcitrant due to chemical reactions between N and phenolic compounds, such as lignin (Waksman, 1936; Nömmik and Nilsson, 1963; Nömmik and Vahtras, 1982; Berg, 1986; Axelsson and Berg, 1988; Johnson, 1992; Stevenson, 1994; Sollins *et al*., 1996). Such reactions have been shown to occur in laboratory studies. Kelley and Stevenson (1996) showed that amino acids could be bound to aromatic rings. Schulten *et al*. (1997) studied organic N in acid hydrolysates and hydrolysis residues from 6M HCl hydrolysis of mineral soils and found heterocyclic N compounds such as pyridine and pyrrole. However, these stabilization reactions require pH values near or above 7 (Nömmik, 1970; Clinton *et al*., 1995) in order to convert NH_4-N to NH_3-N (Nömmik and Vahtras, 1982; Stevenson, 1994). Nömmik (1970) added amino acid-N to limed spruce humus and found that the organic N was dissociated to NH_3-N at pH 9 followed by complexation to the humus. That is probably the reason why these complexes between N and humus seem to be uncommon in forest soils under natural conditions.

Heterocyclic N compounds have not been detected in forest soils to an extent that this reaction could be considered as a major stabilization process during humification (Almendros *et al*., 1991; Knicker, 1993; Clinton *et al*., 1995; Knicker and Lüdemann, 1995; Knicker *et al*., 1997; Knicker, 2000). Knicker and Lüdemann (1995) used solid-state ^{15}N NMR on ^{15}N-enriched composted organic matter at pH varying between 5-9. They found no heterocyclic forms of N (such as pyridine N and pyrrolic N). The organic ^{15}N was mainly detected as amide-^{15}N (*i.e.* ^{15}N-amino acid groups bound to each other) as a peak close to -256 ppm in the spectrum. The remaining N was found as free amino acids and NH_4 ions. This was also the case in native SOM at natural abundance levels of ^{15}N (Knicker *et al*., 1993). Knicker and Hatcher (1997) studied organic-rich sediments of an algal

sapropel from Mangrove Lake, Bermuda, containing brackish water. They found that amide-N mainly originated from proteinaceous material and concluded that formation of heterocyclic N compounds was not likely. Sjöberg (2000) studied immobilization of $^{15}NH_4$-N and $^{15}NO_3$-N in forest floor material collected from the Swedish N fertilization field experiment Skogaby using solid-state CPMAS ^{15}N NMR and could confirm previous ^{15}N NMR findings that most N in SOM is present as amide-N. Added NH_4-N is therefore likely to be incorporated as amino acids and amino sugars in forest floor materials (Johnsson *et al*., 1999).

Dissolved organic matter compounds

The microorganisms in the forest floor also produce dissolved organic matter (DOM) during decomposition of plant residues (Cronan and Aiken, 1985). DOM can be measured as DOC and DON. These compounds can leach through the forest floor and further down in the soil profile. Liquid-state Cross Polarization Magic Angle Spinning ^{13}C Nuclear Magnetic Resonance (CPMAS ^{13}C NMR) spectroscopy has shown that DOC contains carboxylic C, carbohydrates and lignin-derived compounds (Guggenberger and Zech, 1994). McCarthy *et al*. (1997) analyzed ocean water using CPMAS ^{15}N NMR and found that DON mainly contained amide-N. This fraction was also found to be the major component in DON from spruce forest floors (Michalzik and Matzner, 1999). Some researchers have shown that fresh litter is the most important source of DOC (for instance Park *et al*., 2002), while others have shown that the main source is the more decomposed material in the Oe and Oa layers (Solinger *et al*., 2001; Fröberg *et al*., 2003). DON is believed to be a waste product after microbial decomposition (Kalbitz *et al*., 2000). It has been shown that the leaching of DOC and DON may be partly discoupled since the high-molecular hydrophobic fractions, having higher C-to-N ratio, are more easily adsorbed in mineral layers during leaching than the more low-molecular N-rich hydrophilic fractions (Andersson *et al*., 1999).

Lignin degrading microorganisms

Berg *et al*. (1982) and Berg and Ekbohm (1991) among others have previously shown that lignin is a major component of the decomposition of recalcitrant SOM at later stages. Fungi are the dominating microorganisms in the forest floor that are responsible for the decomposition of SOM (Reichle, 1977). Lignin is considered to be one of the refractory SOM components. Kirk and Farrell (1987) have shown that fungi do not use lignin as a C or energy source for their growth. Fungi will therefore only degrade lignin if other C sources (such as cellulose and hemicellulose) are present.

The fungal species that are able to degrade wood can be divided into brown-rot, soft-rot and white-rot fungi. Lignin degradation by bacteria such as *Streptomyces* sp. (Crawford *et al*., 1983) and Actinomycetes (Kirk and Farrell, 1987) occurs as an oxidation similar to the degradation performed by white-rot fungi. However,

these bacteria only have the ability to modify and degrade parts of the lignin molecules.

Brown-rot and soft-rot fungi

Brown-rot fungi predominantly belong to Basidiomycetes (*i.e.* fungi having spores externally on a basidium). They are a minority among wood-decaying species. However, they still contribute to wood decay in coniferous forests since they decompose polysaccharides and modify the structure of lignin (Eriksson *et al*., 1990). They lack ring-cleaving enzymes but can demethylate aromatic methoxyl groups into phenols (Aromatic-OCH_3 → Aromatic-OH). Other minority wood-decaying fungi are the soft-rotters, mostly belonging to Ascomycetes, which means that they have spores in tubular sacs on an apotheticum. They are most common in hardwoods where they soften the wood surface layers. They have the ability to cleave side chains and aromatic rings in lignin structures but can only partly degrade lignin (Kirk and Farrell, 1987).

White-rot fungi

A complete degradation of lignin can only be performed by the dominating group among the wood-decaying fungi, namely the white-rot fungi (Kirk and Farrell, 1987). White-rot fungi are found both in the Basidiomycetes and the Ascomycetes groups. They are able to produce lignolytic enzymes that oxidatively cleave the phenylpropane units, demethylate the methoxylic groups into phenols (Aromatic-OCH_3 → Aromatic-OH), oxidize aldehydes to carboxylic acids (Aromatic-CHO → Aromatic-COOH) and cleave the aromatic rings within the lignin structure (Kirk and Farrell, 1987; Eriksson *et al*., 1990). For instance, the ether-linkages between the phenylpropane units, which build up lignin, will therefore be broken releasing compounds such as vanillic acids (Fig. 2).

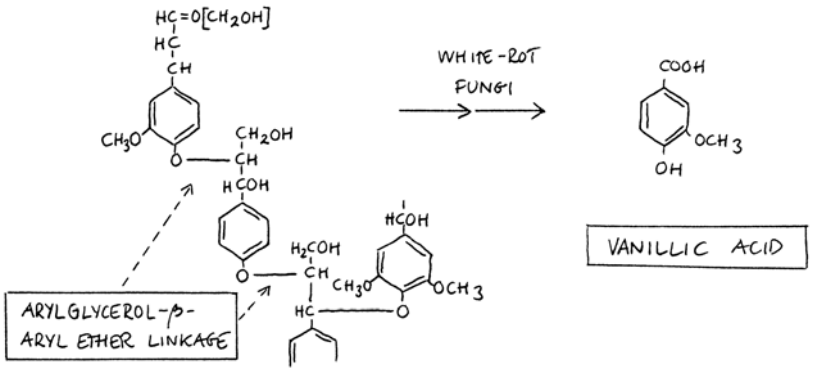

Figure 2. Ether linkage within the lignin structure and formation of vanillic acid.

The dominating linkage in coniferous lignin is the arylglycerol-β-aryl-ether, also expressed as β-O-4 linkage or β-aryl-ether linkage (Adler, 1977; Whitehead *et al*., 1981; Kögel and Bochter, 1985). *Marasmius androsaceus* is a white-rot fungus common in Swedish coniferous forests (Ryman and Holmåsen, 1984). It is an efficient colonizer and competitor as regards the decomposition of newly fallen needle litter (Frankland, 1998). According to Frankland (1998), this white-rot fungus is concentrated in the litter layer, whereas the white-rot fungus *Mycena galopus* is more restricted to mor humus layers.

Synthetic lignin, referred as DHP (dehydrogenation polymerizate), is believed to contain the same type of linkages as found in natural lignin (Kirk *et al*., 1975). Kirk *et al*. (1975) measured the lignolytic activity by studying the degradation of synthetic ^{14}C-lignin and the evolution of $^{14}CO_2$. By labelling different C atoms within the lignin structure, they found that the methoxylic C (-O$^{14}CH_3$) was the first to be degraded to $^{14}CO_2$, followed by aldehyde C (-^{14}CHO), carboxylic C (-^{14}COOH) and finally ^{14}C within aromatic ring structures. Studies have been carried out concerning the effects of NH_4^+ on DHP lignin degradation by white-rot fungi. For instance, Keyser *et al*. (1978) and Fenn *et al*. (1981) showed that the lignolytic enzyme activity of *Phanerochaete chrysosporium* was suppressed in the presence of NH_4^+. One explanation could be that the production of lignin-degrading enzymes becomes reduced in the presence of inorganic N, resulting in a reduced evolution of $^{14}CO_2$. Organic N in the form of glutamate has also a negative impact on lignin degradation. Fenn *et al*. (1981) and Fenn and Kirk (1981) found that addition of glutamate suppressed the lignin degradation by *Phanerochaete chrysosporium*. Furthermore, Reid (1991) showed that the ability of the fungus *Phlebia tremellosa* to degrade DHP lignin was negatively affected by glutamate. However, it is still not thoroughly demonstrated how lignolytic acitivity is affected by N addition, since Leatham and Kirk (1983) found that some species of white-rot fungi were unaffected and Kaal *et al*. (1993, 1995) showed that the white-rot fungi *Bjerkandera adusta* and *Pleurotus ostreatus* had an enhanced lignolytic enzyme activity in the presence of both organic N (peptone) and NH_4^+.

Methods for studying lignin

Klason lignin method

The insoluble organic residue after extraction with water and an organic solvent followed by hydrolysis in concentrated H_2SO_4 is called Klason lignin (Bethge *et al*., 1971; Berg *et al*., 1982). The Klason method is the standard method for determining the lignin content of wood (Swift *et al*., 1979). For needle and root litters, the method is considered less appropriate for determining lignin because cutin and suberin, found in needles and roots, are included in the Klason lignin fraction (Zech *et al*., 1987; Preston *et al*., 1997). A modification of the Klason method, including determination of "total dietary fibre" (*i.e.* non-starch polysaccharide residues + Klason lignin), is the Uppsala method (Theander and Westerlund, 1986; Theander *et al*., 1995). Neutral sugars (polysaccharide

residues) are then determined by gas-liquid chromatography, uronic acid residues (pectin and hemicellulose) by colorimetry and Klason lignin gravimetrically.

Acid detergent lignin method

By using an acid detergent solution consisting of cetyltrimethyl ammonium bromide (CTAB) and 72% sulphuric acid, the acid detergent lignin (ADL) fraction is obtained. This method was first developed by Van Soest (1963) and has thereafter been modified. It is commonly used in animal science and agronomy for estimating the lignin content of animal feeds. Rowland and Roberts (1994) investigated whether the method was suitable for woody heather and spruce litter materials and concluded that it was adequate for determining their lignin as well as cellulose content.

Alkaline CuO oxidation method

The alkaline CuO oxidation method is commonly used for studying the degree of lignin degradation in different plant materials since it yields simple phenolic products with preserved chemical structures from the original lignin polymer. The method was used as early as the 1940s and 1950s by wood chemists. For instance, Pearl and Beyer (1959) used CuO on wood and could detect degradation compounds from wood lignin. Pepper *et al*. (1967) compared CuO oxidation with nitrobenzene treatment and claimed that CuO was a milder and more selective oxidant and less harmful to the environment. The CuO oxidation method has also been used on other materials such as pine craft chlorolignin (Van Buren and Dence, 1970), fresh-water sediments (Hedges and Parker, 1976; Hedges and Ertel, 1982; Lobo *et al*., 2000), humic and fulvic acids extracted from peats (Hänninen and Niemelä, 1991), waste water from pulp mills (Hyötyläinen *et al*., 1995), forest humic materials (Kögel and Bochter, 1985; Kögel, 1986) and coniferous needle litters (Johansson *et al*., 1986; Sanger *et al*., 1996).

Using CuO oxidation, intact lignin structures can specifically be characterized since this method yields simple phenolic compounds originating from lignin (Ertel and Hedges, 1985; Kögel and Bochter, 1985; Kögel, 1986; Kögel-Knabner *et al*., 1988). The yield of lignin products after CuO oxidation is about 10% of the total content of spruce lignin (Kögel, 1986). The rest is condensed parts of the lignin structure, such as phenylcoumaran and pinoresinol (Kögel and Bochter, 1985; Kögel-Knabner *et al*., 1988). Therefore, the content of lignin derived by CuO oxidation is far from the total content of lignin, but the CuO method gives valuable information about the degree of lignin degradation. The CuO-derived products can be detected either by gas chromatography (Hedges and Mann, 1979; Hedges and Ertel, 1982; Hetherington and Anderson, 1998) or by liquid chromatography (Steinberg *et al*., 1984; Kögel, 1986; Hyötyläinen *et al*., 1995; Lobo *et al*., 2000). According to Kögel (1986), Ziegler *et al*. (1986), Kögel-Knabner *et al*. (1988) and Kögel-Knabner (1992), the phenolic compounds derived by CuO oxidation can be divided into the three major phenylpropane groups (Fig. 3); vanillyls (V), syringyls (S) and cinnamyls (C). These groups are

linked together by β-aryl ether linkages in particular. Vanillyls are mostly bound within the lignin structure by C-C and ether bonds, whereas cinnamyls are bound with relatively labile ester linkages. The CuO oxidation procedure breaks these linkages releasing vanillyls, syringyls and cinnamyls. The mass ratio between the acid (Ac) and the aldehyde (Al) components (Fig. 3) of the vanillyls ($(Ac/Al)_V$) reflects the side chain oxidation stage of the phenylpropane groups (Hedges and Ertel, 1982; Kögel, 1986; Ziegler *et al*., 1986; Kögel-Knabner *et al*., 1988; Kögel-Knabner, 1992). With increasing degradation, more acid lignin-derived compounds are released (Kögel-Knabner, 1993). Therefore, the $(Ac/Al)_V$ ratio increases with increasing decomposition, expressing the degradation degree of lignin. Ratios of about 0.2 are typical for lignin from undecomposed or slightly decayed vascular plant material (Ertel and Hedges, 1984), whereas SOM has ratios varying between 0.6 and 0.8 (Ertel and Hedges, 1985). Another way of expressing lignin degradation based on the CuO oxidation method is the acid-to-aldehyde ratio of syringyls, expressed as $(Ac/Al)_S$. This ratio also increases due to decomposition and is especially used when analyzing angiosperm lignin. The ratios S/V and C/V have also been used to study the origin of the organic material and its decomposition (Kögel, 1986; Ziegler *et al*., 1986).

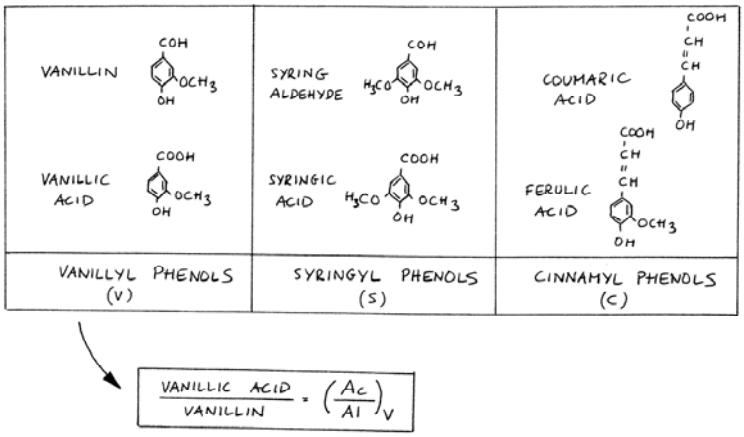

Figure 3. The main CuO oxidation products divided into 3 main groups (Vanillyls, Syringyls and Cinnamyls). The degradation degree of lignin can be expressed as $(Ac/Al)_V$ for spruce lignin.

Solid-state CPMAS NMR spectroscopy

Cross Polarization Magic Angle Spinning Nuclear Magnetic Resonance (CPMAS NMR) spectroscopy is commonly used to characterize SOM composition (Mathers *et al*., 2000). Neyroud and Schnitzer (1972) were among the first to use NMR to characterize soil humic substances. The method allows studies of the changes in organic C composition (Table 1) without any pre-treatment of the dried and ground soil samples. CPMAS ^{13}C NMR is based on a combination of techniques,

such as Cross Polarization (CP) and Magic-Angle Spinning (MAS). Using CP between ^{1}H and ^{13}C, a signal of the ^{13}C through the ^{1}H is obtained (Wilson, 1987). The MAS technique is used for eliminating dipolar interaction effects by rotating the sample at a magic angle (Andrew, 1972). About 1 atom-% of total C (natural abundance) in SOM consists of ^{13}C (Skjemstad *et al*., 1997).

Table 1. *Signal assignments for peaks in a CPMAS ^{13}C NMR spectrum (Kögel-Knabner, 1997; Kögel-Knabner, 2002)*

Chemical shift range ***(ppm)***	**Some of the assignments from ^{13}C**
200-160	Amide R-$CONH_2$, carboxyl COOH, carbonyl C=O
160-140	Aromatic CO-R, aromatic CN-R
140-110	Aromatic C-H, alkene/olefin C=C, guaiacyl C,
110-90	Anomeric C of carbohydrate –$(CHOH)_n$CHO, Syringyl C, ether R-O-R
90-60	Carbohydrate-derived structures in hexoses, higher alcohols R-OH
60-45	Methoxyl OCH_3 in aliphatic rings and chains
45 to-10	Methyl CH_3 groups in aliphatic rings and chains, alkyl-CH_2, terminal CH_3

According to Kögel-Knabner (1992, 1997) and Skjemstad *et al*. (1997) among others the ^{13}C NMR spectrum can be divided into four main regions (Fig. 4). The relative distribution of C in the different C regions is determined by integration of the signal intensities in various chemical shift regions. For instance, litter material contains about 5-10% carboxylic C, 10-30% aromatic C, 50-70% O-alkyl C and 10-30% alkyl C.

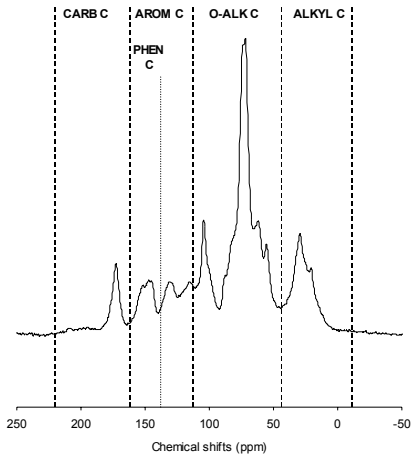

Figure 4. ^{13}C NMR spectrum of unfertilized Oi layer at Skogaby (Paper II). The spectrum is divided into the main C groups (carbonyl C, aromatic C, O-alkyl C and alkyl C). The phenolic C group is included in the aromatic C group.

Wilson (1987) and Kögel-Knabner (1997) discussed the problems in using NMR quantitatively. Problems that are important to be aware of when using NMR are for instance the presence of paramagnetic components (such as Fe), side bands and base line distortion (*i.e.* time loss at the start of the signal detection). These problems can create lower and more broadened NMR signals that result in an underestimation of the C content (Kögel-Knabner, 1997). Conte *et al*. (2002) used CPMAS ^{13}C NMR quantitatively on humic substances by using a technique called Variable Contact Time (VCT). They assumed that the ^{13}C was representative for the whole C content.

Tannin and lignin are the main compounds that give signal intensities in the aromatic C region in a CPMAS ^{13}C NMR spectrum. Signal intensities from tannins overlap with those from lignins (Hatcher, 1987). By combining the dipolar dephasing (DD) technique with CPMAS, there is an opportunity to investigate the signal intensities from tannin and lignin separately. The DD technique differentiates C at the same chemical shift but with differences in molecular motions or substitution levels (Wilson, 1987). The technique is based on a pulse sequence making the spinning of a specific nucleus interact with the spinning of a proton (^{1}H) during detection. It gives a first estimate about the contribution of a nucleus with weak or strong ^{1}H dipolar coupling. Directly after contact time, but before data acquisition, the high power ^{1}H decoupling of CPMAS is interrupted for a certain delay time (*i.e.* dipolar dephasing time, t_{dd}). In the spectrum obtained, the specific nucleus that is affected by strong ^{1}H dipolar coupling (*i.e.* strongly interacts with ^{1}H), exhibits a loss of intensity with increasing t_{dd}. Nuclei without directly attached ^{1}H experience weak ^{1}H dipolar coupling. Therefore, the ^{13}C that is not bound to ^{1}H does not have strong enough interactions for a complete disappearance in the spectrum. For instance, tannins giving signal intensities at 106, 144 and 154 ppm can still be detected (Lorenz *et al*., 2000). From the intensity loss of the signal at 106 ppm, as a function of t_{dd}, an index for the tannin content was calculated (Paper II). Therefore, the calculated tannin value has to be regarded as only an index rather than an absolute value of the tannin content.

Solid-state CPMAS ^{15}N NMR spectroscopy on natural abundance of ^{15}N has been used for instance by Knicker *et al*. (1993). Almost all signal intensity was from the amide-N, giving a rather broad signal in the spectrum obtained. By using ^{15}N-labelled material (Knicker and Lüdemann, 1995; Knicker *et al*., 1997), the amide-N peak could be accounted for being of a higher proportion of the total intensity in the spectrum. The distribution of ^{15}N at natural abundance between different N-containing groups is shown in Table 2. The natural abundance of ^{15}N in SOM is generally about 0.4% (Knicker and Lüdemann, 1995). Spectra from samples with low N contents (<1%) will therefore not give satisfactory detection because of low resolution and low signal-to-noise ratios (Knicker *et al*., 1993).

Table 2. *Signal assignments for peaks in a CPMAS ^{15}N NMR spectrum (Knicker et al., 1997: Kögel-Knabner, 1997)*

Chemical shift range ***(ppm)***	**Some of the assignments from ^{15}N**
25 to -25	Nitrate, nitrite, nitro -NO_2 groups
-25 to -90	Imine, phenazine, pyridine
-90 to-145	Purine, nitrile –CN groups
-145 to -220	Chlorophyll N, purine/pyrimidine, substituted pyrrole
-220 to -285	Amide/peptide R-$CONH_2$, unsubstituted pyrrole, indole, carbazoles
-285 to -325	NH in guanidine, -NH_2, -NH-R, -N-R_2
-325 to -375	Free amino groups in amino acids $CHNH_2COOH$ and amino sugars, NH_4^+

Materials and methods

Study sites

Stråsan

Until the middle of the 1950s, the vegetation at the Stråsan experimental site (lat 60°55'N; long 16°01'E) was dominated by Norway spruce (*Picea abies* (L.) Karst.). The trees were felled and the area was subjected to burning followed by plantation of Norway spruce seedlings (Tamm *et al*., 1974). The soil is classified as a Haplic Podsol according to FAO (1988). The atmospheric deposition is about 5 kg N ha^{-1} yr^{-1}, the annual mean temperature is +3.2 °C and the annual mean precipitation is 740 mm (Tamm, 1985). An N fertilization experiment started in 1967 (Tamm *et al*., 1974). There are 2 randomized blocks and the size of each plot is 30 x 30 m. The N-fertilized plots have received NH_4NO_3 at average rates of 35 (N1), 73 (N2) and 108 (N3) kg ha^{-1} yr^{-1}. NH_4NO_3 was spread manually once a year in early June. Applications ended in 1989 (N2) and 1992 (N3), but were still continuing in N1 at the time of sampling. The N treated plots have a closed canopy as a result of the increased tree growth. Mosses, lichen and grasses with patches of dwarf shrubs dominate the ground vegetation in the control plots. In the N1 plots, some ground vegetation still occurs, while the N2 and N3 treatments have no ground vegetation at all.

Skogaby

Skogaby is situated in south-western Sweden (lat 56°33'N; long 13°13'E). A Norway spruce stand (*Picea abies* (L.) Karst.) was planted in 1966, replacing a Scots pine stand from 1913 (Bergholm *et al*., 1995; Nilsson and Wiklund, 1995). The land was previously used for cattle grazing for hundreds of years, and during this period the vegetation was dominated by heather (*Calluna vulgaris*) and various grass species. Regular burning was performed to improve the grazing conditions. The soil is classified as a Haplic Podsol according to FAO (1988). The

Table 3. *Overview of Papers I-III and the Complementary Study. Treatments are defined C and NS (0 and 100 kg N ha^{-1} yr^{-1} as $(NH_4)_2SO_4$) at Skogaby and C, N1, N2 and N3 (0, 35, 73 and 108 kg ha^{-1} yr^{-1} as NH_4NO_3) at Stråsan. The tree stands at both sites are Norway spruce*

	Paper I	**Paper II**	**Paper III**	**Complementary Study**
Sampling site	Skogaby, Stråsan	Skogaby	Skogaby	Skogaby
Material	Oe, Oa	Oi, Oe, Oa	Fresh needles	Fresh roots (2-5 mm)
Sampling year	1995	1998	1997	1995
No of years with added N	Skogaby: 8 Stråsan: 24-29	Skogaby: 11	Skogaby: 9	Skogaby: 8
Experimental design	Column incubation and continuous leaching at 15°C for 49 days	Collected field samples	Incubation in closed laboratory conditions at 15°C for 559 days[1]	Litterbag study for 853 days[2]
Main methods and variables measured	CO_2 DOC DON NH_4-N NO_3-N C/N	CuO oxidation CPMAS ^{13}C NMR CPMAS ^{15}N NMR C/N	CuO oxidation CPMAS ^{13}C NMR Klason lignin Cellulose Hemicellulose CO_2[1] Mass loss[1] C/N[1]	CuO oxidation Acid detergent lignin CPMAS ^{13}C NMR Mass loss[2] C/N[2]

[1] Karlsson (2000)
[2] Majdi (submitted)

annual means of temperature and precipitation are 7.5 °C and 1100 mm yr^{-1}, respectively (Bergholm *et al*., 1995; Lövblad *et al*., 1995). The annual N deposition was 24 kg N (NH_4^+ + NO_3^-) ha^{-1} yr^{-1} (Lövblad *et al*., 1995). The N fertilization experiment started in 1988 with applications of 100 kg N and 114 kg S ha^{-1} yr^{-1} as $(NH_4)_2SO_4$ divided into 3 annual applications in May, June and July (Nilsson and Wiklund, 1992). There are 4 randomized blocks. Each plot has an area of 45 x 45 m. Scattered mosses are the dominating component of the ground vegetation. The unfertilized plots are denoted C and the N-fertilized NS.

Sampling description

- Mor humus samples (Paper I) from Skogaby (C and NS) and Stråsan (C, N1, N2 and N3) were collected using steel cylinders in November 1995 from 10 randomized sampling spots within each plot. In the laboratory, the 10 samples were divided into Oe and Oa layers and bulked into one composite sample per plot and placed in columns.

- Sampling of litter and mor humus (Paper II) was carried out at Skogaby in November 1998 (C and NS) using similar steel cylinders to those in Paper I. The 10 samples collected were divided into Oi, Oe and Oa layers and bulked into one composite sample per plot.

- Fresh needle litter (Paper III) was collected at Skogaby (C and NS) on fibre cloth sheets placed on the ground for a period of 2 weeks in May 1997. The needles were bulked into one sample for the control and one for the N-fertilized plots, air-dried and cleaned of twigs, cones and green needles. Thereafter, the bulk samples were divided into 3 subsamples (Karlsson, 2000).

- Fresh living roots (Complementary Study) with a diameter range of 2-5 mm were collected in November 1995 from the unfertilized (C) and N-fertilized (NS) mor humus layers (Oe + Oa) at Skogaby (Majdi, submitted). In June 1996, the roots were placed in litterbags in the humus layers at Skogaby with C roots in C plots and NS roots in NS plots.

Laboratory methods and analyses

Measurements of DOC and DON (Paper I)

Each column was leached with an artificial throughfall solution once a week. Measurements of DOC (Shimadzu TOC-5000 A, Japan), total dissolved N (Mitsubishi TN-05, Japan), NH_4-N and NO_3-N (FIA Star 5010, Tecator, Sweden) were made on the percolates at each sampling event. The amount of DON was calculated as the difference between total dissolved N and inorganic N (NH_4-N + NO_3-N).

CO_2 evolution (Papers I and III)

The CO_2 evolution from the mor humus incubated in the columns (Paper I) was measured regularly. At each CO_2 sampling event, a known volume of air was removed from each column with a syringe and analyzed on a gas chromatograph. The air sampling was performed the day before addition and collection of the artificial throughfall and leachate. The needle litter (Paper III) was incubated in plastic jars and gas samples were taken repeatedly to determine CO_2 evolution on a gas chromatograph (Karlsson, 2000). Details of the measurement techniques are given in Papers I and III.

Klason lignin and carbohydrate analysis (Paper III)

Hemicellulose, cellulose and Klason lignin in the needle litter were analyzed at the Swedish Pulp and Paper Research Institute (STFI). The content of hemicellulose was calculated as the sum of arabinose, xylose, mannose and galactose whereas the cellulose was set equal to the total amount of glucose. The individual carbohydrates were analyzed by gas chromatography. The amount of Klason lignin was gravimetrically determined according to Theander and Westerlund (1986) and Theander *et al*. (1995) as an ash-free acid-insoluble residue. To obtain Klason lignin, the samples were extracted with water and organic solvent solutions, treated with sulphuric acid in two steps and heated. Thereby polysaccharides were removed and the final fraction that was lost during ashing was the Klason lignin.

Acid detergent lignin (Complementary Study)

Acid detergent lignin (ADL) was determined on the root litter samples (sampled on Day 474 and Day 853) at the EAU Laboratory of Plants in Tartu, Estonia. The acid detergent fibre method has been thoroughly described by Van Soest (1963), Van Soest (1967) and Robertson and Van Soest (1981) and is based on extraction with cetyltrimethyl ammonium bromide (CTAB) dissolved in sulphuric acid. To obtain the ADL content, a second extraction step with sulphuric acid followed by ashing was performed (Ryan *et al*., 1990).

CuO oxidation and HPLC analysis (Papers II, III and the Complementary Study)

The alkaline CuO oxidation was performed according to Hedges and Ertel (1982), and Kögel (1986). The oxidation procedure was based on addition of Mohr's salt ($(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$), CuO powder, glucose and NaOH followed by heating to 170 °C for 2 hours in sealed vials. CuO is the oxidizing substance. Glucose acts as a blocking material on sorption sites of various plastic materials used during the oxidation procedure in order to have an optimum recovery of the lignin-derived phenols. Fe^{2+} buffers the redox potential so that no oxidation of aldehydes to carboxylic acids can occur. The CuO oxidation products derived from lignin (Papers II-III and the Complementary Study) were analyzed using reversed-phase HPLC (described in Paper II). A C18 column and a phenyl-hexyl column were used for separation and detection at 280 nm. Acetonitrile was used for extracting lignin phenols during the Solid Phase Extraction (SPE). Vanillin was used for

recovery tests both during the CuO oxidation procedure (vanillin dissolved in NaOH) and during the SPE procedure (vanillin dissolved in HCl). The results of the recovery test varied between 3-8% loss of added vanillin for the CuO oxidation whereas the range was 2-6% for the SPE procedure (Papers II-III and the Complementary Study). In Paper II, the reproducibility between two CuO oxidation events was tested and showed an average difference of 5%.

CPMAS NMR (Papers II, III and the Complementary Study)

CPMAS ^{13}C NMR (Bruker DSX 200) spectroscopy was performed at the Technical University in Munich (TUM), Germany. Solid-state ^{13}C NMR spectra were obtained at a frequency of 50.3 MHz and the CPMAS technique was applied with a frequency of 6.8 kHz. A ramped ^{1}H pulse was used during a contact time of 1 ms. The ^{13}C chemical shifts were calibrated relative to tetramethylsilane and glycine. Pulse delays between 800 and 300 ms were used, 5000 and 8000 scans were accumulated and a line broadening of 50 Hz was applied. The relative distribution of C in different C groups was determined by integration of signal intensity in various chemical shift regions (Fig. 3). The phenolic C (chemical shift region of 140-160 ppm) was included in the aromatic C (110-160 ppm) region.

The dipolar dephasing (DD) ^{13}C technique was used in Paper II by applying the cross polarization (CP) technique and the DD technique. The ^{1}H decoupler was turned off between CP and acquisition of the CPMAS pulse sequence. An 180^{o} refocusing pulse was also inserted at ½ t_{dd} to assist phasing. The relative contribution of non-protonated C to the signal intensity between 110 and 90 ppm was calculated (equation 1 in Paper II). This number was taken as an index for the contribution of tannins to the sample.

In Paper II, solid-state ^{15}N NMR (Bruker DMX 400) spectroscopy was also used to test for the presence of heterocyclic N compounds. The spectrometer operated at 40.6 MHz and applied a contact time of 1 ms, a 90° pulse width of 5.8 µs, a pulse delay of 200 ms, and a line broadening of 100 and 150 Hz. Between 5 and 20 x 105 scans were accumulated at a magic-angle spinning speed of 5.5 kHz. The chemical shifts were standardized to the nitromethane scale and were adjusted with ^{15}N labelled glycine.

Results

Decomposition rates and DOM production

In the incubation study of Oe and Oa layers from Skogaby and Stråsan (Paper I), the CO_2 evolution rate in the Oe layer of the NS treatment at Skogaby was lower than in the control during the 49-day incubation (Fig. 5). A similar pattern could be seen at Stråsan, where the Oe layer of the N3 treatment had the lowest CO_2 evolution and the control Oe layer the highest. No indication of a treatment effect was found in the Oa layer at Skogaby, whereas a higher CO_2 evolution rate was indicated in the unfertilized Oa layer at Stråsan compared to the three N treatments during the incubation. The cumulative amount of CO_2 was significantly lower in the NS Oe layer at Skogaby than in the control ($p<0.01$) and also lower in the Oe layer of the N3 treatment at Stråsan than in the control ($p<0.05$). Overall, the Stråsan layers tended to have higher levels of cumulative CO_2 than those from Skogaby. The N-treated needles at Skogaby had initially a higher CO_2 evolution rate than the control needles (Karlsson, 2000; Paper III) but after about 100 days the rates became lower and after the 559-day incubation the cumulative C mineralization was lower in the needle litter from the N-treated plots. In the collected leachates from the incubated Oe and Oa layers (Paper I), the DOC and DON productions were not significantly affected by N addition. However, at Skogaby the leaching of DOC tended to be lower from the N-fertilized Oe layer than from the control throughout the incubation. The cumulative amount of DON was not affected by the N additions at the two sites.

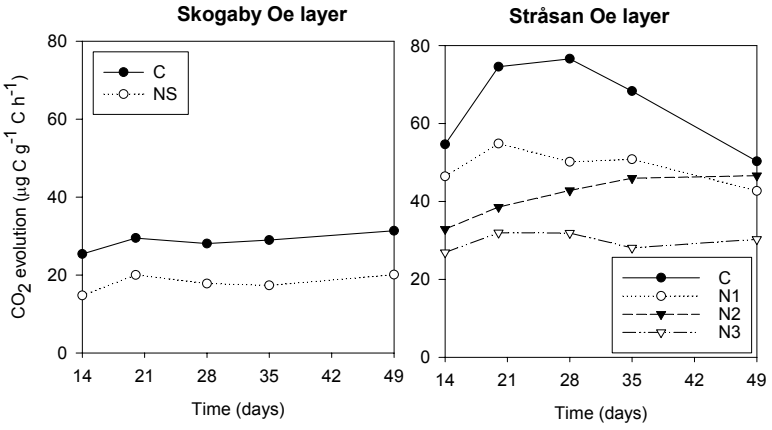

Figure 5. CO_2 evolution (μg C g^{-1} C h^{-1}) from the Oe layers at Skogaby and Stråsan (Paper I).

Chemical changes in the organic C and N

The contents of C and N, lignin degradation determined by the CuO oxidation method and ^{13}C NMR results (Papers II-III and the Complementary Study) are

summarized in Table 4 a and b. The changes in organic C composition with depth at Skogaby (Paper II) and time during decomposition of needle litter (Paper III) and root litter (Complementary Study) are partly comparable since the depth gradient in an intact mor layer also to some extent represents an age gradient. However, in incubation studies no input of fresh organic material occurs. The C-to-N ratios reported in Paper II were significantly lower in the Oi and Oe layers in the N-fertilized plots at Skogaby than in corresponding layers in the control plots.

The degradation degree of lignin, expressed as $(Ac/Al)_V$, did not increase to any major extent with either depth or time (Papers II-III). Only slight increases were found with depth, from the Oe layer to the Oa layer (Paper II), and with time (Papers III). No significant treatment effect on $(Ac/Al)_V$ was found. The sum of CuO oxidation products, expressed as VSC, generally decreased with depth (Paper II). In the decomposing needles (Paper III) an initial increase in VSC was observed in the control treatment followed by a decrease by the end of the incubation. The vanillyls (V) and the coumaryls (C) increased with decomposition time in both NS and control needle litters. This seems contradictory to the forest floor study (Paper II), which showed decreasing V and C contents with depth. The syringyl (S) content in the fresh needles was initially high (13-16 mg g^{-1} initial C) but vanished entirely with time. In the Complementary Study, VSC increased with time since the collected roots contained 72 and 60 mg g^{-1} initial C (C and NS, respectively) and after 853 days the contents were 85 and 92 mg g^{-1} initial C, respectively (Table 4 a). The degradation degree of lignin ($(Ac/Al)_V$) increased from 0.22 to 0.24 in the NS roots and became significantly ($p<0.05$) higher than in the control roots after 853 days of decomposition. V increased with time in both treatments whereas S vanished almost entirely. The third group, C, was rather constant throughout the root decomposition.

The intensity distribution obtained from solid-state ^{13}C NMR (Table 4 b) showed a treatment effect (Paper II) with significantly more aromatic C (and phenolic C) in the fertilized Oi layer than in the unfertilized ($p<0.05$). A similar trend was indicated in Paper III as the NS needles contained more aromatic C than the control needles throughout the incubation. Polysaccharides (O-alkyl C) decomposed with time. The NS forest floor materials (Paper II) and the NS needles (Paper III) seemed to have less O-alkyl C than the control samples. Alkyl C (such as waxes and lipids) tended to increase with both depth and decomposition time (Papers II-III).

Decomposing needle litter (Paper III) showed a decreasing Klason lignin content with time in both treatments (Table 6). Klason lignin in the C needle litter decreased from 783 to 631 mg g^{-1} initial C. Significantly ($p<0.05$) lower Klason lignin content was found at Day 179 in the NS needle litter (727 compared to 767 mg g^{-1} initial C). The Complementary Study of decomposing root litter showed an increase in acid detergent lignin (ADL) content due to N fertilization, whereby ADL increased from 454 to 462 mg g^{-1} initial C between Days 474 and 853 (Table 6). Significantly ($p<0.05$) more ADL was obtained in NS roots on Day 474 (454 compared to 429 mg g^{-1} initial C).

Table 4 a. *Overview of C/N ratios and CuO oxidation data from Papers II-III and the Complementary Study. Non-detected CuO oxidation products are marked with n.d.*

Material	Treatment	C/N	$(Ac/Al)_V$	VSC	V	S	C
				mg g^{-1} initial C	*mg g^{-1} initial C*	*mg g^{-1} initial C*	*mg g^{-1} initial C*
Fresh needles[1]	C	55	0.21	58	41.8	15.5	0.6
Fresh needles[1]	NS	23	0.21	57	42.9	13.0	0.6
Needle litter (179 days) [1]	C	43	0.22	62	49.7	9.6	2.4
Needle litter (179 days) [1]	NS	19	0.21	57	52.4	n.d.	4.7
Needle litter (559 days) [1]	C	34	0.23	54	49.9	n.d.	4.4
Needle litter (559 days) [1]	NS	17	0.22	54	49.0	0.1	4.7
Fresh roots[3]	C	66	0.21	72	67.1	2.6	2.1
Fresh roots[3]	NS	47	0.22	60	56.1	2.0	1.7
Root litter (474 days) [3]	C	73	0.24	88	85.9	0.4	1.8
Root litter (474 days) [3]	NS	43	0.23	84	82.5	n.d.	1.8
Root litter (853 days) [3]	C	55	0.21	85	82.5	0.3	1.8
Root litter (853 days) [3]	NS	39	0.24	92	89.9	n.d.	1.8
Oi[2]	C	34	0.35	56	52.4	n.d.	3.4
Oi[2]	NS	24	0.34	59	55.7	n.d.	2.8
Oe[2]	C	27	0.34	48	43.6	1.3	3.3
Oe[2]	NS	23	0.33	49	44.5	1.3	2.7
Oa[2]	C	28	0.37	40	37.5	n.d.	1.5
Oa[2]	NS	23	0.43	28	26.4	n.d.	0.7

[1] Paper III

[2] Paper II (The unit is mg g^{-1} C since no decomposition period was investigated)

[3] Complementary Study

Table 4 b. *Overview of CPMAS ^{13}C NMR data from Papers II-III and the Complementary Study. Phenolic C is included in Aromatic C (chemical shift region of 160-110 ppm). The roots from Day 474 were not analyzed by ^{13}C NMR and are marked with – signs*

Material	Treatment	Carbonyl C	Aromatic C	Phenol C	O-alkyl C	Alkyl C
		%	*%*	*%*	*%*	*%*
Fresh needles[1]	C	5	16	6	62	17
Fresh needles[1]	NS	6	17	6	60	17
Needle litter (179 days) [1]	C	4	15	4	61	20
Needle litter (179 days) [1]	NS	6	18	5	54	22
Needle litter (559 days) [1]	C	6	18	5	55	21
Needle litter (559 days) [1]	NS	8	19	6	50	23
Fresh roots[3]	C	5	22	8	64	9
Fresh roots[3]	NS	6	22	8	62	11
Root litter (474 days) [3]	C	-	-	-	-	-
Root litter (474 days) [3]	NS	-	-	-	-	-
Root litter (853 days) [3]	C	8	23	8	63	8
Root litter (853 days) [3]	NS	6	24	8	62	9
Oi[2]	C	7	18	6	57	18
Oi[2]	NS	8	21	8	52	19
Oe[2]	C	9	21	8	50	20
Oe[2]	NS	9	22	8	49	20
Oa[2]	C	10	24	9	47	18
Oa[2]	NS	12	21	8	45	22

[1] Paper III
[2] Paper II (The unit is mg g^{-1} C since no decomposition period was investigated)
[3] Complementary Study

Condensed tannins were detected in all forest floor layers at Skogaby except for the Oa layer of the unfertilized plot. An increasing tannin index with depth was indicated in the Oi and Oe layers. (Paper II). The ^{15}N spectra showed that most N was bound in amide structures (Fig. 6), whereas heterocyclic N compounds could not be detected (Paper II).

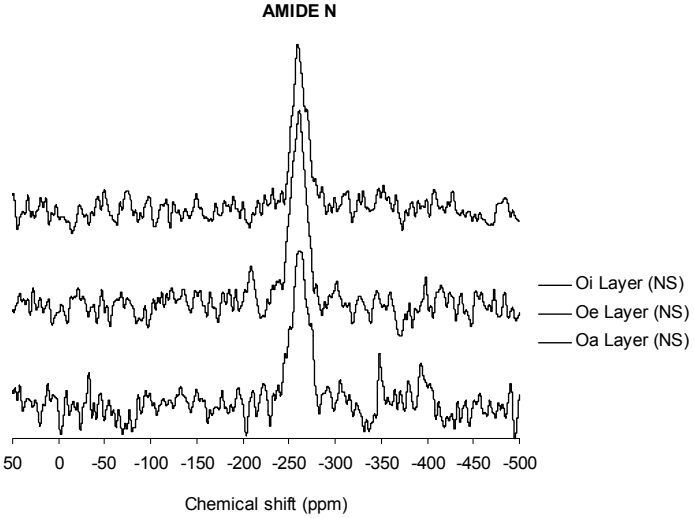

Figure 6. CPMAS ^{15}N NMR spectra of fertilized (NS) Oi, Oe and Oa layers at Skogaby. Amide N gives signal intensity at -256 ppm (Paper II).

Discussion

Reduced CO_2 evolution and impact on DOM production

The studies in Papers I and III confirm that long-term addition of N reduces the decomposition rate of SOM. In Paper I, we were able to show that the fertilized Oe layer at Skogaby had a lower CO_2 evolution rate than the unfertilized layer (Fig. 5). The Oe layer at Stråsan behaved in a similar way since the lowest CO_2 evolution was obtained in the highest N treatment. In Paper III, CO_2 evolution was measured from decomposing needle litter from Skogaby according to Karlsson (2000). Initially, there was a higher evolution of CO_2 from the fertilized needle litter but the cumulative amount of CO_2 was significantly lower in the fertilized litter after 170 days of incubation. Calculated mass losses of needle litter and root litter decomposition were done by Karlsson (2000) and Majdi (submitted). It turned out that the mass loss development of root litter showed another pattern than the mass loss of needle litter. The decomposition of needles initially gave a higher mass loss in the fertilized needles than in the unfertilized. Later, the highest mass losses were obtained in the unfertilized needle litter (Karlsson, 2000). This is

in accordance with calculated mass loss development of needle litter in the field (Nilsson *et al*., 2001). However, concerning root litter decomposition, the NS roots had a higher mass loss than the control roots after 853 days of decomposition in litterbags (Majdi, submitted).

Concerning the production of DOC, no clear N effects in the Oe and Oa layers at Skogaby could be seen (Paper I). There was a tendency for a lower DOC leaching in the N-fertilized Oe layer than in the control. This contradicted the hypothesis put forward by Fog (1988) as well as previous results by Guggenberger (1994) and Zech *et al*. (1994). Fog (1988) hypothesized that the reason for an increased DOM leaching from recalcitrant organic matter after addition of N could be reduced white-rot fungal activity. Water soluble, partially decomposed lignin degradation products would therefore accumulate with time. Guggenberger (1994) and Zech *et al*. (1994) investigated the effects of varying N deposition on DOC leaching in spruce forest ecosystems. They both found increasing levels of DOC at high N deposition. Zech *et al*. (1994) hypothesized that this was caused by an increased mineralization of the organic matter and/or suppressed ligninase activity. Partly degraded water-soluble lignin compounds would thereby accumulate, as previously postulated by Fog (1988). Our findings concerning DOC production are supported by other studies such as Currie *et al*. (1996) and McDowell *et al*. (1998). Currie *et al*. (1996) studied forest floors in the Harvard Forest experiment in the U.S.A. and found no significant effect on DOC leaching after long-term additions of N. McDowell *et al*. (1998) sampled in the Harvard Forest experiment and showed that after 4 years, there were still small changes in DOC. Our results showing unaffected DON leaching after N addition somewhat contradict the findings by McDowell *et al*. (1998) and Park *et al*. (2002). McDowell *et al*. (1998) showed large increases in DON concentration and Park *et al*. (2002) documented a long-term increase in DON after addition of NH_4NO_3 in their laboratory leaching experiment with forest floor material. They argued in a similar way as Fog (1988) and stated that an incomplete degradation of the SOM and formation of water-soluble N compounds could explain the increased DON-release.

We were able to demonstrate a positive relationship between the release of CO_2 and DOC when combining control and N treatments in the Oe layer at Stråsan (Paper I). Similar results have been obtained by Michel and Matzner (1999) who incubated Oa materials from European Norway spruce stands. Park *et al*. (2002) obtained a weak correlation and hypothesized that there was a partial discoupling of CO_2 evolution and DOC production. CO_2 is believed to mainly originate from polysaccharides whereas DOC to a large part originates from ligno-cellulose.

Organic C and N composition

At Skogaby, the forest floor depth trends (Paper II) and the decomposition of needle litter (Paper III) and root litter (Complementary Study) are compared with each other since the depth gradient also represents an age gradient to some extent. The C-to-N ratios decreased both with depth and time. The N-treated needle and root litter as well as Oi and Oe materials had higher N contents than in the

controls. However, the Oa layer seemed to be unaffected by the N fertilization in the time perspective of about 10 years. This indicates that the N content in the forest floor is mainly dependent on the litterfall (residence time of needles is 6-10 years in the canopy) followed by a subsequent effect on underlying horizons.

Phenolic compounds derived from lignin by CuO

The CuO oxidation method provided us with information concerning the degradation degree of lignin ($(Ac/Al)_V$) as well as the sum of the CuO derived phenolic compounds (VSC). Using this method, we were able to show that the degradation of lignin in the forest floor at Skogaby had a similar pattern to previous findings on humification patterns with depth (Kögel, 1986; Kögel-Knabner *et al*., 1988). VSC generally decreased with depth and increasing lignin degradation. The almost unchanged lignin degradation degree in the forest floor materials of the N treatments compared to the controls (Table 4 a) indicated that no major conservation or recalcitrance of lignin caused by N had occurred.

The CuO oxidation data of the decomposing needles after 559 days (Paper III) were somewhat comparable to the Oi layer (Paper II). The 559 days incubation in the laboratory corresponded to 4 years decomposition in the field (Seyferth, 1998). For instance, the content of VSC was in the same range (54 compared to 56-59 mg g^{-1} initial C in needles and Oi layer, respectively). However, the $(Ac/Al)_V$ was 0.3 in the Oi layer compared to 0.2 in the needles. When comparing the forest floor field samples (Paper II) with the two litter decomposition studies (Papers III and the Complementary Study), there turned out to be differences between VSC concerning the time patterns (Table 4 a, Fig. 7)). The decomposition studies showed a trend for an increase in VSC, both for needles (Paper III) and N-fertilized roots (Complementary Study). The root material yielded higher values of VSC lignin than needles and forest floor materials (Table 4 a). VSC in roots was in the range 60-92 mg g^{-1} initial C compared to needles (54-62) and forest floor material (28-59).

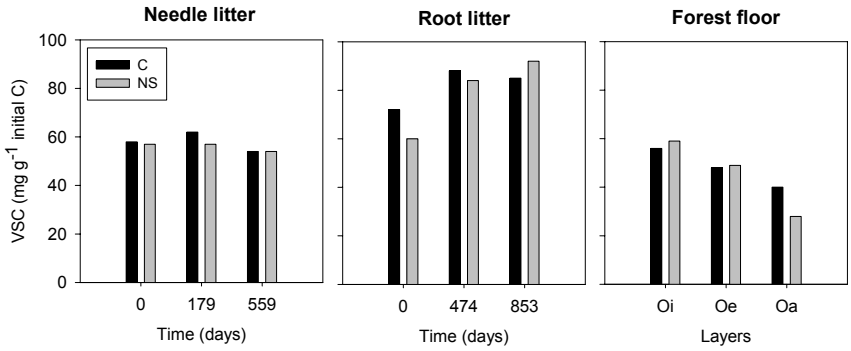

Figure 7. Sum of the derived CuO oxidation products, VSC (mg g^{-1} initial C), from forest floor (Paper II), needle litter (Paper III) and root litter (Complementary Study) materials. The unfertilized materials are denoted as C and the N-fertilized as NS. The forest floor shows a depth trend, whereas the needle and root litters are based on decomposition times in laboratory and litterbags, respectively.

The vanillyls (V) dominated among the phenolic VSC compounds (Table 4 a). The highest content of V was found in the root material (Complementary Study) compared to the other substrates (Papers II-III). The root litter contained 56-90 mg g^{-1} initial C compared to needles (42-52) and forest floor material (26-56). The vanillyls actually increased with time in both decomposition studies (Papers III and the Complementary Study). Within vanillyls, vanillin generally dominated (Paper II), which is specific for gymnosperm lignin (Kögel *et al*., 1988; Sanger *et al*., 1996; Sanger *et al*., 1997). In the forest floor (Paper II), both the vanillin and the vanillic acid contents decreased from the Oi layers to Oe layers in both treatments. However, in the incubation study of needle litter (Paper III), the absolute contents of both vanillin and vanillic acid increased with increasing decomposition time in both treatments. For instance, in the unfertilized needle litter, vanillin increased from 36 to 40 mg g^{-1} initial C and vanillic acid from 7 to 9 mg g^{-1} initial C. Hedges *et al*. (1988) carried out a laboratory experiment to study the degradation of the CuO-derived oxidation products from birch wood by adding species of both white-rot and brown-rot fungi. They found an increase in mass-normalized yields of vanillic acid from 0.15 to 0.20 weight-% after 12 weeks incubation with the white-rot fungus *Phlebia tremellosus*. The fungi had thereby increased the absolute yield of vanillic acid above its initial level. The authors explained this yield of vanillic acid as being produced without the oxidative pathway through vanillin (Aromatic-CHO → Aromatic-COOH) causing the $(Ac/Al)_V$ of the initial contents to be higher than expected. They concluded that the side chains of vanillyls could be microbially oxidized without affecting the ring structures.

The slight increase in cinnamyls (C) with increasing decomposition time of the incubated needles (Paper III) contradicts findings by Johansson *et al*. (1986) and Hedges and Weliky (1989). One explanation for this increase in cinnamyls in the decomposing needle litter (Paper III) could be the cutin content in the needles (Zech *et al*., 1987). Both lignin and cutin can give rise to cinnamyls. Cutin is a polyester of fatty acids and contains esterified coumaric and ferulic acids. It is connected to lignin by ether and ester linkages (Kolattukudy, 1980). In the decomposing root litter (Complementary Study), cinnamyls could be formed from suberin. Suberin is a polyester component found in plant roots having a phenolic structure similar to lignin (Kolattukudy, 1980). Suberin contains hydroxycinnamic acids and can therefore be found within the cinnamyl group (Bernards, 2002).

The syringyls (S) disappeared almost entirely during the decomposition studies (Paper III and Complementary Study), indicating that they were quickly decomposed. This confirms findings by Ander *et al*. (1984) and Hedges and Weliky (1989). One hypothesis is that syringaldehyde becomes demethoxylated (Aromatic–OCH_3 → Aromatic–H) with time. Demethoxylation of syringyls, containing two OCH_3 groups, could theoretically also give an increased amount of vanillyls, containing one OCH_3 group (H. Knicker; pers. comm.).

Analytical considerations concerning the CuO oxidation method

Kögel-Knabner *et al*. (1988) obtained lower values of VSC in forest floor material (25-30 mg g^{-1} C) than in our study (28-59 mg g^{-1} C) in Paper II. Therefore, I compared my procedure of CuO oxidation and HPLC analysis with the procedure of CuO oxidation and GC analysis used at the Technical University in Munich, Germany. It turned out that the values for VSC and the degradation index $(Ac/Al)_V$ were lower when using a GC procedure compared to my procedure described in Paper II (Table 5).

One explanation for this discrepancy could be differences in the extraction procedure. The extraction time needed for the phenolic compounds before analysis with GC is longer than for HPLC. Instead of extracting with ethylacetate and pyridine (Kögel-Knabner *et al*., 1988), I used acetonitrile and a shorter extraction time before the HPLC analysis. The phenolic compounds were possibly not stable enough and degraded during the extraction procedure before the GC analysis. Another possibility could be that the volatile phenolic compounds were lost. Losses of volatile vanillyls may have caused the lower values obtained with GC. For future studies, it might be better to use ethylvanillin as a standard for recovery tests instead of vanillin since this compound was not detected in the samples that I studied (Papers II-III and Complementary Study). Vanillin, which is the dominating compound, is probably not optimal for recovery tests.

Table 5. *Comparison of CuO oxidation products (VSC) and degree of lignin degradation ($(Ac/Al)_V$) determined by either HPLC or GC (mg g^{-1} C). The samples originated from unfertilized (C) and fertilized (NS) litter and mor humus (Oi, Oe and Oa). Number of replicates was n=4 (HPLC procedure; Paper II) and n=1 (GC procedure)*

Layer	Treatment	HPLC[1]		GC[2]	
		VSC (*mg g^{-1}C*)	$(Ac/Al)_V$	VSC (*mg g^{-1}C*)	$(Ac/Al)_V$
Oi	C	55.9	0.35	26.6	0.25
	NS	59.0	0.34	31.9	0.19
Oe	C	48.2	0.34	25.4	0.27
	NS	48.5	0.33	30.4	0.20
Oa	C	39.5	0.37	18.3	0.36
	NS	27.8	0.43	18.4	0.34

[1] Paper II

[2] The CuO oxidation procedure and the GC analysis were performed at TUM in Germany

Solid-state ^{13}C and ^{15}N NMR

By using CPMAS ^{13}C NMR on forest floor material, needle litter and root litter from Skogaby, we were able to characterize the organic C composition. Therefore it was possible to investigate the hypothesized changing pattern of the lignin content (phenolic C region). The NMR studies (Papers II-III and Complementary Study) did not show any major changes concerning lignin that could be attributed to the N addition, either with depth or decomposition time. Treatment effects of N were only found in the Oi layers (Paper II) with a significantly ($p<0.05$) higher

phenolic C content in the NS Oi layer. It is important to notice that CPMAS ^{13}C NMR spectroscopy is believed to be a rather insensitive method giving just a "fingerprint" of the SOM. Other methods, such as Near Infrared Spectroscopy (NIR), give a more precise estimation of the different C compounds in SOM but does not show their identity.

The bulked decomposed needles at Day 559 (Paper III) and the Oi layer (Paper II) had a similar organic C composition (Table 4 b). For instance, the aromatic C content (phenolic C is included in the aromatic C region) increased from 16 to 18% in the control during the incubation of the needles, which was comparable to the aromatic C content of 18% in the control Oi layer. The O-alkyl C content was also comparable, since the content in the decomposing needles decreased from 62 to 55% compared to the Oi layer value of 57% (Table 4 b). The needle litter at 559 days and the Oi layer were therefore comparable and showed similar values both with respect to CuO oxidation and ^{13}C NMR. However, the O-alkyl C in the root litter was unchanged after about 2.5 years decomposition in litterbags.

The root litter contained slightly more polysaccharides (*i.e.* O-alkyl C) than the needle litter (Table 4 b, Fig. 8). The O-alkyl C content ranged between 62-64% in the roots (Complementary Study) compared to 50-62% in the needle litter (Paper III). During decomposition of the root litter, the O-alkyl C content did not change either in the NS roots or the control roots (Table 4 b), which was unexpected since the calculated mass loss indicated a high initial decomposition rate of the root litter. Whether this lack of a decreased O-alkyl C content was caused by the fact that the polysaccharides were shielded with lignin forming ligno-cellulose complexes is only a speculation. It seems as if needle litter and root litter have different decomposition patterns since polysaccharides behaved differently. Despite high initial mass loss of both needle and root litter (Karlsson, 2000; Majdi, submitted), the polysaccharides in root litter might not be in such an easily degradable form as the polysaccharides in needle litter.

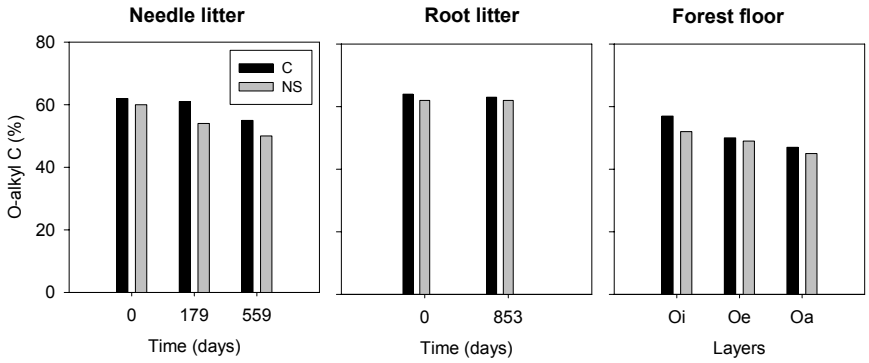

Figure 8. O-alkyl C (%) obtained by CPMAS ^{13}C NMR spectroscopy on forest floor (Paper II), needle litter (Paper III) and root litter (Complementary Study). The unfertilized materials are denoted as C and the N-fertilized as NS. The forest floor shows a depth trend (Oi, Oe and Oa), whereas the needle and root litters are based on decomposition times in laboratory (O, 179 and 559 days) and in field litterbags (0 and 853 days).

Our needle study (Paper III) showed alkyl C values of 20-23% and the forest floor study (Paper II) showed 18-22% alkyl C (Table 4 b, Fig. 9). Root litter contained much less alkyl C (8-11%). The alkyl C in the decomposing needles and the forest floor increased with time and depth (Fig. 9). However, the alkyl C in the root litter material had a decreasing time trend. Alkyl C originates from fats, waxes, lipids, cutin, suberin and proteins (Kögel-Knabner *et al*., 1989; Kögel-Knabner, 1992). About 50% is extractable lipids (*i.e.* fats, waxes), 20% bound lipids and the rest is mainly cutin and suberin (Ziegler, 1989). Previous solid-state ^{13}C NMR studies have shown an alkyl C content of around 15-20% in needle litter (Kögel *et al*., 1988) and a content of about 25-40% in an Oa layer (Kögel-Knabner *et al*., 1988). Cutin and suberin, found in needles and roots, contribute to the alkyl C signal.

Solid-state ^{15}N NMR spectroscopy on bulked forest floor samples (Paper II) indicated that most of the N was bound in amide structures (Fig. 6). The ^{15}N spectra did not show any detectable amounts of heterocyclic or aromatic N compounds (such as pyrolic N, pyridine N and aromatic amine N) in the N-fertilized forest floor material studied. Condensation products due to reactions between N and phenolic compounds (Nömmik and Vahtras, 1982; Berg, 1986) could therefore not be found to the extent that they would be important for the humification process. This has also been shown by Knicker (1993, 2000) and Knicker *et al*. (1997). In Paper III, the pH rose to almost 8 during the incubation because of formation of ammonia. If these samples had been investigated using ^{15}N NMR it could, hypothetically, have been possible to detect heterocyclic N compounds since complexation between ammonia and phenolic compounds can occur at such high pH (Nömmik, 1970).

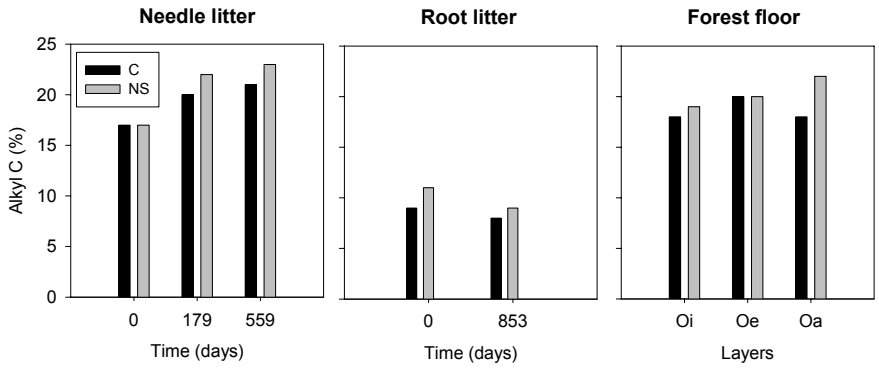

Figure 9. Alkyl C (%) obtained by CPMAS ^{13}C NMR spectroscopy on forest floor (Paper II), needle litter (Paper III) and root litter (Complementary Study). The unfertilized materials are denoted as C and the N-fertilized as NS. The forest floor shows a depth trend (Oi, Oe and Oa), whereas the needle and root litters are based on decomposition times in laboratory (O, 179 and 559 days) and in field litterbags (0 and 853 days).

Comparison between the CuO oxidation method and ^{13}C NMR spectroscopy

The VSC derived with CuO oxidation and the relative intensity of the signal in the phenolic C region of the ^{13}C NMR spectra did not show any correlation in the forest floor at Skogaby (Fig. 10). This was unexpected due to the assumption that a decrease in lignin content would be followed by a decrease in phenolic C. DeMontigny *et al*. (1993) studied woody and non-woody forest floor horizons from Salal-Moss ecosystems on the Northern Vancouver Island in Canada. They sampled forest floor material from both an undisturbed old-growth phase and a younger growth phase. They obtained a positive correlation between lignin derived from CuO oxidation and lignin determined with ^{13}C NMR. One possible explanation for this difference could be the presence and accumulation of tannins during decomposition (Paper II). A slight increase in the calculated tannin index with depth was obtained using DD solid state ^{13}C NMR in the Oi and Oe layers. However, whether tannins could cause the trends for a decrease in VSC with depth and an increase in phenolic C with depth is still a hypothesis.

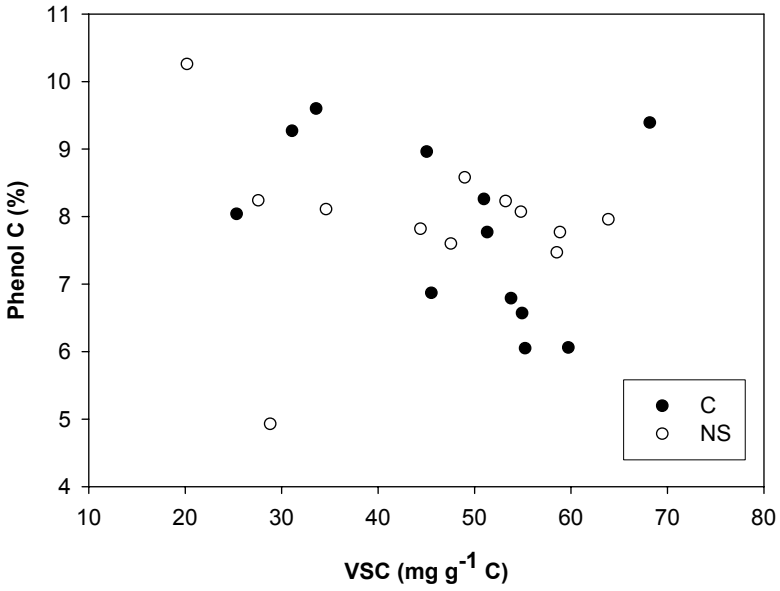

Figure 10. Relationship between VSC (mg g^{-1} C) and phenol C (%) in unfertilized (C) and fertilized (NS) litter and mor humus layers (Oi+Oe+Oa) at Skogaby.

Other methods for determining lignin

In Paper III, we compared Klason lignin with CuO lignin. The relationship between the two methods was positive, though not significant. The CuO oxidation did not indicate any major lignin degradation in the decomposing needle litter.

However, Klason lignin showed a decline (Table 6). As for ADL in the Complementary Study, the lignin content in the roots was higher in the NS treatment than in the control at both Day 474 and Day 853 (Table 6).

Both the Klason lignin method and the ADL method are based on hydrolysis with sulphuric acid. The major differences between the two methods are the acid concentration and temperature during the steps of hydrolysis. The Klason lignin method is initially based on a high acid concentration under low temperature conditions followed by a low acid concentration at high temperature. The ADL method is opposite in the way that it is based on a low acid concentration at high temperature followed by a high concentration at low temperature. Klason lignin has been shown to have a similar molecular composition to ADL but shows 2-5 times higher concentrations than ADL (Hatfield *et al*., 1994). Furthermore, Jung *et al*. (1999) showed that Klason lignin was more accurate for estimating the lignin content than ADL and that the underestimation of lignin when using ADL may be due to loss of acid-soluble lignin fractions. The Klason lignin method is mostly used for woody materials and is not as suitable as the ADL method for forage materials due to the high content of protein, especially in legume forages (Van Soest, 1967). The reason for analyzing ADL in the root material instead of using the Klason lignin method was that it was a cheaper way of getting a comparison for the lignin obtained by the CuO oxidation method.

Table 6. *Mean contents (mg g^{-1} initial C) of Klason lignin (Paper III) and Acid Detergent Lignin (Complementary Study) from unfertilized (C) and fertilized (NS) treatments. Number of replicates was n=2 (needles) and n=3 (roots). Different letters for the same period indicate significant differences (p<0.05)*

Material	Treatment	Klason lignin	Acid detergent lignin
		mg g^{-1} initial C	*mg g^{-1} initial C*
Fresh needles[1]	C	783	
Fresh needles[1]	NS	802	
Needle litter (179 days)[1]	C	767 **a**	
Needle litter (179 days)[1]	NS	727 **b**	
Needle litter (559 days)[1]	C	631	
Needle litter (559 days)[1]	NS	698	
Root litter (474 days)[2]	C		429 **a**
Root litter (474 days)[2]	NS		454 **b**
Root litter (853 days)[2]	C		424
Root litter (853 days)[2]	NS		462

[1] Laboratory incubation (Paper III)

[2] Litterbag decomposition (Complementary Study)

Altered biological activity after N application

The reduced CO_2 evolution of the decomposing needle litter (Paper III) and the Oe layers (Paper I) after long-term N addition at Skogaby, could not be explained by any major differences in organic C chemistry. Neither CuO oxidation nor ^{13}C NMR could show any major change in the chemistry of lignin and the remaining organic C content. The ^{15}N NMR analysis of the fertilized forest floor did not indicate any heterocyclic N compounds. The question therefore remains as to why there are reduced decomposition rates of SOM after long-term N addition. Could it be that N directly alters the decomposer community?

It seems as though the explanation for a reduced biological activity in the upper forest floor could to same extent be a direct effect of N on the decomposers. For instance, the ligninase enzyme activity among the white-rot fungi could have been suppressed (Carreiro *et al*., 2000; Sinsabaugh *et al*., 2002). Keyser *et al*. (1978) and Fenn *et al*. (1981) found a suppressed lignolytic activity after N additions.

Ågren *et al*. (2001) used data from various experimental studies of N addition in simulation models to find out why long-term effects of added N caused a decreased decomposition of litter. They hypothesized that the main explanation for a reduced decomposition rate in N-rich litter is a change in the microbial community towards the presence of microorganisms that are more efficient in assimilating N while still having a higher N demand. These decomposers were assumed to utilize available N at a lower C mineralization rate. The composition of fungal species within the microbial community has actually been shown to change due to N addition (Bååth *et al*., 1984; Arnebrant *et al*., 1990). Schimel and Weintraub (2003) also used modelling to clarify whether N limits microbial growth. They also simulated the impact enzymes have on microbial C and N availability. The conclusion was that addition of labile C sources to N-limited systems increase respiration, whereas the opposite occurs when N is added. The reason could be, according to the authors, that microbial growth is N-limited. N addition would stimulate a C immobilization, resulting in an increased microbial biomass and less microbial C used for "overflow metabolism" associated with the decomposition of organic macromolecules into smaller compounds.

Nilsson *et al*. (2001) investigated whether the surrounding environment or the chemical composition of the litter could cause a decreased decomposition of spruce needle litter at Skogaby. The authors placed fresh control needle litter samples in the control and NS litter layers and fresh NS needle litter in the control and NS litter layers. The calculated mass loss after 4 years of decomposition in litterbags showed the highest values for control and NS needle litters (60 and 59 %) placed in the control plots. The mass loss of control litter placed in the NS plots was 48 %. The conclusion was that the surrounding environment mostly affected the decomposition of the needle litter at Skogaby rather than the chemical composition of the litter itself.

Conclusions

With this work I have been able to conclude that there was:

- Reduced heterotrophic respiration in N-fertilized forest floors compared to unfertilized.

- No major qualitative changes in the organic C and N composition. The CuO oxidation method and the solid-state ^{13}C NMR spectroscopy indicated that no major changes had occurred concerning the lignin content. No heterocyclic N compounds could be detected by solid-state ^{15}N NMR either in the unfertilized or the N-fertilized forest floors. This indicated that no major conservation or recalcitrance of the SOM had occurred as a result of N additions.

- Similar lignin degradation patterns were found for the needle litter and root litter. The comparison between needle litter and root litter decomposition showed results such as that the CuO oxidation products tended to increase during decomposition while they decreased in the forest floor horizons. This was mainly due to the fact that the CuO-derived compounds vanillyls and cinnamyls in the decomposing needles and roots increased with time.

- Direct effects on the microorganisms and their enzyme production, composition and assimilation efficiency may have caused the reduced biological activity after long-term N addition. As for future research, I would recommend an investigation into the biological effects of N addition. For instance, studies concerning the enzyme activities of white-rot fungi would be interesting, since it has been shown that the enzyme activity could be suppressed in the presence of high N levels. This could be one of the explanations for the increasing amount of less degraded lignin in forest floors after long-term addition of N.

Svensk sammanfattning (Swedish summary)

Till södra Sveriges skogsekosystemen sker en hög tillförsel av kväve (N) i form av atmosfärisk deposition. Höga N-halter innebär ökad trädtillväxt vilket i sin tur leder till ökad mängd fallförna till skogsmarkens ytskikt. Långsiktigt kan N reducera nedbrytningshastigheten av markens humifierade organiska material. Lignin är en aromatisk kolförening som har stor betydelse för nedbrytning av det organiska materialet på lång sikt eftersom de mer lättnedbrytbara kolföreningarna såsom cellulosa redan har börjat brytas ned. Förklaringen till en långsiktig reducerad nedbrytningshastighet på grund av N skulle kunna vara att (1) enzymaktiviteten hos ligninnedbrytande vitrötesvampar hämmas (2) det organiska materialet kvalitativt förändras genom bildning av stabila heterocykliska N-föreningar (3) den mikrobiella sammansättningen i marken förändras.

Syftet med den här studien var att studera de långsiktiga effekterna av N-tillförsel på ligninnedbrytningen i granskogsekosystem. Bland annat mättes och bestämdes koldioxidavgivningen, ligninets nedbrytningsgrad samt eventuella strukturella förändringar av markens organiska kol- och kväveföreningar. Metoder som användes var CuO-oxidationsmetoden och NMR-spektroskopi. Med hjälp av CuO-oxidationsmetoden kunde ligninets nedbrytningsgrad beräknas. Markens olika organiska kol- och kväveföreningar studerades med hjälp av NMR-spektroskopin. Färska barr, nedbruten barrförna, mårhumus (L-, F- och H-skikten), färska rötter samt nedbruten rotförna hämtades från det långsiktiga N-gödslingsförsöket Skogaby i sydvästra Sverige (Halland). De färska barren bröts ned till barrförna genom inkubation under laborativa förhållanden. De färska rötterna bröts ned till rotförna i förnapåsar utlagda i fält. Mårhumusen inkuberades i kolonner under laborativa förhållanden varefter koldioxidavgivningen samt produktionen av det lösta organiska materialet mättes med tiden.

Ogödslade och N-gödslade prover studerades utifrån skikt (L, F och H) och olika nedbrytningstid (laborativt för barrmaterialet och fält för rotmaterialet). Det visade sig att hastigheten av koldioxidavgivningen var lägre i det N-gödslade F-skiktet i Skogaby jämfört med det ogödslade F-skiktet. Däremot fanns inga tydliga behandlingseffekter på nedbrytningen av lignin i de olika materialen. Inga heterocykliska N-föreningar kunde detekteras i Skogabys mårhumus med hjälp NMR spektroskopi. Sammanfattningsvis kunde den här studien inte påvisa några stora kvalitativa förändringar av markens organiska material (barrförna, mårhumus och rotförna) som kunde förklaras av den långsiktiga N-tillförseln. Den lägre koldioxidavgivningen från det N-gödslade F-skiktet kunde därmed inte förklaras vara en effekt av att ett stabilare organiskt material skulle ha bildats efter lång nedbrytningstid. Kanske finns förklaringen i att själva ”markmiljön” för mikroorganismer påverkats genom tex förändring av mikrobiell sammansättning och enzymproduktion.

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Acknowledgements

My sincere gratitude to colleagues and friends for all support during these years that has gone by to finally finish this thesis. Especially:

- **Ingvar Nilsson**, my supervisor, for guiding and encouraging me to continue at moments when I have nearly given up. Ingvar, thanks for always being there for me when I have needed your help and support!
- **Dan Berggren**, my assisting supervisor, who has always given me valuable and enthusiastic comments concerning results. You were actually the one that initially came with the idea for the project of studying lignin degradation.
- **Heike Knicker**, my assisting supervisor at the Soil Science Department at TUM in Freising (Germany). Thanks for running the NMR spectroscopy. I feel honoured to have been able to work together with you! Thanks also for your hospitality. I really enjoyed our evenings at pubs and "bier garden" in Freising. Not to mention Christian´s brilliant cooking of delicious Bavarian and Austrian meals!
- **Ingrid Kögel-Knabner**, who has written a whole lot of inspiring literature concerning lignin degradation! Thanks for welcoming me to the laboratory at TUM (Germany) and for introducing me to the CuO oxidation method.
- **Tryggve Persson and Patrik Karlsson**, for giving me the opportunity to study lignin degradation in your decomposing needle litter. Tryggve, I really appreciated that you were willing to become my assisting supervisor this final year. Thanks for all inspiring discussions that we have had!
- **Bosse Bergkvist** at LU for field sampling the forest floor material and also for our discussions concerning the column incubation.
- **Hooshang Majdi** for letting me take part of your enormous litterbag study concerning root litter decomposition at Skogaby.
- **Björn Lindahl** for the interesting discussion concerning white-rot fungi.
- **Clemens Engelke** at TUM for our discussions (in Swedish!) about tannins.
- **Lennart Mattson and Stefan Andersson**; the SAS experts at our Department!
- **Ernst Witter, Olle Andrén and Martin Larsson** for giving your valuable comments on my thesis!
- **Mary McAfee** for correcting my English writing.
- **Morgan Zaar** who helped me with scanning the drawings.
- **All colleagues** at the Department of Soil Sciences. In particular Helena, Petra, Anke, Anna, Katarina, Stefan, Lasse, Karin, Minh Ha, Anne, Mats, Magnus... During the years, we have had many cheerful and memorable moments together. Without you folks, I would never have succeeded. No tears without laughs!
- **Friends** all around Sweden (and Norway): Anja, Helen, Sven, Anna, PJ, Pål, Sophie, Barbro, Ann, Jop, Hacke, Gunilla, Kristine… Hopefully, I will have more spare time now to spend with all of you!
- **My family!** Mum, Marianne, for always being there for me. You are like a mentor for me through life! Mikael and Håkan, for being my loving brothers. My stepparents, Gunilla and Lennart, for endless help and support. My sincerest gratitude is dedicated to my beloved Fredrik, Viktoria and Sebastian.