Chapter 2
Decomposition as a Process: Some Main Features

2.1 Litter Decomposition: A Set of Different Processes Including Synthesis

Decomposition of plant litter involves a complex set of processes including chemical, physical, and biological agents acting upon a wide variety of organic substrates that are themselves constantly changing. Due to the immense diversity of possible factors and interactions, decomposition in a natural setting can be described in general terms only. In spite of this complexity, several major processes are involved and general trends can be outlined. However, new promising research has created new views that in part may seem contradictory to the traditional one.

Litter is in its simplest state when shed by the plant. From this initial state, litter composition changes, with some litter components disappearing rapidly, some slowly and some begin to disappear only after a time delay. Perhaps non-intuitive, but very significant, is the fact that some substances, particularly nutrients, are imported into the decomposing substrate, and new organic compounds are synthesized during decomposition. Due to the heterogeneity in both litter composition and factors influencing decay, decomposition of litter is far more complex than decay of, for example, a radioactive isotope.

A complication in the analysis and understanding of these processes has been the chemical analytical methods for the main organic components. Gravimetric methods for lignin determination have included not only native lignin but newly formed products, to some extent similar to lignin, chitin from fungi as well as ash, unless that has been analyzed. A recent development using ^{13}C-NMR has allowed us a new view on plant litter chemistry during decomposition and given more detailed as well as more specific, and correct information. Further, it allows us to draw new conclusions about the main process, giving us a new starting point for our thinking. Some recent work is that of for example Preston et al. (1997, 2009b), Ono et al. (2009, 2011), and De Marco et al. (2012).

The bulk of plant litter consists of varying amounts of several major classes of organic compounds. The relative proportions of these compounds vary with plant part (for example, leaves, stems, roots, and bark) and among species (see Chap. 4).

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These major groups of compounds can be classified according to their molecular size, their solubility, and their primary constituents. Some materials, notably sugars, low molecular weight phenolics, and some nutrients, are readily lost from litter through dissolution and leaching combined with the action of rapidly growing opportunistic microorganisms. Larger macromolecules, including cellulose, hemicelluloses, and lignin, are degraded more slowly. During decay, condensation of phenolics and lignin-degradation products, combined with the import of nutrients, results in the net accumulation of newly formed substances. The relative magnitudes of the main flows (Fig. 2.1) are thus different not only among litter types and species but are influenced by litter chemical composition (see Chap. 6).

We regard 'litter mass loss' or 'decomposition' as the sum of CO_2 release and leaching of compounds, including both C compounds and nutrients. Leaching is simply the loss of nutrients and incompletely decomposed organic compounds transported out by water from the remains of decomposing litter (see Glossary).

The interpretation of mass-loss data during the initial stages of decay may be influenced by a high leaching rate of water-soluble material that is not physiologically modified by microorganisms until after leaving the litter (McClaugherty 1983). These dissolved materials may be lost from litter to subsequently sequestered by humus or clay particles. In such cases, the materials are lost from a particular substrate but are retained in the soil ecosystem.

Under aerobic conditions, microbial decomposition results in a release of CO_2 that leaves the system. Under more anaerobic conditions, such as a temporarily waterlogged organic matter layer, anaerobic decomposers may produce organic acids instead of CO_2. This may also happen with aerobic decomposers that suffer

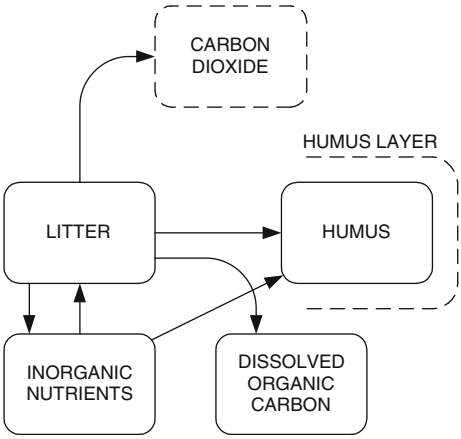

Fig. 2.1 Generalized pathways for transformation of litter to humus and inorganic C. When the litter is shed and its decomposition has started, the microorganisms begin forming carbon dioxide, and soluble compounds that are initially present may be leached out. Newly formed compounds that are stable but water-soluble are also leached out (dissolved organic carbon—*DOC*), and long-term stable remains, including newly formed products form humus

from a lack of oxygen. For example, acetic acid may be released instead of CO_2 and either be decomposed outside the cell or be stored and fulfill another role (see Sect. 10.3.3).

In some cases, the rate of decomposition approaches zero. In 1974, Howard and Howard estimated limit values for the decomposition of some species of leaf litters that were incubated in an animal-free environment. Using litter decomposition data from nutrient-poor forest systems, Berg and Ekbohm (1991) also estimated such limit values, indicating a stage at which the decomposition rate nears zero (Fig. 2.2).

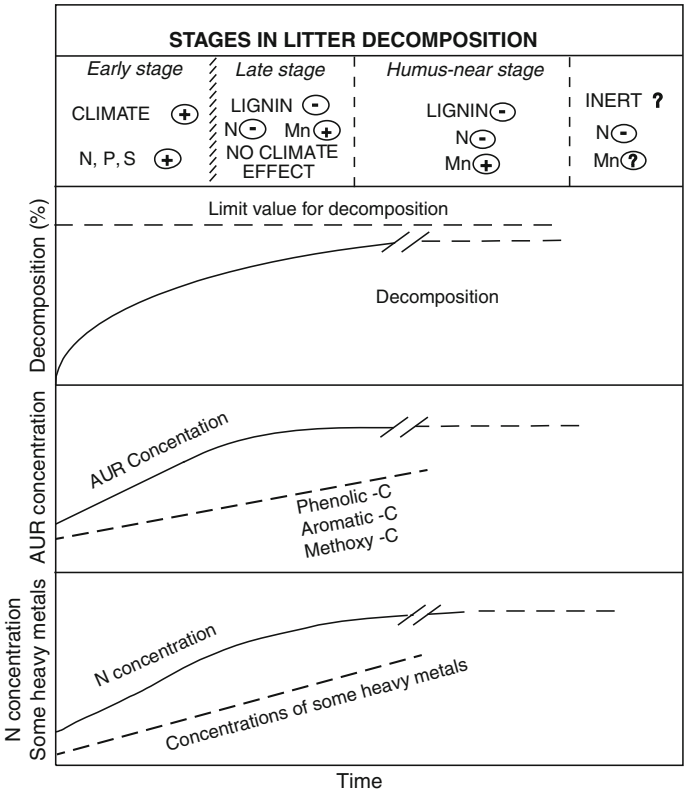

Fig. 2.2 As decomposition proceeds and the accumulated mass loss increases, the rate may decrease and a limit value for decomposition is reached which often is less than 100 % mass loss. Please note - that the rate at the limit value is calculated to be close to zero does not imply that the formed humus is biologically undegradable. The limit value rather identifies a litter fraction that may decompose very slowly. With decomposition, some main chemical changes take place. Thus, concentration of nitrogen increases, which appears to be general over most litter species. The concentration of AUR (gravimetric lignin) increases, in part possibly due to new products registered as gravimetric lignin. Also, concentration of some specific lignin-related bonds increases (cf Appendix III). Further, some heavy metals (e.g., Fe, Al, Cu, Pb) generally appear to increase in concentration

This chapter describes the principal microbial processes associated with litter decomposition that result in mass loss or CO_2 release. We present basic principles about the microbial degradation of the main components of litter such as cellulose, different hemicelluloses, and lignin/AUR using information mainly from Scots pine needle litter and the genus *Pinus*. We thus focus on litter from Scots pine and pine spp. to develop an existing conceptual model. Two main analytical approaches are being used, an earlier, using gravimetric and gas chromatographic determinations and a new, using ^{13}C-NMR, resulting in somewhat different pictures of the process, and we cannot exclude that we in part may need to reevaluate existing decomposition models that are based on older analytical techniques (below).

We also comment on Acid Unhydrolyzable Residue (AUR, 'gravimetric lignin'), degradation of native lignin and synthesis of new products, which increase in concentration and in later stages become more abundant in the residue. These are probably synthesized in the early stages of decomposition.

The process of litter mass loss is described in its main, general features, thus what appears to be patterns in common for the so far studied foliar litter species with focus on *Pinus* spp. We may call these basic and general subprocesses in decomposition. Details in decomposition are different, not only as regards methods but also on the level of species. In the later chapters, we will analyze more in-depth process patterns for different species/genera as far as information exists. We organize our description using a conceptual model that separates the decomposition process into different phases. As litter passes through these phases, the factors that regulate the decay process change. The model connects the developmental processes that occur, beginning with newly formed litter and continuing to the formation of humus. In this chapter, we have included a section discussing the synthesis of new products and their role in the existing models as well as in a new hypothetical model. Differences in the importance of substrate properties (for example, the influence of Mn and N) as well as the possible roles of native lignin and new compounds as the decay process unfolds are emphasized. Recent development has allowed us to revise and develop a former model (Fig. 2.3) into a somewhat more detailed one. We develop this discussion in Chap. 6 introducing studies on specific litter species and genera.

2.2 Definition of Litter Decomposition

Litter decomposition may in part be defined by the method used to study it. A very common method is the litter bag, used for incubations in the field or in laboratory microcosms. Another variety of direct incubation is tethered litter. A further one is the mass of 1.000 m of litter, specific for needle litter (Kurz et al. 2005). With these kinds of measurements, decomposition is measured as loss of mass and studies normally do not distinguish between what is respired as carbon dioxide and what is leached out of the litter or lost due to fragmentation, unless those processes

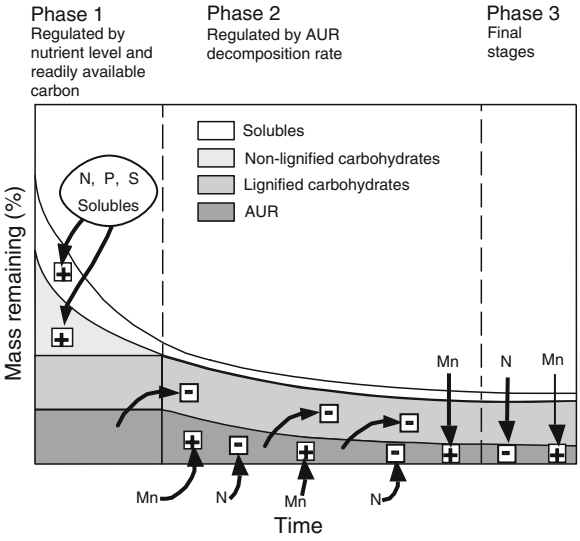

Fig. 2.3 Some main stages in decomposition of pine needle litter. The decomposition of water-soluble substances and unshielded cellulose/hemicellulose is stimulated by high levels of the major nutrients (early stage—phase 1). When degradable solubles and unshielded holocellulose are decomposed, only lignin-encrusted holocellulose and lignin remain as well as newly formed stable products. The early phase has been observed to last up to about 25–30 % accumulated mass loss in Scots pine needle litter. In the late stage (phase 2), the degradation of lignin (often measured as AUR) rules the litter decomposition rate. Nitrogen hampers the degradation of lignin, and higher N concentrations suppress the decomposition whereas higher Mn concentrations appear to have a stimulating effect on the degradation of lignin. Finally, in the humus-near stage (phase 3), the accumulated mass loss reaches its limit value at which the litter decomposition rate is close to zero. Figure modified from Berg and Matzner (1997)

are investigated separately. In terms of mass, net release of mobile nutrients such as K and Mn is also part of the loss. The ingrowth of microbial biomass and the transport of nutrients into the litter result in the movement of mass into the litter that was not there originally. Thus, what is often called 'litter mass loss' or 'decomposition' is a *net mass loss* although the ingrowth of mycelium normally is negligible from the point of view of mass. As we will see later, the in situ incubation of intact litter is, from several points of view, preferred to laboratory incubation methods, but it still to some extent may be compared with laboratory studies.

When litter decomposition is measured as respiration, only part of the mass-loss process is quantified. No specific term has been suggested for this more specific process, but terms such as 'release of CO_2,' 'C-mineralization,' and 'litter respiration' are used. We will use 'litter CO_2 release' (see Glossary in Appendix I) in this book. Thus, the two processes of 'litter CO_2 release' and 'leaching' together should correspond to 'decomposition' as the term is used today (Fig. 2.1), although loss to fragmentation could influence mass-loss measurements.

Distinguishing litter-based respiration from other respiration in the field is difficult. Methods to separate other sources of respiration, for example, root and faunal respiration which is not directly associated with litter decomposition need further development although some first steps have been taken (cf. Högberg et al. 2001).

In boreal forest systems, microorganisms carry out more than 95 % of the litter decomposition (Persson et al. 1980). Before litter falls, some microorganisms are present on the litter. Most of these are not involved in decomposition unless they are pathogens. After litter fall, fungi are generally the first invaders, penetrating the leaf through openings and thus invading the fresh substrate. The less mobile bacteria come later and there is also a succession of fungal species with different physiological properties depending on the decomposition stage and thus the substrate quality of the litter.

2.3 Ash Dynamics

The ash content of litter can vary between litter types and over time. Ash content in, for example, needle litter of Scots pine and of other pine species is initially low, often around 1 %, and in the course of decomposition, it may increase to 2 %. In a study, ash contents in sugar maple leaf litter were initially 11.3 % of dry matter, increasing to 19.5 % after 1 year of decay, and to 26.6 % after 10 years of decay (McClaugherty unpublished). Ash is normally defined as the fraction of matter that stays after heating a sample to, for example, 400 °C for 2–3 h, which means that all organic C and N disappears and components that are neither burnable nor volatile remain.

The concept ash is complex and ash may include, for example, silicates and nutrients such as Ca, Mg, K, and P. It may also include particles such as clay that have entered the litter during incubation. If not considered, high ash contents could affect the calculation of the percentage of mass loss, and concentration of N and other substances relative to that of less ash-rich litter types. Mass-loss and litter-nutrient contents should thus be related to the litter organic matter, rather than to the whole litter, something that is done inconsistently.

2.4 Degradation of the Main Groups of Organic Compounds in Litter

Although there are differences among litter species and genera as regards, chemical composition and arrangements of compounds in fibers there appear to be some general patterns that may be organized, and at present, we may see one main pattern for pine spp. as regards mass-loss and rate-regulating factors. Organic compounds may be degraded in a sequence, which possibly may differ among

litter species and we have organized the sections below accordingly. However, we have made separate presentations for proximate analysis and those based on ^{13}C-NMR (see also Appendix III).

2.4.1 Degradation and Leaching of Soluble Organic Substances

Foliar litter may contain considerable levels of soluble substances. For example, concentrations of water-soluble substances between a few percentage in lodgepole pine needle litter and c. 30 % in gray alder leaves have been recorded (see Chap. 4). Part of these substances may be leached out of the litter (Bogatyrev et al. 1983; McClaugherty 1983) and part may be degraded in the litter structure. So far we have seen four principal groups of soluble organic material in litter: sugars, phenolics, hydrocarbons, and glycerides. The soluble sugars are predominantly mono- and oligosaccharides that were involved in metabolic processes of the plant. The soluble phenolics are low molecular weight compounds that serve either as defensive agents against herbivory, lignin precursors, or waste products; hydrolyzable tannins are a common example of soluble phenolics. Phenolics are highly variable in their solubility and many have a tendency to condense into less soluble forms or to react with larger molecules (Preston et al. 2009a,b). A nutrient like N may be found in soluble and insoluble organic compounds and may be insolubly bound into organic complexes such as condensed phenolics (Fig. 2.4).

Few attempts have been made to follow the degradation of simple soluble components in litter and it should be pointed out that most studies describe net disappearance only. The soluble fraction is challenging to study, due to the complexities of tracing the formation of new solubles during decomposition and the disappearance of the same solubles due to leaching or metabolism. For example, glucose, which is present initially in newly shed litter, is also produced from decomposing remains of starch and from cellulose and is thus found even in

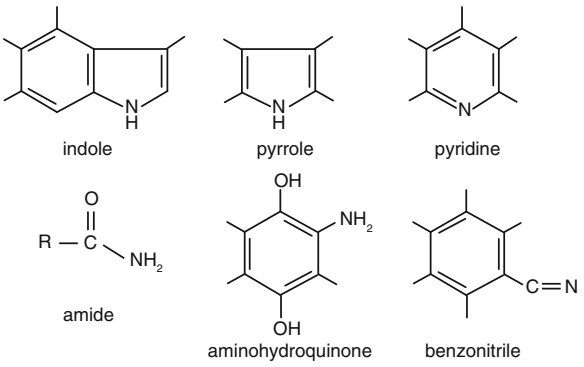

Fig. 2.4 Products detected by ^{15}N-NMR after reaction of ^{15}N-labeled ammonium hydroxide with humic material after oxidative ammonolysis of lignin model compounds. Figure from Knicker (2004)

Table 2.1 The time for onset of net mass loss of different organic chemical components and their relative degradation rates in decomposing Scots pine needle litter

	AUR	Cellulose	Mannans	Xylans	Galactans	Arabinans
Onset (days)	726	376	376	545	Immediate	Immediate
Rate (% day^{-1}) (0–726 days)	–	0.1041	0.0647	0.1077	0.0633	0.1461
Rate (% day^{-1}) (>726 days)	0.0418	0.0393	0.0526	0.0461	0.0375	0.0449

The rates given refer to two stages of decomposition, namely the early stage when the unshielded polymer carbohydrate components and solubles are degraded independently of AUR degradation and the late stage when the degradation of carbohydrate components is regulated by the degradation of AUR, and all components have similar rates (cf. Fig. 2.5). Data from Berg et al. (1982a)

the later stages of decomposition. The same applies to the simple sugars of hemicelluloses. Also several phenolic substances are found in newly shed litter, but are also produced during the degradation of both native lignin and AUR.

Berg et al. (1982a) found that compounds such as simple sugars, for example, glucose and fructose, or ones related to simple sugars such as glycosides and pinitol, were also degraded very early and at a high rate. Also groups of triglycerides and hydrocarbons disappeared quickly whereas fatty acids and diterpene acids remained.

2.4.2 Degradation of Non-Lignified Organic Substances

The ingrowth of microorganisms, mainly fungi, into the litter, may begin prior to litter fall, but the ingrowth of decomposers takes place when the litter has reached the ground. The more fast-growing microorganisms start invading the litter, with part of the litter C becoming microbial biomass and part CO_2. Although the microorganisms degrading the polymer carbohydrates and lignin may be partly the same, the physiology of the degradation of celluloses and lignin is different as are the induced enzyme systems, as previously described. It would therefore be reasonable to use this physiological background for a definition of different steps in the decomposition process, based on substrate and nutrient availability. Raised nutrient levels, in particular N, P, and S, that are normally the main limiting nutrients for microbial growth, stimulate microbial degradation of cellulose, hemicelluloses, and many solubles.

The degradation of solubles and the early degradation of hemicelluloses and cellulose are rather rapid processes, and the measured early-stage rates in a field experiment were at least twice as high as in the late stage. The relative degradation rates of the polymer carbohydrates are relatively high and range from 0.063 to 0.146 % day^{-1} (Table 2.1). In the late stage, the rate of decomposition of the same components can range between 0.038 and 0.053 % day^{-1}. The higher rate of disappearance of arabinan may be due to this hemicellulose being more easily

hydrolyzed and/or less protected. Thus, the cellulose and hemicelluloses were degraded rather quickly until the unshielded portions were consumed. Investigations of the changing patterns of enzyme activities in decomposing litter also support this division in phases (Fig. 2.2). The cellulolytic enzymes appear relatively early, reach a maximum, and decrease before peroxidase (part of the lignolytic system) appears.

The majority of studies on litter decomposition present results from the stage when the litter is recently shed, where normally positive relationships are seen between litter concentrations of N, P, or S and factors such as the mass-loss rate or litter CO_2 release (Taylor et al. 1989; Berg et al. 1997). Also, climatic factors have a strong influence on the turnover rate in newly shed litter (Jansson and Berg 1985; Berg et al. 1993a). For fresh litter with only lignified tissue, this phase should not really be distinguishable.

The simplified but incorrect picture is that climate regulates the rate of decay in early stages on a regional scale and substrate quality on a local one (Chap. 7). This picture holds in a few cases but is far from general. We discuss the basic model with this reservation, while recognizing that local climate and nutrient availability appear to dominate the early stage of decomposition.

2.4.3 A Pattern of Degradation of the Main Organic Compounds in Pine Needle Litter

Some general and common patterns. It appears today that although there are differences among litter species as regards decomposition patterns, there are some main common features, which may be best described using a conceptual model (Fig. 2.2; see also Chap. 6) and we give some main points below. We start with some observations and groups of components that are not affected by the two analytical approaches and continue with these in separate sections.

Decomposition often follows a sequential pattern with different classes of organic compounds dominating the decay process as it proceeds and it appears that this may be related not only to the relative composition of compounds such as cellulose and lignin but also to the synthesis of new compounds as the decomposition process proceeds. Further, it appears that we may be able to describe models, specific for litter genera or groups of genera and species. Such differences among models may be due to a high variability not only in initial chemical composition but also in environment.

The degradation pattern probably is related to the arrangement of the components in the fiber (Fig. 4.2). Microorganisms may first attack and degrade those carbohydrates that are located on the more available and exposed outer structures. Whether this is observed or not may be related to intensity in sampling and analysis/analytical approach.

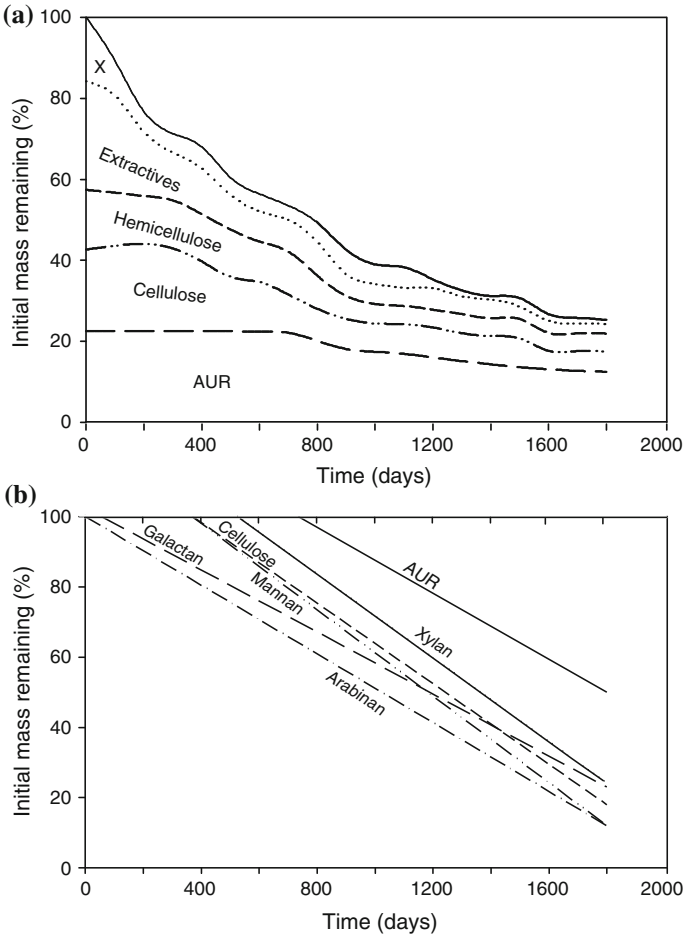

Fig. 2.5 The disappearance of some main organic components from decomposing needle litter. **a** Remaining amounts in decomposing Scots pine needle litter. **b** Onset of decomposition of the different polymer carbohydrates and AUR, showing the different rates of decomposition in the early and the late phases. Decomposition rates are given in Table 2.1. Data from Berg et al. (1982a)

A practical consequence of this is that after onset of decomposition, the degradation of different components may start at different times (Fig. 2.5). Further, the relative degradation rates of the different components may be different at least initially.

Some chemical changes that may be in common for foliar litter over species/genera. As decomposition proceeds, the mass-loss rate often decreases and the accumulated mass loss approaches a limit value, often less than 100 %. With several mass-loss determinations over time, such limit values may be determined with precision (Fig. 2.2; see Chap. 9).

A general phenomenon is that litter N concentration increases, often even linearly to accumulated mass loss (see Sects. 5.3.1, 5.4.2) both for litter with low initial concentrations, for example, 0.3 % as well as for those with higher initial levels, even 3 %. We may expect that N is in the form of microbial biomass, proteins, nucleic acids, and to a certain extent in newly formed compounds (Thorn and Mikita 1992).

Several heavy metals appear generally to increase in concentration (e.g., Fe, Al, Pb, Cu; see Sect. 5.3). In all decomposition studies we have found concentrations of Pb and Cu increase with accumulated litter mass loss. These patterns have been developed for litter in mainly boreal and temperate regions.

AUR. Analyses of AUR ('gravimetric lignin') so far have given one main pattern (Fig. 2.2), namely an increase in concentration. Recent results using ^{13}C-NMR technique have provided new information on the behavior of true lignin, which may resolve and develop the pattern created by AUR. We present available data followed by an attempt to synthesize the information.

2.4.4 Pattern for Main Organic Compounds Based on AUR: Gravimetric Analyses of Lignin

The conceptual 3-stage model (Fig. 2.3) illustrates a typical pattern. When a net loss of AUR begins, there may be little or no easily available carbohydrate and the microbial community must change to one that degrades the AUR complex (see Glossary). The net loss of AUR appears to start after the other groups of compounds have started to degrade. In our example (Fig. 2.5; Table 2.1), the rate of net loss of AUR from the start of year two until the end of year five was about 0.04 % day^{-1}. When we compare to the degradation rates of the carbohydrates during the same period, they were similar in magnitude to that of AUR. This suggests that once the AUR degradation has started, these components are degraded at the same rate because they are so well mixed in the fiber structure that they cannot be degraded separately. Newly formed recalcitrant products may support this pattern.

It thus appears that we may see at least two different groups of carbohydrates: those for which degradation starts immediately after litter fall, namely hemicelluloses dominated by arabinans and galactans; sometimes remaining starch may be included in this group; the second made up of mannans, cellulose, and xylan for which degradation starts later. This could mean that the second group of components is less available than those in the first group as a result of being more dependent on AUR mass loss. So far, there is just one reported detailed pattern (Scots pine) and we cannot exclude a different order or pattern at least for genera other than pine.

Using AUR (sulfuric-acid lignin) and gas chromatography, it was possible to see that onset of decomposition, and the degradation of different components may start at different times (Fig. 2.5). Further, the relative degradation rates of the

different components are different at least initially (Table 2.1). Part of this observation may change using the ^{13}C-NMR approach.

AUR (*gravimetric lignin*) increases in concentration as decomposition proceeds. This is a consequence of its late start and the preferred decomposition of other compounds. The AUR may include some newly synthesized products. We may note that the concept AUR is based on different groups of compounds, namely native lignin, cutin, suberin, waxes as well as recombination products formed during the decomposition process (e.g., Preston et al. 2009a).

2.4.5 ^{13}C-NMR Analysis Applied onto Decomposing Foliar Litter

Recent publications using ^{13}C-NMR have given an alternative analytical approach to apply on decomposing litter. This approach may be used to describe chemical composition and to quantify the disappearance of chemical components in decomposing litter. Some recent work, for example, Preston et al. (1997, 2009a, b), Ono et al. (2009, 2011) and De Marco et al. (2012) have followed decomposing litter and analyzed for changes in chemical composition as well as disappearance of specific bonds (Fig. 2.6). A difference to traditional analytical approaches is that the ^{13}C-NMR does not identify specific molecules such as glucose, arabinose, or native lignin but rather types of chemical bonds, specifically C bonds. To identify a specific compound, we thus may need to identify at least one and more likely several specific bonds belonging to a given compound (Appendix III). Thus, we will obtain information on concentration or amount of, for example, the O–C–O (*Di-O-alkyl-C*) bonds or the O–C (*O-alkyl-C*) bond typical for carbohydrates, which means that cellulose and some hemicelluloses give a common peak or signal (Preston et al. 2009b).

A certain terminology has developed relating to the compounds of which the bonds are part. Thus, the term *O-alkyl-C* mainly encompasses carbohydrate carbon, namely cellulose and hemicelluloses, but it also gives side chains of lignin (those going from carbon 1; see Fig. AIII.2b). At the present development stage, the change in concentration or decomposition of a chemical compound is given as concentration or amount of a certain C-bond. A bond is identified as response in a certain frequency interval (Fig. AIII.3; cf Table AIII.1) and we may expect such responses to be complex. For example, bonds in native lignin molecules may respond to different areas of the spectra (*aromatic-C, phenolic-C, methoxy-C, and alkyl-C*; cf Table AIII.3).

Alkyl-C. Also called aliphatic-C. This frequency interval indicates long chains with $–CH_2–$ units. Further, a side chain, in hemicellulose, namely an acetate group belongs here as well as C in side chains of lignin.

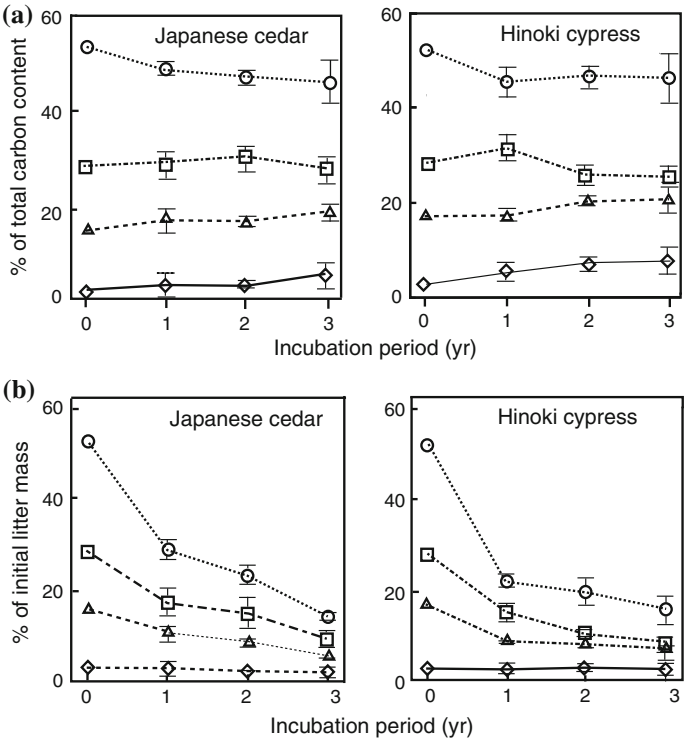

Fig. 2.6 Decomposing needle litter of Japanese cedar and Hinoki cypress investigated using ^{13}C-NMR, litter sampled over time. **a** Concentrations of some C bonds. **b** Remaining amount of C bonds as related to remaining carbon. (◇) carbonyl-C, (Δ) aromatic-C, (◯), O-alkyl-C, (□) alkyl-C. From Ono et al. (2011)

O-alkyl-C mainly encompasses carbohydrate carbon, namely in cellulose and hemicelluloses, but also side chains of lignin (those going from carbon 3, Appendix III). Further, some signals from tannins come in this interval.

Di-O-alkyl-C mainly encompasses cellulose plus hemicelluloses, thus carbohydrate carbon but shows no difference between the different carbohydrates.

Methoxy-C shows the methoxyl carbon in lignin (cf Fig. 3.3; Appendix III, Fig. AIII.2a). This frequency interval also includes the alkyl carbon bound to N in proteins.

Aromatic-C. Also called Aryl-C. The intensity in this interval (112–140 ppm) comes from the aromatic carbon in both lignin and condensed tannins. It may also show the guaiacyl group of lignin.

Phenolic-C. The intensity in this interval comes from phenolic-C (140–165 ppm) in both lignin and condensed tannins. It may also show the syringyl group of lignin.

Carboxyl-C. This region includes carboxylic acids, amides, and esters.

The ratio alkyl-C/O-alkyl-C is sometimes used as an index for degradation of decomposition and is increasing with accumulated litter mass loss. Some authors call it 'humification.'

An investigation by Ono et al. (2011) encompassing Japanese cedar and Hinoki cypress gives concentrations and amounts of 4 types of bonds (Fig. 2.6). A heavy decrease in amount of O-alkyl-C may mean a fast decomposition of cellulose and hexose-based hemicelluloses (Fig. 2.6b). Some signals from tannin may be part of the loss. We may see that also the concentration decreases (Fig. 2.6a). A less fast decrease in aliphatic-C (alkyl-C) may mean a loss of mainly fatty acids, but may also include a side chain in lignin. We may see a decrease in amount of aromatic-C from the start, which indicates that aromatic structures are degraded. This may mean tannins as well as lignin. Tannins are more readily degraded than lignin and may be degraded from the start of the incubation. Further, they do not have the methoxy group (methoxy-C), which makes them possible to distinguish from lignin.

2.5 Factors Regulating Degradation of Lignin/AUR

2.5.1 Potential Effects and Possible Interactions on Lignin/AUR Degradation

Some nutrients have been found to influence the degradation and dynamics of lignin and AUR. We may apply findings from fungal and bacterial physiology as well as from laboratory studies and we discuss a few such ones below. The relationships we discuss on the level of litter decomposition are ones that we have applied and confirmed in the sense of significant relationships as far as data allow. As such we may rather consider them as theories, as an investigated specific relationship between, for example, litter mass loss and a nutrient does not automatically exclude influences of other ones. Still, the relationships we forward in this chapter for mainly Scots pine needle litter have been confirmed in that sense. As we will discuss later, influences like those from Mn and N may have opposite effects (e.g., Fig. 2.2) and interact. However, what is possible today is just to present and comment on the potential effects of single nutrients. We may remember that if an effect is shown for native lignin, it does not automatically apply to the more crude AUR fraction, which is based on several components. As effects on AUR/lignin degradation have been related to both nutrients, we discuss both of them here, well aware of that the effect is potential and may vary among litter species. What we may rely on for support is the shown effects on the AUR fraction. Interaction effects between Mn and N may need to be confirmed in future studies. We have related effects of both nutrients to mass loss for Scots pine litter and to that of AUR.

2.5.2 *Effects of Litter Mn Concentration on Lignin/AUR Degradation and Litter Mass Loss*

The discovery of the enzyme manganese peroxidase (MnP) was important for the science of litter decomposition. The enzyme appears to be produced by the majority of all wood-degrading basidiomycetes which cause white-rot, as well as by various soil-litter-colonizing saprotrophic fungi. Among the lignolytic enzymes, MnP is probably the most widely spread peroxidase produced by these fungi (Hofrichter 2002).

Manganese is essential for the activity of MnP, and enhances its production (Perez and Jeffries 1992). Mn is also involved in the regulation of other lignolytic enzymes, including laccase (Archibald and Roy 1992) and lignin peroxidase (Perez and Jeffries 1992). In a large number of laboratory studies, it has been shown that this nutrient is essential for formation and activity of lignin-degrading enzymes and thus for degradation of lignin.

Manganese peroxidase is a glycosylated heme protein, which is secreted by the fungi into their environment. It oxidizes Mn^{2+} ions, which are found in plant residues, wood and soil, to highly reactive Mn^{3+} ions. These ions in turn are stabilized by organic acids also produced by these fungi. Organic acids, such as oxalic or malic acid, chelate Mn^{3+} ions and prolong their lifetime until the enzymes attack the phenolic structure of lignin or humic acids.

How we should measure the effect of Mn on lignin degradation as part of litter decomposition is still not clear. Annual litter mass loss for several species has been related to Mn concentrations, but the variation in its concentration in litter, both during decomposition and among litter species, has so far not allowed any general conclusions. That there is an effect of Mn on the degradation of pine litter in late stages of decomposition has been demonstrated using litter in the late stages of decomposition (Fig. 2.7).

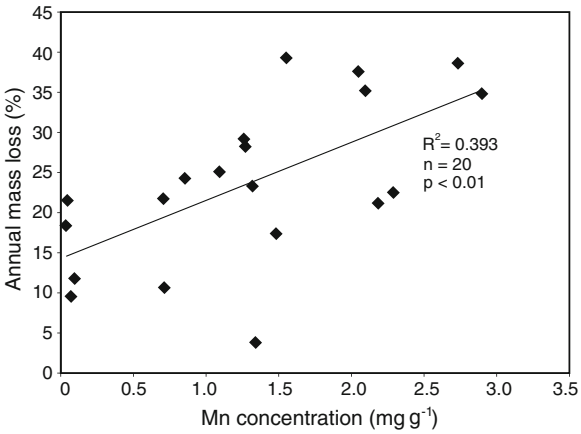

Fig. 2.7 Annual mass loss for pine needle litter (*lodgepole* pine, *Aleppo* pine) in the late stage plotted versus Mn concentration at the start of each one-year period. Data from Berg et al. (2007)

2.5.3 *Effect of N on Lignin/AUR and Late-Stage Litter Degradation*

As litter decomposes and the level of AUR/lignin increases, that of total N also increases (Fig. 2.2). This is a well-known and general phenomenon in decomposing litter. We emphasize this because of the important role of N in the degradation of lignin and the formation of humus (Stevenson 1982; Nömmik and Vahtras 1982; Eriksson et al. 1990; Keyser et al. 1976). In more recent papers, Thorn and Mikita (1992) and Knicker (2004), using NMR, report numerous compounds formed as a consequence of reactions between fulvic and humic acids and ammonia/ammonium.

For the increase in nitrogen concentration, there is no really clear border between the early, the late, and the humus-near or the limit-value stage of decomposition. The rate-retarding effect normally ascribed to the increasing AUR/lignin concentrations (e.g., Fogel and Cromack 1977; Berg and Lundmark 1987) is probably in part due to the associated high concentration of N which, (1) may have a suppressing effect on formation of ligninase and thus on lignin mass loss (see below, Biological mechanisms) and (2) may form complexes with organic compounds (Stabilized compounds, below). Thus, it may not be the lignin concentration per se that is rate retarding, but lignin in combination with N concentrations above a certain and so far unknown level.

We may, as an extreme case, imagine that with low enough N levels and thus no suppressing effect, a higher degradation rate for lignin and for litter may result and probably no raised lignin concentration. There is indirect evidence for this based on experiments in wood decay as wood is extremely low in N. Based on litter-bag studies in Black Hawk Island (WI, USA), wood chips of red maple and white pine lost more mass (88 %) than any of ten other litter types including leaf litter, needle litter, bark, and fine roots after 10 years of incubation on the forest floor. The initial N concentration in these wood samples was 0.09 % for red maple and 0.04 % for white pine (Aber et al. 1990; McClaugherty unpublished). A similar finding was made in the CIDET project using wood blocks (Preston et al. 2009a).

Much remains unknown about the roles of AUR/lignin and N during the late and final stages of decomposition. We do not know whether the rate-retarding effect ascribed to lignin, or the combination of lignin and N in aging litter is due to the level of N, the number of different recombination products or the formation of specific compounds. Several suggestions exist (below). What is the role of the initial N in litter and of that taken up during decomposition? The N transported through fungal mycelia into the litter is one easily available source of N from outside the decomposing substrate (e.g., Berg 1988), but inputs from atmospheric deposition may become increasingly important in some systems. Possibly both internal and external sources are influential, but this requires further investigation.

An evident question is whether the declining rate of decomposition observed in the late stage of decay is due to the suppression caused by N or a combination of (high) N and (low) Mn. The suppressing effect of N on the degradation of lignin, as

well as on the decomposition of whole litter, has been observed in studies with different levels of resolution. The suppression of ligninases in white-rots has been described in reviews by Eriksson et al. (1990) and Hatakka (2001). An effect, possible to ascribe to N has been observed directly, producing different AUR-degradation rates in decomposition experiments (Berg and Ekbohm 1991). That study did not exclude other possible agents, though. Recent studies using N additions to decomposing litter has shown a clear suppression (Sect. 6.3.2; Hobbie et al. 2012; Perakis et al. 2012).

Stabilized compounds. Stabilization of litter residues is a consequence of an enrichment of stabilized compounds, for example, macromolecules. Some different pathways have been suggested (e.g., Knicker 2004).

The N-fixation process involves ammonia, not the ammonium ion, and therefore, the reaction is faster at higher pH values. Also, amino acids react at higher rates at higher pH values for the same reason. Broadbent and Stevenson (1966) demonstrated that close to pH 9 the reaction was 10–20 times as fast as at pH 6 and below. The higher the level of N (or the higher the degree of humification) the lower was the NH_3-fixing capacity of the organic matter studied. For Scots pine needle litter, Axelsson and Berg (1988) largely confirmed these findings and estimated a fixation rate three times higher at pH 9 as compared to pH 5. They also found that Scots pine needle litter that had reached a higher accumulated mass loss and thus higher N concentrations, adsorbed/fixed less ^{15}N. In a more recent investigation, Thorn and Mikita (1992) found that N concentration in a natural fulvic acid preparation increased from 0.88 to 3.17 % after fixation with ammonia. This may be a potential increase.

Using ^{15}N-NMR and natural fulvic and humic acids, it has been possible to follow *pathways for mineralized N*. Thus, Thorn and Mikita (1992) found an array of new compounds, namely after recovering the ^{15}N in a set of compounds (Fig. 2.4). In contrast to earlier investigations, they did not find any quinones. This may be a first step in the humification process. Of refractory N compounds in soils, melanins have been suggested as a precursor.

An alternative suggestion is the *depolymerization—recondensation pathway*. Naturally occurring macromolecules are degraded to smaller units. A small fraction of these may recombine by random reaction (condensation) to insoluble and refractory structures with a more long-term stability. An example on stabilization for N compounds suggests that carbonyl (C=O) in lignin reacts with NH_2 groups of proteinaceous material forming Schiff bases (see the review by Knicker 2004).

The selective preservation pathway suggests that refractory biopolymers may resist biodegradation and accumulate. Such compounds are produced by plants and by bacteria.

Thorn and Mikita (1992) identified and listed 24 compounds, mainly aromatic ones, found in 3 fulvic and humic acid samples. Such chemical transformations may result in structures that are not easily degradable by the soil microorganisms.

Several pathways have been suggested for the reaction of NH_3 with, for example, humic and fulvic acids and several mechanisms are reasonable, possibly related to the nature of the organic matter and the ecosystem, although several

mechanisms are reasonable. Methoxyl groups are removed from the lignin aromatic ring, forming phenolic groups which then may react with and bind NH_3. A fixation mechanism has been suggested involving quinones, which are formed during lignin degradation as side products from laccase or peroxidase acting on diphenol rings (Nömmik and Vahtras 1982). These latter, by reacting with ammonia, could be transferred to heterocyclic polymeric compounds finally resulting in a polymerization into quinones. A newer study (Fig. 2.4) gives a more varied spectrum of compounds than the quinones found by Lindbeck and Young (1965).

In their review, Nömmik and Vahtras (1982) point out that prolonged exposure of organic matter to NH_3 under aerobic conditions leads to degradation of humic acid polymers by hydrolytic and oxidative processes, which results in the formation of soluble low molecular weight soluble compounds (see also Sect. 6.5; Guggenberger 1994).

Biological mechanisms. Raised N levels may suppress the formation of lignin-degrading enzymes, the degradation of lignin (Keyser et al. 1978; Eriksson et al. 1990; Hatakka 2001) and consequently also the decomposition rate of litter (Berg et al. 1987; Berg and Ekbohm 1991; Berg and Matzner 1997). This simply means that the higher the level of available N, the stronger the repression of the formation of lignolytic enzymes in the population of lignin-degrading organisms (see also Chap. 3). The ability of several fungal species to degrade lignin was heavily suppressed when N was added to the culture medium at concentrations of 2.6–7.8 mM, corresponding to 0.0036–0.0109 %. The level of N in solution in a pure fungal culture is not directly comparable to those in litter, where the N will be bound in different compounds and will be much less mobile than in solution. However, trends apparent in culture may stimulate speculation as to possible mechanisms in litter. With an N concentration of 0.4 % in our case-study litter, N concentration is 100-fold greater than in the fungal laboratory culture system. In both cases, the status of the N changes over the course of the experiment. In liquid pure culture, the N becomes bound in microbial biomass and thus less available. In the litter substrate, there may be a mineralization and degradation of proteins, thus converting a fraction of the bound N to more available N, possibly in concentrations high enough to suppress decomposition.

2.6 Proposed Model for Decomposition from Newly Shed Litter to the Humus Stage

In the section above, we demonstrated that the decomposition patterns for organic chemical components not embedded in lignin (early stage) were different as compared to those of the same components in tissue that was either lignified already in the newly shed litter or had become embedded in newly formed resistant products in the late stage. This may be a general basis for considering rate-regulating factors, but the fraction of lignified tissue may vary among litter species

in the same genus and possibly also within our model species, for example, among stands and locations.

We cannot exclude that rate of formation as well as properties of newly formed resistant products may vary with species and environment. A given litter may have only a certain fraction of its holocellulose embedded in lignin, while that of another species may have its holocellulose completely embedded, which may change both decomposition pattern and rate-regulating factors.

We used information obtained from pure culture and physiological studies on microorganisms, compared this to the degradation of different components and organized a three-stage conceptual model based on AUR analysis (Fig. 2.3). It has been recently shown that as further litter species are investigated, the original three-stage model (based on AUR analysis; Berg and Matzner 1997) can be further developed and modified, and we can now see differences in length of the early stage, possibly due to the level of lignification of the holocellulose. We develop this in Chap. 6.

It is possible that we in the future will see separate models related to plant genera or to groups of genera. In the present case, we give explanations for the different stages, which are connected to in situ decomposition experiments for litter and humus. Although each stage can be uniquely described, the process is more accurately described as a continuum in which transition points cannot be defined precisely.

The reasons for dividing the decomposition process into different stages are rather straightforward. On the level of plant cells, the polymers lignin, cellulose, and hemicelluloses are structurally organized (see Fig. 4.2) and the main part of the cellulose and hemicelluloses are found in the primary cell wall, whereas lignin is distributed in the secondary wall and in the middle lamella (Eriksson et al. 1990). A result of the distribution in the cell wall is that there is a separation of carbohydrates into those that are not lignified and those that are encrusted in native lignin. Microorganisms that are not lignolytic may degrade only the former.

We discuss the possibility (Chap. 4) that newly shed foliar litter of some litter species may have a structure with a much higher level of lignified tissue, which may explain the very short early phase and the fact that the litter cannot decay to a significant extent until the onset of lignin degradation, often recorded as AUR degradation. Thus, the early stage is missing or simply too short for us to measure. Still today, it is not clear if this lignification is due to species-related differences or properties related to, for example, the balance of nutrients in the soil that may influence, for example, the formation of lignin (cf Sect. 4.7).

The model is divided into three main phases, describing the decomposition of litter toward humus. The process may be divided into functionally defined stages: (1) newly shed litter—early decomposition stage; (2) late stage for partly decomposed litter; and (3) humus-near stage or limit-value stage, where litter is close to becoming stable humus. These are the main stages that we have described and connected. The model considers the effects of climate, the effects, and roles of nutrients in the early phase and of AUR, N, and Mn in different substages of the late phase, as well as effects of Mn concentration on the level of the limit value. It

also accounts for theories for formation of a stable fraction (humus formation-humus-near stage, Sect. 2.6.3). In recent work, it has been possible to subdivide the stages by adding a transition stage, in which the effects of nutrients are less clear (B. Berg and J. Kiønaas, unpublished).
We will discuss these stages more in detail, referring to the denominated model stages including what factors that may regulate the mass-loss rate (Fig. 2.2).

2.6.1 The Early Stage

The model (Fig. 2.2) was constructed using Scots pine as a model substrate and is a development of the original 3-stage model (Berg and Matzner 1997). Information so far available has indicated that the early phase may encompass different fractions of litter mass, for Scots pine ranging from c. 25 to 28 % accumulated mass loss, and for lodgepole pine, about 20 %. For black pine, De Marco et al. (2012) suggested 28 % accumulated mass loss as the end of the early stage.

The duration of the early stage measured as accumulated mass loss is likely to vary, possibly in some proportion to lignin/AUR concentration. It has been documented that raised concentrations of, for example, N, P, and S are positively related to an increased litter mass loss (e.g., Berg and Staaf 1980). Also increased site MAT or AET has a positive effect, (e.g., Johansson et al. 1995) on mass-loss rate. The existence of an early phase may depend on our ability to measure it. Thus, a very short phase of say 10 % accumulated mass loss or less may simply pass unnoticed in an environment with a high mass-loss rate (see also Sect. 6.3.1).

The definition of this early stage was originally 'the amount of organic matter that is not lignified,' viz. the material that could be decomposed without any degradation of AUR/lignin. We may revise this somewhat to 'the fraction of organic matter the degradation of which is not dominated by that of lignin.' We cannot exclude that ^{13}C-NMR analysis may give mainly the same result but that the fraction of lignin will be smaller than that for AUR, which again may alter the definition (see also Sect 6.3.2).

2.6.2 The Late Stage May Have Substages

An intermediate stage. We may not expect a sharp transition from the early stage to the late one. Using Scots pine needle litter, Berg et al. (201Xa) calculated annual litter mass loss using litter that had decomposed to >30 % accumulated mass loss and thus was at the end of the early stage. They subdivided the dataset (75 annual mass-loss values) into groups of annual mass loss, based on accumulated mass loss subdivided into 10 %-units, namely 30–40, 40–50, 50–60, 60–70, and 70–80 % accumulated mass loss. Using annual mass loss (see the Glossary) for litter in each one of these groups, they investigated for any significant

relationship between factors potentially regulating lignified tissue and annual mass loss, namely MAT, MAP, AUR, N, and Mn.

They identified a zone in which there was a certain influence of MAT ($p < 0.1$), and none of N, AUR, or Mn and interpreted this as a transition zone between the early and late stages. There was no significant effect of anyone of the four factors.

Further substages or simultaneous influences of Mn and N? We have commented on the effect of Mn and on different effects of N in the late stage. One question is whether these two nutrients exert their effect on degradation of lignin and litter simultaneously over the late stage or in a sequence. Using the approach of annual mass loss grouped as described above, Berg et al. (201Xa) found a simultaneous effect of Mn and N throughout the late stage with Mn-stimulating and N-retarding decomposition. Whether the relative effects were related to the relative concentrations of the two nutrients is today not known. At this late stage, there was no relationship to climate. Berg et al. (201Xa) used data from a climate gradient with MAT ranging from −0.7 to 6.8 °C.

2.6.3 The Humus-Near or Limit-Value Stage

Literature that describes the functional transfer from partially decomposed litter to a stable phase or humus is rare. Nonetheless, moderately decomposed litter, the humus-near stages, and humus have at least some properties in common. One example including pine needle litter was that the estimated N concentration at the limit value was found to be almost the same as that in the FH or H layer of the same stand (Berg et al. 1999b). Further, Couteaux et al. (1998) measured rates of litter CO_2 release close to the limit value and in the humus layer from the same Scots pine forest (Table 2.2) and found them to be very similar.

We may also connect litter mass-loss rate to the concept humus. It has been possible to adapt mathematical functions to accumulated mass loss of litter and with good statistical precision estimate how far the decomposition should proceed before we may estimate the rate zero. The rate zero may be found at different

Table 2.2 Compartments of different stability in decomposing Scots pine needle litter and humus in a Scots pine forest

Labile comp. (%)	K_L (% day^{-1})	Intermediate comp. (%)	K_{IN} (% day^{-1})	Recalcitrant comp. (%)	K_R (% day^{-1})
Needle litter incubated in litter layer for 16 months					
4.09 (0.39)	0.124	17.01 (2.41)	0.0087	78.52	<0.0001
Brown needle litter from forest floor					
4.67 (0.61)	0.124	21.91 (1.54)	0.0087	74.93	<0.0001
H layer particles <2 mm					
0.00	0.124	9.80 (1.32)	0.0087	91.20	<0.0001

The sizes of the compartments were estimated and the rate constants were based on respiration measurements. Standard deviation in parentheses. (Couteaux et al. 1998)

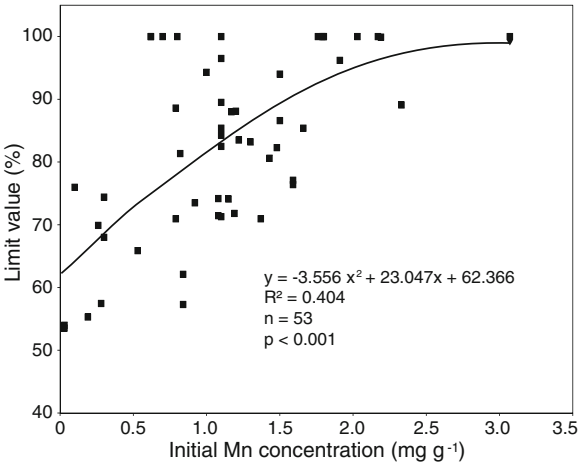

Fig. 2.8 The level of the limit value, given as accumulated mass loss. The limit value for needle litter of pine spp. appears to be related to litter Mn concentration. Decomposition studies from four litter species (mainly Scots pine) were combined into a climatic gradient and limit values estimated using litter accumulated mass loss. A backward elimination procedure removed insignificant factors. Of MAT and MAP and eight substrate-quality factors, Mn was selected as the single significant factor. Data from Berg et al. (2010)

values for accumulated litter mass loss (Fig. 2.8), which also means that a variable fraction of very slowly decomposing material is left (cf Fig. 9.2).

A number of such investigations have been carried out using our case-study litter (Scots pine). Within a given forest plot, there is a certain repeatability of limit values. For example, Berg and Ekbohm (1993) found some homogeneity among limit values within groups of studies on decomposing litter of Scots pine and lodgepole pine. They also found that the limit values of the two groups were significantly different. Berg et al. (1999b) published data for 11 studies on Scots pine litter decomposition in one forest system and found that the limit values ranged between 76.0 and 93.2 %, giving an average of 84.7 % (SE 1.57). In other words, on average, 15.3 % of the initial litter became stabilized as very slowly decomposing residue. For the same litter type, Couteaux et al. (1998) using release of carbon dioxide calculated a mass-loss rate that was less than 10^{-4} % day^{-1} for the recalcitrant fraction (above; Table 2.2).

The variation in limit values using litter from different pine species (genus *Pinus*) was investigated over a larger region using 56 decomposition studies and litter from four pine species with Scots pine dominant. Eight litter-quality factors such as initial concentrations of nutrients and AUR were evaluated as well as MAT and MAP and resulted in Mn as the single significant factor (Berg et al. 2010; Fig. 2.8). With the support of the causal relationship for the role of Mn in degradation of lignin, the result appears reasonable. In that study, they used local litter, which means that the litter was incubated at the same site as where it was

harvested. Further, they identified limit values for pine spp. litter ranging from 100 % accumulated mass loss to c. 50 %. Using backward elimination and available substrate-quality factors, they found that Mn was the sole remaining factor. They used mainly Scots pine needle litter as a model substrate, and the average limit value for Scots pine was 82.7 % accumulated mass loss. We may use part of the dataset of Berg et al. (2010), namely that for just Scots pine ($n = 34$) and comment on that also for this limited dataset, a quadratic function was significant ($p < 0.05$) and similar to that in Fig. 2.8.

The role of animals in the decomposition of litter toward a limit value is unclear. Most of the studies that are used to estimate limit values for pine litter have been carried out in forest soils containing relatively small numbers of animals that would influence litter decomposition. However, other studies have indicated that decomposition may have a limit-value pattern also in systems in which soil animals are found in higher numbers. At present, there appears not to be any study in boreal and temperate forests actually showing an influence of animals on the limit value. We cannot exclude that the existence of limit values and their levels in these studies may in part be ascribed to the absence of soil animals.

Although it is possible to estimate significant limit values for litter decomposition, we do not conclude that such limit values necessarily indicate a completely recalcitrant residue in the humus-near litter (e.g., Table 2.2). The estimated values may illustrate a fraction that is stabilized and thus decomposed at a very low rate or possibly not at all. Even if this is the case, the phenomenon is no less interesting or useful, especially if we can connect this resistance or recalcitrance in litter to its properties, for example, to the concentration of lignin or some nutrient, or climate. We cannot exclude that a limit value may indicate a point of transformation from decomposing litter to stable humus.

Just the fact that allophanic (see Glossary) humus exists shows that an 'eternal' storage is possible. Although allophanic organic material may be regarded as an extreme case, the level of stabilizing components (for example, aluminum and iron ions) necessary to stop the decomposition process is not known (Paul 1984). The fact that the use of limit values allows us to reconstruct a humus buildup over a period of 3,000 years also indicates that at least in some forest systems, the litter has a long-term stability (below). We may speculate about what factors that could disturb the litter/humus accumulation process. That wild fire will cause a severe disturbance appears clear, especially if repeated with some frequency. Forest management such as soil preparation is a factor as well as drainage.

What further influences may cause the decomposition to cease? Although Mn was well related to the limit value, the model suggested by Berg et al. (2010) could 'explain' less than 35 % of the variation. Although we have presented results from a study dominated by Scots pine that excluded other factors than Mn, we cannot conclude any generality, not even within the genus *Pinus* (Fig. 2.8) and we discuss their influence in this sense. They are thus potential factors also for pine litter. Although 8 substrate-quality factors were investigated (Berg et al. 2010), none of them was a heavy metal such as Pb, Cu, Hg, or Cd. Further, all factors were substrate-quality ones and there was none related to the soil.

Heavy metals. We must consider these as a group for which there are too few studies to allow any conclusion about their effect on decomposition under ambient conditions.

It appears that the effect of heavy metals on litter mass-loss rate in non-polluted environments has been little studied and few studies have recorded dynamics of the main heavy metals. Some appear to increase in concentration with accumulated mass loss (e.g., Pb and Cu) as judged from available information, whereas other ones are highly variable. For example, Cd mobility is related to pH. Still, there are several unknowns about increasing *vs* decreasing concentrations for different heavy metals. We have seen for Scots pine needle litter that Fe, Zn, Cu, Pb, and Cd increase heavily (Laskowski and Berg 1993). Such a general increase may influence decomposition in the late stages and at the limit value.