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Effects of nutrient cycling through litter of different broadleaved deciduous tree species on soil biochemical properties and the dynamics of carbon and nitrogen in soil

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List of abbreviations and acronyms

Ah-horizon	humic top horizon
AIC	Akaike information criterion
Al	aluminum
Al^{3+}	aluminum cation
Al-horizon	lessivied top horizon
a.s.l.	above sea level
BS%	base saturation
C	carbon
^{12}C	stable carbon isotope, mass of 12 g mol^{-1}
^{13}C	stable carbon isotope, mass of 13 g mol^{-1}
Ca	calcium
Ca^{2+}	calcium cation
$CaCl_2$	calcium chloride
CEC	cation exchange capacity
CFE	chloroform fumigation extraction
C_{fum}	organic carbon extracted from fumigated soil
$CHCl_3$	chloroform
C_{MB}	microbial biomass carbon
C_{non}	organic carbon extracted from nonfumigated soil
CO_2	carbon dioxide
C_{org}	organic carbon
DBH	diameter at breast height
DM	dry matter
DOC	dissolved organic carbon
EA-IRMS	elementar analyzer isotope ratio mass spectrometer
Fe	iron
Fe^{2+}	iron cation
g	gravity acceleration
GC	gas chromatograph
H^+	proton, hydrogen cation
HF	heavy density fraction (>1.8 g cm^{-3})
H_2O_{dem}	demineralized water
ICP-OES	inductively coupled plasma optical emission spectrometer
IRMS	isotope ratio mass spectrometer
K	potassium
K^+	potassium cation
KCl	potassium chloride
KOSI	Kompetenzzentrum für stabile Isotope (Center for stable isotope research and analyses)
K_2SO_4	potassium sulfate

LF	light density fraction (<1.8 g cm^{-3})
L-horizon	fresh litter horizon
LL_{ash}/LL_{total}	proportion of ash leaf litter to total leaf litterfall
$LL_{(tree\ species)}/LL_{total}$	proportion of a leaf litter type to total leaf litterfall
M	molar
MB	microbial biomass
Mg	magnesium
Mg^{2+}	magnesium cation
ML-ash	variant with 1:1-mixture of isotopically labeled ash litter and unlabeled beech litter
ML-beech	variant with 1:1-mixture of isotopically labeled beech litter and unlabeled ash litter
Mn	manganese
Mn^{2+}	manganese cation
N	nitrogen
n	number
^{14}N	stable nitrogen isotope, mass of 14 g mol^{-1}
^{15}N	stable nitrogen isotope, mass of 15 g mol^{-1}
N_2	molecular nitrogen
Na^+	sodium cation
NH_4^+	ammonium
NH_4Cl	ammonium chloride
N_{MB}	microbial biomass nitrogen
N_2O	nitrous oxide
NO_3^-	nitrate
N_t	total soil nitrogen
OF	decayed organic layer
OH	humic organic layer
O-horizon	organic horizon
OL	organic litter layer
P	phosphorus
p	significance level
PDB	belemnite of the PeeDee formation, standard for stable carbon analyses
PL-ash	variant with pure isotopically labeled ash litter
PL-beech	variant with pure isotopically labeled beech litter
PLFA	phospholipid fatty acid
ppm	parts per million
PU-ash	variant with pure isotopically unlabeled ash litter
PU-beech	variant with pure unlabeled beech litter
PVC	polyvinyl chloride

***q*CO$_2$**	metabolic quotient, unit of CO_2 produced per hour and unit of microbial biomass
***q*MB**	percentage of microbial biomass at the organic carbon content
R²	coefficient of determination
ref	reference
rpm	returns per minute
SOC	soil organic carbon
SOM	soil organic matter
SPT	sodium polytungstate
V-PDB	new standard for stable carbon analyses, Vienna-PDB

Zusammenfassung

Baumarten beeinflussen bodenchemische Eigenschaften über den Eintrag von Nährstoffen und Protonen mit dem Bestandesniederschlag, dem Stammabfluss, dem Streufall, der Wurzelatmung und/oder der Ausscheidung von Wurzelexudaten. Wie sich Nadelbäume im Vergleich zu Hartholz-Bäumen, wie z.B. Buche (*Fagus sylvatica* L.) verhalten, ist weitestgehend erforscht. In jüngerer Zeit wurde der Fokus vermehrt auf Untersuchungen zum Einfluss verschiedener Laubbaumarten auf die Bodeneigenschaften gelegt und es zeigte sich, dass sich auch unterschiedliche Laubbaumarten in ihrem Einfluss auf Bodeneigenschaften, wie z. B. den C- und N-Haushalt, deutlich unterscheiden können. Allerdings sind in den meisten Studien verschiedene Einartbestände miteinander verglichen oder Pflanzexperimente durchgeführt worden. Untersuchungen in einem ausgewachsenen Mischwald sind selten. Noch seltener sind vergleichende Untersuchungen der Kohlenstoff- und Stickstoffverteilung während der Streuzersetzung verschiedener Baumarten. Mögliche artenspezifische oder mischungsrelevante Unterschiede sind daher weitgehend unbekannt. Vor diesem Hintergrund entstand die vorliegende Arbeit, die sich aus den drei im Folgenden beschriebenen Studien zusammensetzt:

(1) Die „Cluster-Studie“ wurde in einem artenreichen Mischbestand des Nationalparks Hainich durchgeführt. Der Bodentyp war eine Parabraunerde aus Löss über Muschelkalk. Es wurden kleinräumig drei in einem Dreieck zueinander stehende Baumgruppen, die sogenannten „Cluster“, gewählt. Die Cluster bestanden aus jeweils einer oder zwei der folgenden Baumarten: Buche, Esche (*Fraxinus excelsior* L.) und Linde (*Tilia cordata* Scop. oder *Tilia platyphyllos* Mill.). Der Streufall, die Humusauflage und der Oberboden (0-10 cm und 10-20 cm) wurden auf ihre chemische Zusammensetzung hin untersucht.

(2) Die „Mesokosmen-Studie“ wurde in einem bodensauren Buchenwald des Nationalpark Hainich durchgeführt. Der Bodentyp war eine Parabraunerde aus Löss über Muschelkalk. Der Abbau von und die C- und N-Verteilung aus $^{13}C/^{15}N$-markierter Buchenblatt- und Eschenblattstreu wurde in Rein- und Mischvarianten verglichen. Hierzu wurden Bodensäulen ausgestochen, in PVC-Zylinder überführt und in die Probenahmestelle zurückgeführt. Die ursprüngliche frische Streu wurde durch die jeweils zu untersuchende isotopisch markierte Streuart bzw. Streumischung ersetzt. Die gesamte und die streubürtige CO_2-Respiration wurden zweiwöchentlich über einen Zeitraum von zwölf Monaten mittels geschlossener Hauben erfasst. Nach fünf und zehn Monaten Versuchslaufzeit wurde der Masse-, C- und N-Verlust der ursprünglichen Streu erfasst, die gesamten und streubürtigen

C- und N-Gehalte im O-Horizont und im Mineralboden (0-4 cm) sowie in der mikrobiellen Biomasse (0-4 cm) bestimmt.

(3) Die „Mikrokosmen-Studie“ wurde bei konstanter Lufttemperatur und Bodenfeuchte in einer Klimakammer durchgeführt. Boden-Streu-Gemische wurden über 206 Tage inkubiert. Die C-Verteilung im Zuge des Abbaus von ^{13}C-markierter Blatt- oder Wurzelstreu von Buche und Esche wurde in Rein- oder Mischvarianten verglichen. Die CO_2-Emission wurde täglich erfasst. Die ^{13}C-Messungen im CO_2 wurden anfangs alle drei Tage und später alle sieben Tage durchgeführt. Gesamte und streubürtige Gehalte an gelöstem organischem Kohlenstoff (DOC) wurden an den Tagen 9, 29 und 206 gemessen. Die Bestimmung von gesamten und streubürtigen C-Gehalten in der mikrobiellen Biomasse sowie der leichten und schweren Dichtefraktion erfolgte an Tag 206.

Die wichtigsten Erkenntnisse aus den drei Studien werden im Folgenden kurz darstellt.

(1) Clusterstudie: Baumarten beeinflussten die chemischen Bodeneigenschaften im Oberboden (0-10 cm) kleinräumig, während in 10-20 cm Tiefe der Einfluss der Baumarten hinter den Einfluss des Tongehalts zurücktrat. In 0-10 cm Bodentiefe waren die Basensättigung, der pH-Wert und der Vorrat an austauschbarem Mg^{2+} am höchsten unter reinen Eschen- (98%; 5,1; 135-137 kg ha^{-1}) und am niedrigsten unter reinen Buchenclustern (88%; 4,3; 70-76 kg ha^{-1}). Der Anteil an austauschbarem Al^{3+} an der Kationenaustauschkapazität (KAK) war am niedrigsten unter reinen Eschen- (0-0,6%) und am höchsten unter reinen Buchenclustern (3,7-7,8%). Die Bodeneigenschaften unter Lindenclustern waren intermediär. Mischungseinflüsse gab es keine. Als eine wichtige Einflussgröße konnte die Zusammensetzung der Blattstreu nachgewiesen werden. Die Vorräte an austauschbarem Mg^{2+} und Ca^{2+} im Oberboden korrelierten positiv mit den jährlichen Einträgen des jeweiligen Nährstoffes über die Blattstreu. Sie waren am höchsten in der Eschenblattstreu und korrelierten positiv mit dem Anteil der Eschenblattstreu am Gesamtblattstreufall. Außerdem hatte der Anteil der Eschenblattstreu am Gesamtstreufall einen positiven Einfluss auf den Boden-pH und die Vorräte an organischem C und Gesamt-N im Mineralboden, was vermutlich an der schnelleren Zersetzbarkeit im Vergleich zur Buchenblattstreu lag, die im Gegenzug zu höheren C-Vorräten in der Humusauflage führte.

(2) Mesokosmenstudie: Eschenblattstreu wird schneller abgebaut als Buchenblattstreu, was sich vor allem in einer schnelleren Mineralisation der Eschenblattstreu in den ersten 5 Monaten widerspiegelte (höhere streubürtige CO_2-Emissionen als bei Buchenblattstreu). Der Masseverlust der Streu korrelierte positiv mit dem Ca-Gehalt und negativ mit dem Lignin-Gehalt der Eingangsstreu.

Das Lignin:N-Verhältnis spielte keine Rolle, da beide markierten Streuarten hohe N-Konzentrationen aufwiesen und sich nur geringfügig unterschieden. Die Mineralisation der Eschenblattstreu wurde in Mischung mit Buchenblattstreu beschleunigt, weitere Mischungseffekte wurden nicht nachgewiesen. Unterschiede in der Verteilung von streubürtigem C und N im Boden und der mikrobiellen Biomasse zwischen den Varianten wurden nicht nachgewiesen. Insgesamt fanden sich 7-20% des streubürtigen C im O-Horizont und 1-5% in 0-4 cm des Mineralbodens wieder. Weniger als 1% des streubürtigen C wurde in den oberen 4 cm des Mineralbodens in die mikrobielle Biomasse eingebaut. Die Verlagerung des streubürtigen N in den O-Horizont (9-35%), den oberen Mineralboden (<8%) und die mikrobielle Biomasse (<1%) war vergleichbar mit der Verlagerung des streubürtigen C.

(3) Mikrokosmenstudie: Vergleichbar mit den Ergebnissen der Mesokosmenstudie war die Mineralisation (geschätzt über die streubürtige CO_2-Emission) der Eschenblattstreu höher (34% nach 206 Tagen) als die der Buchenblattstreu (24%) und wurde in Mischung mit letzterer zudem beschleunigt (39%). Ebenso wurde mehr C aus Eschenwurzeln (29%) als aus Buchenwurzeln (23%) mineralisiert. Die Höhe der Mineralisation korrelierte negativ mit dem Lignin:N-Verhältnis der Eingangsstreu und war der Hauptpfad des Streuabbaus. Die Freisetzung von DOC war vernachlässigbar und ging zudem mit der Versuchslaufzeit stark zurück, was auf eine Mineralisation, Ausfällung oder Assoziation an die Minerale schließen lässt. An die Minerale wurden 4-12% des streubürtigen C gebunden und es gab keinen Hinweis auf einen Art- oder Mischungseffekt. Die mikrobielle Biomasse baute weniger buchenstreubürtiges (0,2-0,4%) als eschenstreubürtiges C (0,7-1%) ein, wobei sie nicht zwischen Wurzeln und Blättern unterschied.

Zusammenfassend lässt sich feststellen, dass die Baumarten die Bodeneigenschaften kleinräumig beeinflussen können. Eine wichtige Steuergröße ist hierbei die Blattstreu. So hängen die Nährstoffvorräte im Oberboden linear mit der Nährstoffrückführung mit der Streu zusammen. Die Unterschiede im C-Vorrat des Oberbodens unter Buche und unter Esche konnten nicht auf eine unterschiedlich starke Umverteilung von Blatt- oder Wurzelstreu-C in den Mineralboden bzw. an die Minerale nach 10 Monaten Abbau zurück geführt werden. Das bedeutet, dass der positive Einfluss der Eschenblattstreu auf den C-Vorrat im Oberboden im Vergleich zur Buchenblattstreu ein langfristiger Effekt ist. Außerdem können Standortunterschiede, wie z. B. die Bodenazidität und die Zusammensetzung und Abundanz der Bodenfauna, ebenfalls unterschiedliche Ergebnisse hervorrufen. Letztendlich zeigt sich, dass eine unterschiedlich starke Beimischung von Esche in buchendominierten Beständen zu einer kleinräumigen Diversifikation des Lebensraum Boden führen kann.

Summary

Tree species influence soil chemical properties via the input of nutrients and protons through throughfall, stemflow, litterfall, and root respiration and/ or exudation. The effect of conifers versus hardwood trees on soil properties, such as beech (*Fagus sylvatica* L.), has often been investigated. More recent studies have focused on the influence of different broadleaved tree species on soil properties, and it was found that different broadleaved tree species may significantly influence soil properties, such as the C and N storage. However, most studies compared different mono-species stands or carried out common-garden experiments. Studies in an adult mixed forest are rare. Even fewer studies exist that compared the C and N partitioning during litter decomposition of different species. Species-specific or mixture related differences in the partitioning of C and N are therefore largely unknown. Identifying this gap in knowledge gave rise to the present work, which consists of the following three studies:

(1) The "cluster study" was conducted in a species-rich mixed forest stand of Hainich National Park. The soil type was a luvisol of loess over limestone. In a small area, three trees that were standing in a triangle to each other, so-called “clusters”, were selected. The clusters each consisted of one or two of the following tree species: beech, ash (*Fraxinus excelsior* L.) and lime (*Tilia cordata* Mill. or *Tilia platyphyllos* Scop.). The litterfall, the forest floor and topsoil (0-10 cm and 10-20 cm) were analyzed for their chemical composition.

(2) The "mesocosm study" was conducted in an acidified beech forest of Hainich National Park. The soil type was a luvisol of loess over limestone. The partitioning of C and N from $^{13}C/^{15}N$-labelled beech and ash leaf litter was compared in pure and mixed variants. For this purpose, soil columns were transferred into PVC cylinders and returned to the place of sampling. The original fresh litter (L-horizon) was replaced by the respective isotopically labeled litter type or litter mixture to be examined. Via closed chambers, the total and litter derived CO_2-respiration was measured biweekly over a period of twelve months. After five and ten months, the mass loss and the C- and N-loss of the original litter and the total and litter derived C and N contents in the O-horizon and mineral soil (0-4 cm) and in the microbial biomass (0-4 cm) were measured.

(3) The "microcosm study" was carried out at constant air temperature and soil moisture in a climatic chamber. Soil-litter mixtures were incubated for 206 days. The partitioning of litter C during decomposition of ^{13}C-labeled leaf or root litter of beech and ash was compared in pure and mixed variants. The CO_2-emission was recorded daily. At the beginning, the ^{13}C of CO_2 was measured every three days and later on every seven days. Total and litter derived contents of dissolved organic carbon (DOC) were analyzed on days 9, 29 and 206. Total and litter-

derived C contents in the microbial biomass as well as the light and heavy density fractions were investigated on day 206.

The key findings from the three studies are presented below:

(1) Cluster study: Tree species influenced the chemical soil properties in the topsoil (0-10 cm) on a small spatial scale, while in 10-20 cm depth the clay content was more important. In 0-10 cm soil depth, the base saturation, the pH and the stock of exchangeable Mg^{2+} were highest under pure ash (98%, 5.1, 135-137 kg ha^{-1}), and lowest under pure beech clusters (88%, 4.3, 70-76 kg ha^{-1}). The proportion of exchangeable Al^{3+} to the cation exchange capacity (CEC) was lowest under pure ash (0-0.6%) and highest under pure beech clusters (3.7-7.8%). The soil properties under lime clusters were intermediate. Mixture effects were not detected. An important factor influencing chemical soil properties was the composition of leaf litter. Stocks of exchangeable Mg^{2+} and Ca^{2+} in the topsoil correlated positively with the annual inputs of the respective nutrient with the leaf litterfall. Since these were highest in the ash leaf litter, the stocks of exchangeable Mg^{2+} and Ca^{2+} in the topsoil also positively correlated with the proportion of ash leaf litter to total leaf litterfall. Ash leaf litter also had a positive effect on soil pH and the stocks of organic C and total N in the mineral soil, which was probably due to more rapid decomposition of ash leaf litter than of beech leaf litter, which in turn led to higher C stocks in the humus layer.

(2) Mesocosm study: Mass loss of ash leaf litter was faster than of beech leaf litter, which is reflected primarily in a more rapid mineralization of the ash leaf litter during the first 5 months (higher litter derived CO_2-emissions compared to beech leaf litter). The mass loss of litter was positively correlated with the initial litter Ca-content and negatively with the initial litter lignin-content. The lignin:N ratio was not among the explaining variables, because both litter types contained high concentrations of N which differed only slightly. The mineralization of the ash leaf litter was accelerated in the mixture which contained beech leaf litter. No other mixture effects were detected. Differences in the distribution of litter derived C and N in the soil and the microbial biomass between the variants were not detected. In total, 7-20% of the litter derived C was found in the O-horizon and 1-5% was detected in the first 4 cm of mineral soil. Less than 1% of litter derived C was incorporated into the microbial biomass in the upper 4 cm of mineral soil. The partitioning of litter derived N to the O-horizon (9-35%), the upper mineral soil (<8%) and the microbial biomass (<1%) was comparable with the partitioning of litter derived C.

(3) Microcosm study: Similar to the results of the mesocosm study, the mineralization (estimated by the litter derived CO_2-emission) was higher for ash leaf litter (34% after 206 days) than beech leaf litter (24%). It was further accelerated when mixed with the latter (39%). Similarly, more C was mineralized from ash roots (29%) than from beech roots (23%). The amount of C mineralized was negatively correlated with the initial lignin:N ratio of the litter, and mineralization was the main path of litter decomposition. The release of DOC was negligible. Further, the DOC concentration was strongly declining with time, suggesting that most of it either mineralized, precipitated or associated to minerals. Four to twelve percent of litter derived C associated to minerals and there was no indication for a litter type or litter mixture effect. The microbial biomass incorporated less beech litter (0.2-0.4%) than ash litter derived C (0.7-1%), and did not differ between roots and leaves.

In summary, tree species can affect soil properties on a small spatial scale. An important control variable is the leaf litter. Thus, the nutrient stocks in the topsoil are linearly related to the return of nutrients via the litter. The differences in the topsoil C storage under beech and under ash could neither be related to different partitioning of leaf or root litter C into the mineral soil, nor to the minerals after 10 months of decomposition. This means that the positive influence of the ash leaf litter compared to the beech leaf litter on the C stocks in the topsoil is a long-term effect. In addition, differences in site properties, such as soil acidity and the composition and abundance of soil fauna, also cause different results. Finally, varying proportions of admixture of ash to beech dominated stands can cause a small-scale diversification of the soil habitat.

1 GENERAL INTRODUCTION

Thirty-two percent of Germany's land area is covered with forest (Eurostat 2008), with conifers making up more than half of it (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz 2005). However, the potential natural forest vegetation would be composed of beech (*Fagus sylvatica* L.) dominated (74%) and oak (*Quercus robur* L., *Quercus petraea* Liebl.) dominated forests (18%), while the coniferous forests would cover only about 3%. Therefore, forest policy makers set themselves the goal of increasing the proportion of deciduous trees and mixed forests, using natural forestry techniques to avoid hazards (such as a deterioration of the soil or susceptibility to pests) and to improve the ecological stability of forests (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz 2005). The second National Forest Inventory showed initial success (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz 2005): The proportion of deciduous and mixed forests has increased. Therefore, to understand how tree species and species mixtures affect soil properties is a central focus of current scientific research.

1.1 Soil acidification and nutrients

In humid climates soil acidification is a natural process. The soil acidity is made up by the sum of all solid and dissolved acids that are capable to release protons. The exchangeable H^+ and Al^{3+} ions contribute with the highest proportion to the total soil acidity. Sources of protons are (Blume et al. 2010):

- precipitation
- formation of carbonic acids by soil respiration
- release of organic acids by microbes and roots
- release of H^+- ions by plant roots to charge the neutrality during nutrient uptake
- oxidation of NH_4^+ to NO_3^- during nitrification
- oxidation of soluble Fe^{2+} and Mn^{2+}-Ions and Fe-sulfides

With increasing soil acidification, the cation exchange capacity (CEC) declines, due to pH-dependent loadings at the organic substances (Blume et al. 2010). Further, with increasing acidification (below pH 4.5), the proportion of Al^{3+} to the CEC rises, inducing a decline in the base saturation (proportion of Ca^{2+}, Mg^{2+}, Na^+ and K^+ to the CEC). However, Mg, Ca and K are essential nutrients for plants. Thus, an increasing acidification often results in a reduced growth and vitality of plants. The high concentration of Al in the solution of acidic soils, which acts in equilibrium with the exchangeable Al^{3+}, is toxic to plants and markedly inhibits the growth of roots. This in turn could lead to a phosphate deficiency and an increased susceptibility to drought stress (Blume et al. 2010).

Soil acidification is a major risk in many forest ecosystems, because the deposition of acids is a lot higher than the input of air pollutants onto open land (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz 2009). This is related to the high specific area of leaves, twigs and needles, which intercept water and dust in the first place. These may then reach the forest soil via canopy drip. The infiltration thus is higher compared to the open land, were water runoff and transpiration from the soil may be of higher importance.

Understanding the effect of factors, including tree species litter, on the soil pH, nutrient contents and dynamics is of major importance for forestry. At a given proton input, the speed of acidification depends on the soil`s ability to buffer acid inputs (Blume et al. 2010). All buffers function irreversibly and are accompanied by a leaching of nutrients. This means that if all the buffers are used up, the soil will become impoverished. Along with the well known effect that the parent material (buffering by carbonates), the clay content (buffering by surfaces with constant charge and buffering by silicate weathering) and the content of soil organic matter (SOM) as well as oxides and hydroxides (buffering by surfaces with variable charge) have in determining how fast a soil acidifies, in forests tree species may affect the soil pH by altering the proton charge of throughfall (Talkner et al. 2010) and stemflow (Koch and Matzner 1993). Further, the composition of leaf litter, e.g. the proportion of base cations, affects soil acidity (Augusto et al. 2002). It has been shown that the concentration of nutrients in the leaf litterfall was linearly correlated with the stock of the respective nutrients in the topsoil (Guckland et al. 2009). Thus, with an appropriate choice of tree species, the soil acidification can be slowed and the nutrient status of the soil can be improved, if the acidification is not too advanced.

1.2 Soil organic matter; C and N cycling

Forest soils are an important carbon sink (Goodale et al. 2002). Since tree species alter the SOM storage (Finzi et al. 1998a; Oostra et al. 2006; Vesterdal et al. 2008), the sink function of the forest soil may be increased by the appropriate choice of tree species. Above this, SOM is an important exchanger for cations and anions, with their negative and/or positive charge being pH-variable. Thus, a higher SOM storage also leads to better nutrient conditions for the plants.

Soil organic matter enters the soil via the decomposition of plant materials such as root or leaf litter (Figure 1.1; Schulze 2000).

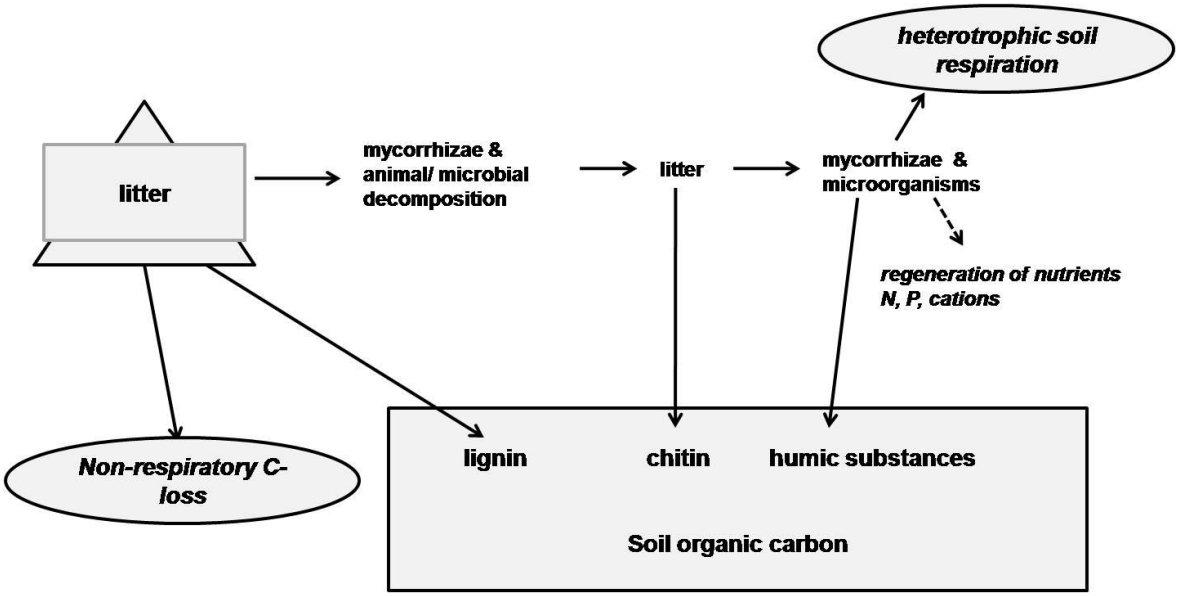


Figure 1.1 The below-ground carbon cycle during litter decomposition. Displayed are the main fluxes (modified after Schulze 2000).

Easily decomposable C compounds such as sugars and cellulose are incorporated by microorganisms or completely mineralized (Figure 1.1), with the end products being H_2O and CO_2. Litter C may enter the soil as (1) dissolved organic and inorganic C (Kalbitz et al. 2003), (2) with the soil fauna (Hättenschwiler et al. 2005; Scheu 1997) and (3) the microbial biomass (Hättenschwiler et al. 2005). Once in the soil, the C may undergo physical or chemical stabilization. The three major stabilization mechanisms referred to in the literature are

(1) selective preservation, i.e. biochemical stabilization due to the molecular structure of the organic matter,
(2) spatial inaccessibility, i.e. by occlusion in aggregates or micropores and
(3) interactions with surfaces and metal ions, i.e. organo-mineral associations or complexation (von Lützow et al. 2006).

Selective preservation may be divided into primary recalcitrance of e.g. lignin and secondary recalcitrance of microbial and faunal products (residues) (von Lützow et al. 2006). Thus, the quality and decomposability (e.g. different lignin contents) of tree species litter affects the C sequestration in the soil (Finzi et al. 1998a; Oostra et al. 2006).

Nitrogen enters the soil via the input of litter (Figure 1.2), deposition through anthropogenic inputs or rainfall, or via fixation or oxidation of N_2 (Schulze 2000).

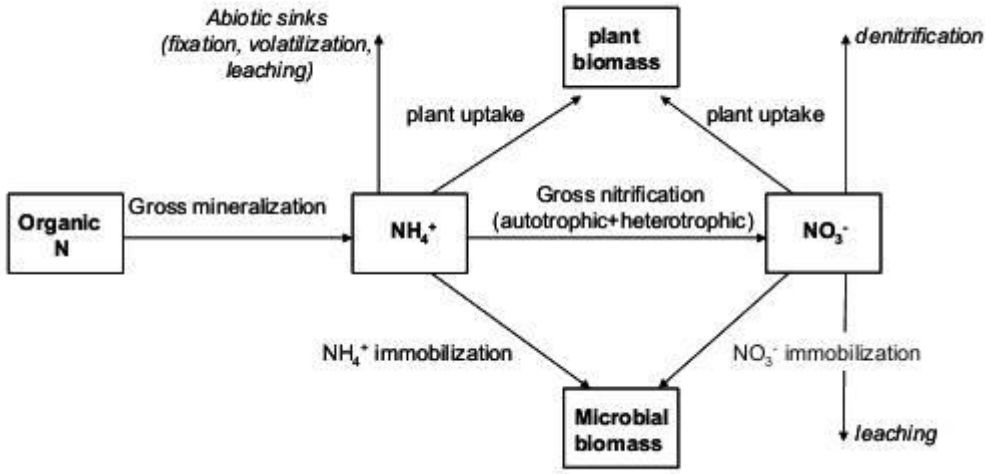


Figure 1.2 The N cycle in the forest soil. Processes in standard letters represent the internal N cycle; those written in Italics belong to the external N cycle (adapted from Corre et al. 2003 and Hart et al. 1994).

The litter-N is mineralized by the microbial biomass to NH_4^+ which may then take one of the following paths (Figure 1.2; Schulze 2000)

- absorbed and immobilized by microorganisms
- fixed in clay minerals
- absorbed by the plants
- further nitrified to nitrate (NO_3^-)

Nitrate is then (Figure1.2; Schulze 2000)

- absorbed by plants
- leached, or
- denitrified with the release of N_2O and N_2

In the Hainich National Park, the release of N_2O plays only a minor role (Guckland et al. 2010). Tree species affect the soil N storage, e.g. through the input of N with their leaf litter (Finzi et al. 1998a; Vesterdal et al. 2008).

1.3 State of knowledge and research gaps

Tree species affect soil chemical and biological properties through many factors, such as the rates and distribution of nutrient and water inputs, outputs and cycling (Binkley and Giardina 1998). The relative influence of conifers, as compared to hardwoods, on soil biochemical properties has often been analyzed (Augusto et al. 2003; Berger et al. 2009b;

Binkley and Valentine 1991) in most cases revealing that soil acidity was higher under conifers than under hardwood species. More recently, the research has focused on the effect of different broadleaved tree species on soil acidification and nutrient reservoir and the main findings are that pH, nutrient and SOM concentrations and base saturation are lower in the topsoil under mullmoder-forming species than under mull-forming tree species (Finzi et al. 1998a, b; Neirynck et al. 2000; Nordén 1994; Oostra et al. 2006; Vesterdal et al. 2008).

Data on the tree species effects on soil properties in mixed stands is scarce and there is a need to clarify the relationships between composition of mixed stands and nutritional properties on a small spatial scale (Rothe and Binkley 2001).

The factors (i.e. lignin content, lignin:N ratio or concentrations of base cations) regulating decomposition rate of leaf and/or root litter are quite well understood (Hobbie et al. 2007, 2010; Melillo et al. 1982). However, to the best of our knowledge, studies that compare the partitioning of litter C between several tree species are scarce (Don and Kalbitz 2005; Fahey et al. 2011; Kalbitz et al. 2006; Trum et al. 2011) and even lacking for N.

In mixtures, decomposition may not be additive (Gartner and Cardon 2004; Wardle et al. 1997). However, to the best of our knowledge studies of temperate tree litter mixtures on the partitioning of litter C and N are missing.

1.4 Use of stable isotopes for tracing litter-derived C and N

Chemical elements can have several isotopes. The isotopes of an element consist of the same number of protons and electrons, but differ in the number of neutrons, which is why they have different atomic weights (Sulzman 2007). Isotopes can be either stable or radioactive. In the current work, the stable isotopes ^{12}C, ^{13}C, ^{14}N and ^{15}N have been used for the study of C and N partitioning in the soil. Naturally, the ^{12}C (98.892%) and ^{14}N (99.635%) isotopes dominate strongly over ^{13}C (1.108%) and ^{15}N (0.365) (Sulzman 2007). The ratio of $^{13}C/^{12}C$ and of $^{15}N/^{14}N$ is specified in relation to an international standard (Dawson et al. 2002):

Equation 1.1: $\partial‰ = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$

with R being defined by equation 1.2:

Equation 1.2: $R = \frac{Number\ of\ heavy\ atoms}{Number\ of\ light\ atoms}$

International standards are used as reference values. Working standards were calibrated against the international standards and are used for daily measurement (Sulzman 2007). The defined international standard for ^{13}C was the Belemnite of the Pee Dee Formation in South Carolina, USA (PDB), but this is no longer available. A new reference standard, Vienna-PDB (V-PDB), has been defined by its relationship to NBS19 (Sulzman 2007). The primary standard for ^{15}N is the atmospheric N (Sulzman 2007) because it has an extremely stable isotope ratio.

In studies with enriched material, often atom% instead of δ‰ is used to define the amount of the heavier isotope (Dawson et al. 2002):

Equation 1.3: $atom\% = \frac{number\ of\ heavy\ atoms}{number\ of\ heavy\ AND\ light\ atoms} \times 100 = 100 \times \frac{R_{sample}}{R_{sample}+1}$

The use of a pool that is significantly enriched or depleted relative to another pool makes it possible to track the flows from one pool to another pool (Dawson et al. 2002). As an example, leaf litter was used in this study, which was artificially enriched with the heavier isotopes ^{13}C and ^{15}N compared to the natural abundance. Thus, this litter had a different isotopic signature than the forest soil. When the litter (pool 1) was decomposed, C and N was partitioned to the soil (pool 2), and the isotope signatures in the soil changed. Thus, it could be calculated how much C and N was transported from the litter (pool 1) into the soil (pool 2). The use of stable isotopes enables a very precise quantification of fluxes between pools.

2 OBJECTIVES AND WORKING HYPOTHESES

This study was conducted as part of the DFG (Deutsche Forschungsgesellschaft) Research Training Group 1086 "The role of biodiversity for biogeochemical cycles and biotic interactions in temperate deciduous forests". By now, 26 PhD-students in two phases examined the influence of tree species diversity on ecosystem functions in a species rich temperate deciduous forest, the Hainich National Park, Thuringia, Germany. My work was related to the influence of litter quality and litter mixture on the chemical soil properties and the dynamics of C and N in the soil. The soil under study was a luvisol developed from loess over limestone.

The objectives of this project were:

(1) To identify species and species mixture related effects on the topsoil acidity, nutrient status and soil organic matter (SOM) content. For this, triangles of three neighboring trees that consisted of either one or two species of European beech (*Fagus sylvatica* L.), European ash (*Fraxinus excelsior*) or lime (*Tilia cordata* Mill. or *Tilia platyphyllos* Scop.) were selected in Hainich National Park and analyzed for their litterfall chemistry. Furthermore, soil physical properties (clay content) and chemical properties of the forest floor and mineral soil (0-10 cm and 10-20 cm), e.g. SOM, nutrient stocks and pH, were investigated. This study is referred to as the “Cluster study”.

(2) To understand how litter type and litter mixture influence the partitioning of litter C and N to the soil via differences in their initial chemistry. One field and one laboratory incubation experiment were established. The field incubation experiment was conducted in a mature beech stand of Hainich National Park, Thuringia, Germany. Soil cores were transferred to plastic cylinders and the original litter was replaced by ^{13}C- and ^{15}N-labeled beech or ash leaf litter, by the respective unlabeled litter as a reference, or by a mixture of beech and ash leaf litter. Emissions of litter derived CO_2-C were measured biweekly and partitioning of litter C and N to the topsoil and microbial biomass was measured five and ten months after the start of the experiment. This study is referred to as the “Mesocosm study”. In the laboratory incubation experiment (litter-soil mixture), the partitioning of litter C during decomposition to CO_2, dissolved organic C, microbial biomass and to light and heavy density fractions was examined. Mesofauna and macrofauna were excluded from the soil. Decomposition of ^{13}C-labeled root and leaf litter of ash and beech was compared. This study is referred to as the “Microcosm study”.

The following hypotheses were tested in the three studies:

(1) Topsoil chemical properties under different tree species (i.e. beech, ash, lime) vary on a small spatial scale and these differences are induced by the chemical composition of the above-ground litterfall. → Cluster study

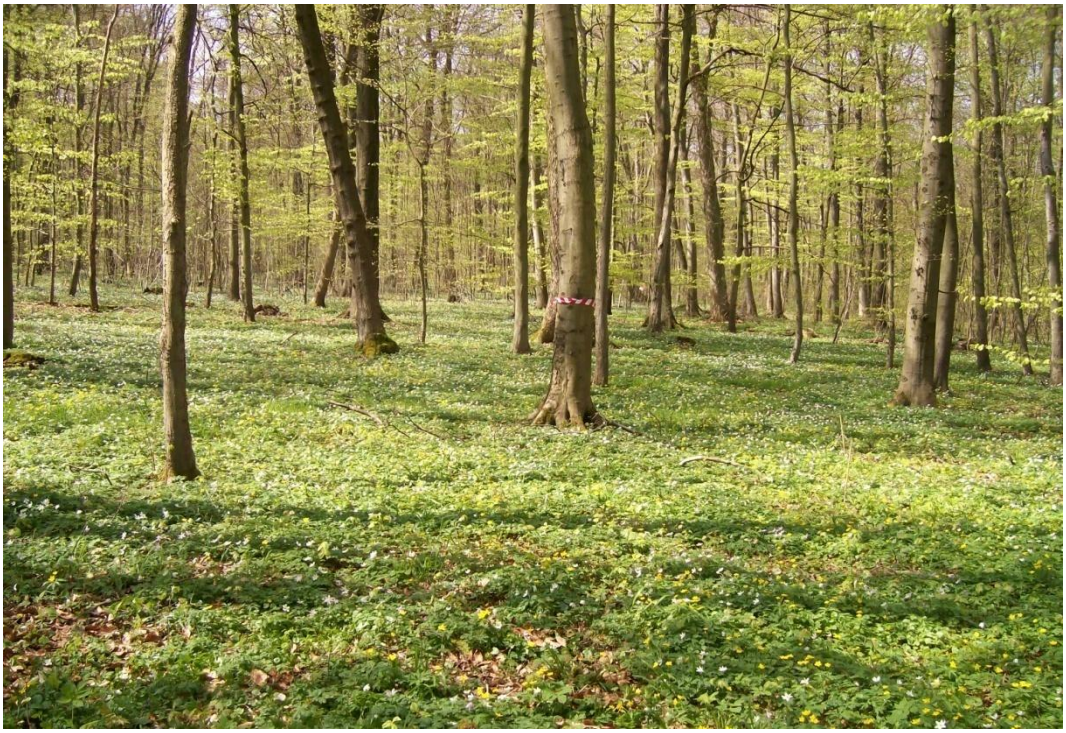
(2) The decomposition of ash leaf litter is faster than of beech leaf litter and the faster decomposition is associated with a greater partitioning of litter C and N to the mineral soil and the soil microbial biomass. →Mesocosm and microcosm study

(3) The decomposition of root litter is slower than of leaf litter, because of a higher content of lignin in roots than in leaves. → Microcosm study

(4) The mixture of beech and ash litter affects the partitioning of C and N from the respective litter type, but the partitioning of litter C and N of the litter mixture are additive. →Mesocosm and microcosm study.

3 EFFECTS OF BEECH (*FAGUS SYLVATICA*), ASH (*FRAXINUS EXCELSIOR*) AND LIME (*TILIA SPEC.)* ON SOIL CHEMICAL PROPERTIES IN A MIXED DECIDUOUS FOREST[1]

Langenbruch C, Helfrich M, Flessa H



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3.1 Abstract

We aimed to determine the influence of the distribution of different broadleaved tree species on soil chemical properties in a mature deciduous forest in Central Germany. Triangles of three neighboring trees (tree clusters) that consisted of either one or two species of European beech (*Fagus sylvatica* L.), European ash (*Fraxinus excelsior* L.) or lime (*Tilia cordata* Mill. or *Tilia platyphyllos* Scop.) were selected and analyzed for their litterfall chemistry and chemical properties of the forest floor and mineral soil (0-10 cm and 10-20 cm). Base saturation, pH-value and the stock of exchangeable Mg^{2+} (0-10 cm) were highest under ash and lowest under beech. The proportion of exchangeable Al^{3+} was smallest under ash and highest under beech. The stock of exchangeable Mg^{2+} and Ca^{2+} correlated positively with the annual input of the respective nutrient from leaf litterfall. Ash leaf litterfall contained highest amounts of Mg and Ca. Beech leaf litterfall showed the highest C:N ratio and lignin:N ratio. Soil pH, stocks of organic C, total N and exchangeable Mg^{2+} and Ca^{2+} correlated positively with increasing proportions of ash leaf litter to total leaf litterfall. Our results indicate that the abundance of ash in beech dominated forests on loess over limestone had a positive effect on soil chemical properties and reduced soil acidification. The intermixture and distribution of ash in beech-dominated stands resulted in an increase of the horizontal and vertical diversity of the soil habitat.

3.2 Introduction

Tree species affect soils through many factors, such as the rates and distribution of nutrient and water inputs, outputs and cycling (Binkley and Giardina 1998). While the relative influence of conifers, as compared to hardwoods, on soil biochemical properties has often been analyzed (Augusto et al. 2002; Berger et al. 2009a,b; Mareschal et al. 2010), research on soil chemical variations under different broadleaved species is a younger and less advanced field. The main findings of studies analyzing soil properties under broadleaved tree species are that pH and base saturation are lower in the topsoil under mullmoder-forming species (including beech) compared to mull-forming tree species (including ash and lime; Neirynck et al. 2000; Nordén 1994; Oostra et al. 2006). Further, Oostra et al. (2006) and Vesterdal et al. (2008) found out that concentrations and stocks of organic carbon (C_{org}) and total nitrogen (N_t) in the forest floor were higher for beech than for ash and lime. (The latter was only analyzed by Vesterdal et al. 2008.) In the mineral soil it was vice versa.

Tree species influence soil chemical properties through differences in the quantity and chemistry of their leaf litterfall (Guckland et al. 2009; Reich et al. 2005;

Vesterdal et al. 2008). In their review, Augusto et al. (2002) ranked tree species in the order of decreasing acidifying ability: conifers ≥ beech, oak and birch ≥ Norway maple, hornbeam, ash and lime. They described several ways by which species can acidify soils, including litter composition, deposition and root exudates. Data on the effects in mixed stands is especially scarce, and there is a need to clarify the relationships between composition of mixed stands and nutritional properties on a small spatial scale (for a review, see Rothe and Binkley 2001).

Recently, Guckland et al. (2009) conducted a field study in a highly diverse broadleaved forest in Hainich National Park in central Germany using a plot design where different diverse 50x50 m stands were compared. They discovered significant differences in various soil properties between pure beech stands and mixed stands of mainly three (European beech (*Fagus sylvatica* L.), European ash (*Fraxinus excelsior* L.) and lime (*Tilia cordata* Mill*. & Tilia platyphyllos* Scop.)) or six tree species (in addition hornbeam (*Carpinus betulus* L.), Sycamore maple (*Acer pseudoplatanus* L.) and Norway maple (*Acer platanoides* L.)). Soil pH, base saturation and cation exchange capacity were found to rise with increasing species diversity and decreasing beech abundance. However, the clay content tended to be lower in beech stands, which could also have been an important factor influencing the above mentioned soil properties. Therefore, Guckland et al. (2009) could not fully differentiate between a possible effect of tree species mixture, a beech gradient effect or a clay content effect.

In this paper, we present the results of a study design, where the effects of European beech (*Fagus sylvatica* L.), European ash (*Fraxinus excelsior* L.) and Lime (*Tilia platyphyllos* Mill*., Tilia cordata* Scop.) on soil chemical properties and nutrient turnover were analyzed at two subsites of differing loess cover in Hainich National Park, the site where Guckland et al. (2009) conducted their research. It was designed as a follow-up to the study of Guckland et al. (2009) and aimed to answer the question they raised concerning whether they detected a beech gradient effect, and to detach clay content effects from tree species effects. In a small area of approximately 90x90 m and 250x120 m, respectively, tree triangles (“clusters”) of three beeches, limes or ashes as well as mixed clusters of two of these species were chosen. With this approach we aimed to detect possible effects of tree species and tree species mixtures on forest soil chemistry, in a fully developed forest with a high diversity in broadleaved tree species. Due to the small scale approach, variations in the clay content, the loess cover or those induced by land use history were reduced to a minimum. We hypothesized that there were differences in soil properties underneath the different cluster variants that were induced by the quality of the leaf litterfall of the cluster trees.

3.3 Material and methods

3.3.1 Study site

The study was conducted in Hainich National Park, which is located in central Germany in Thuringia. With up to 14 tree species per hectare, Hainich National Park belongs to one of the largest and most diverse broadleaved forests in Central Europe. The forest has existed for over 200 years and contains mature trees aged 100 to 200 years. In 1997, this area became National Park (Mölder et al. 2006).

Two subsites were chosen for analyses. They were located at the southeast of Hainich National Park close to a meteorological station (Meteomedia, station Weberstedt/Hainich; 51°06´N, 10°31´E) near the Thiemsburg. The mean annual temperature is 7.5°C, and the mean annual precipitation is 670 mm. The mean elevation of the sites is 350 m a.s.l. The forest grows on a Luvisol developed from loess underlain by Triassic Limestone. At some places, the profile showed stagnic properties. For a detailed site description, see Mölder et al. (2006).

The two subsites differed in the thickness of loess cover. Subsite 1 ("TB 60") had a mean loess cover of 60 cm, ranging from 48 to 77 cm (Table 3.1). The clay content (0-20 cm) averaged 25%. Subsite 2 ("TB 100") had a mean loess cover of 100 cm, ranging from 70 cm to more than 100 cm (Table 3.1). The clay content (0-20 cm) averaged 18%. Tree species under investigation appeared to grow in a random mixture with each other and there was no large grouping of ash and lime within TB 60 and TB 100. The size of TB 60 was approximately 250x120 m and that of TB 100 approximately 90x90 m. The distance between the centers of both subsites was around 565 m.

3.3.2 Selection of tree cluster areas

The impact of three tree species, i.e. European beech (*Fagus sylvatica* L.), European ash (*Fraxinus excelsior* L.) and lime (*Tilia cordata* Mill. or *Tilia platyphyllos* Scop.), and their mixtures on soil chemical properties was analyzed. These species were chosen because they are the most dominant tree species in Hainich National Park. Furthermore, results from former studies suggest that these three species differ in their effects on soil acidification and nutrient availability (e.g. Neirynck et al. 2000; Nordén 1994; Oostra et al. 2006).

At both subsites TB 60 and TB 100, tree clusters, defined as three trees that were standing in a triangle to each other, were chosen for investigation (Figure 1.2). The trees had a mean distance from the cluster centre of 3.5 m, ranging from 2 to 5.5 m. All cluster

trees were mature, having a mean diameter at breast height (DBH) of 31 cm (ash), 39 cm (beech) and 32 cm (lime). The three trees of one cluster had a similar DBH. The canopy in the forest stand was closed. At each site, three or more replicates of the following six cluster variants were selected: (1) pure beech, (2) pure ash, (3) pure lime (except at TB 100, because there were not enough pure lime clusters) or mixture of (4) beech and ash, (5) beech and lime and (6) lime and ash (Table 3.1). The number of beech clusters was higher because beech was the most abundant tree species at both sites and it was difficult to find adequate clusters containing ash and/or lime.

Table 3.1 General soil physical properties and number of replicates of the cluster variants at the two study sites (TB 60 and TB 100); mean with standard deviation in brackets.

Study Site		Cluster Variants	Thickness of loess cover [cm] (min-max)	Soil texture [%] sand/silt/clay		Bulk density [g cm^{-3}]		Number of replicates
				0-10 cm	10-20 cm	0-10 cm	10-20 cm	
TB 60	(1)	Beech	58-73	2/75/23 (0/4/4)	2/76/22 (0/4/4)	1.1 (0.1)	1.4 (0.1)	9
	(2)	Ash	53-54	2/68/30 (0/4/4)	2/67/31 (0/4/4)	1.0 (0.1)	1.4 (0.1)	3
	(3)	Lime	50-65	2/75/23 (1/2/1)	2/76/22 (0/3/2)	1.1 (0.1)	1.4 (0.2)	3
	(4)	Beech-Ash	55-60	2/75/23 (0/2/2)	2/74/24 (0/1/1)	1.2 (0.1)	1.5 (0.1)	3
	(5)	Beech-Lime	53-77	2/71/27 (0/6/6)	2/71/27 (0/5/5)	1.1 (0.1)	1.4 (0.0)	4
	(6)	Ash-Lime	48-65	2/71/27 (0/6/6)	2/71/27 (0/7/6)	1.1 (0.1)	1.4 (0.1)	4
TB 100	(1)	Beech	70-98	2/79/19 (0/2/2)	2/80/18 (0/2/2)	1.0 (0.0)	1.4 (0.1)	5
	(2)	Ash	80-96	2/79/19 (0/2/3)	2/80/19 (0/3/3)	1.0 (0.1)	1.3 (0.1)	4
	(4)	Beech-Ash	79-98	2/79/19 (0/3/2)	2/80/18 (0/2/2)	1.1 (0.1)	1.3 (0.1)	4
	(5)	Beech-Lime	70-97	2/80/18 (1/1/1)	2/80/18 (1/0/0)	1.1 (0.1)	1.4 (0.1)	4
	(6)	Ash-Lime	80- >100	2/82/16 (0/2/2)	2/82/16 (0/2/2)	1.1 (0.1)	1.4 (0.1)	4

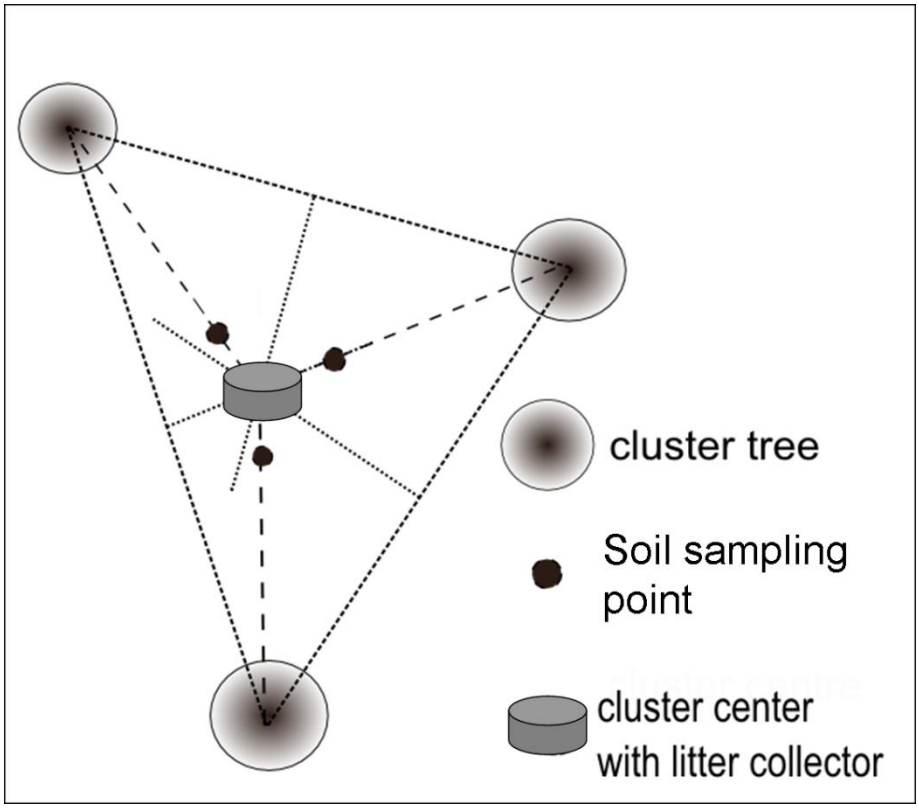

Figure 3.1 Sampling design of soil and litter within a cluster area. Litter was collected from the circumcenter of the cluster while soil samples were taken approximately 50 cm from the circumcenter towards each cluster tree.

3.3.3 Litterfall sampling and preparation for analyses

Litter collectors with a diameter of 64 cm were installed at the center of each cluster between the soil sampling places (Figure 3.1). The litterfall was sampled at four dates (Oct 1st 2008, Oct 23rd 2008, Nov 12th 2008 and March 10th 2009). It was found in former studies that the litterfall was negligible during spring and summer (Jacob, personal communication). The litter samples were separated into fruit and leaves, and these were further divided into the cluster tree species and other dominant species. After separation, the samples were dried at 70°C for four days. The total litterfall (Mg ha^{-1}) in 2008 was calculated for each cluster from the sum of the dry weight of all leaves and fruit from all four sampling dates.

The samples from each date from one cluster were put together as one pooled sample, however still separated into fruit, leaf and species. These samples were ground in a mixer mill (RETSCH MM2, Haan, Germany). Fruit were shredded before grinding (FRITSCH pulverisette Type 15.302, Idar-Oberstein, Germany).

3.3.4 Forest floor sampling and preparation for analyses

According to the morpho-functional classification of humus forms by Zanella et al. (2011), the forest floor was classified as mesomull (OL) under pure ash and pure lime clusters and as dysmull (OL + OF) under pure beech clusters. In mixed clusters the forest floor was either a mesomull or an oligomull (OL + discontinuous OF). The forest floor was sampled from the center of each cluster in June 2008 (Figure 3.1) before the litter collectors were positioned. For collection, an iron cylinder with a diameter of 27.85 cm was placed onto the soil surface. The forest floor in this cylinder was then collected by hand. The samples were dried at 60°C until the weight remained constant. The dry samples were shredded and then ground to fine material in a mixer mill (RETSCH MM2, Haan, Germany).

3.3.5 Soil sampling and preparation for analyses

In May 2008, three soil samples (diameter of 6.4 cm; height of 20 cm) were taken at a distance of 50 cm from the center of each cluster area as shown in Figure 3.1.

Soil cores were cut into the depth increments of 0-10 cm and 10-20 cm. Big roots were removed before weighing the fresh soil material. The soil was then dried at 40°C, passed through a 2 mm sieve, and a sub-sample was ground in a planetary ball mill (RETSCH PM 4000, Haan, Germany).

3.3.6 Laboratory analyses

The following physical parameters of soil were determined: bulk density, gravimetric moisture (mass%) at sampling date, and particle size distribution. Soil bulk density was calculated from the mass of dry soil and the volume of the soil core collected in field. We proved the reliability of this approach by comparing results to those produced by the standard method of determining soil bulk density (taking undisturbed soil cores from a soil-profile pit). No difference was found between the results of the two methods. Particle size distribution was determined using the sieving and pipette method (Schlichting et al. 1995).

The pH of the sieved mineral soil was measured in 1 *M* KCl-solution (10 g soil and 25 ml KCl-solution). Exchangeable cations were extracted from sieved soil by 1 *M* NH_4Cl-solution (König and Fortmann 1996) and then measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Kleve, Germany).

Effective cation exchange capacity (CEC) was calculated as the sum of exchangeable cations. Base saturation (BS%) is the proportion of the sum of base cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) to CEC in percent.

Total cation contents were analyzed after pressure digestion with concentrated nitric acid (Heinrichs et al. 1986) in litterfall and forest floor samples. The solution was measured with the ICP-OES.

C_{org} and N_t were measured in ground material from the mineral soil, forest floor and litterfall by an automated C and N analyzer (Heraeus Elementar Vario EL, Hanau, Germany). All samples were free of carbonates.

Lignin content of leaf litter samples from mono species clusters at TB 60 was determined using the acetylbromide method (Brinkmann et al. 2002 modified after Morrison 1972). Prior to the admixture of acetylbromide, the grounded samples were extracted using the procedure of Brinkmann et al. (2002).

3.3.7 Statistical analyses

Statistica 8.0 (StatSoft, Inc., 2008) was used for statistical analyses. A two-factorial analysis of covariance with “site” and “cluster variant” (excluding lime clusters) as factors and clay content as co-variable was used to detect significant differences between variants. To detect the influence of lime clusters, a simple analysis of covariance was additionally done. The Scheffé-Test was used for post-hoc comparisons in cases of significance ($p<0.05$). When the residuals were not at least approximately normally distributed and/or the variances were not homogenous and correlated positively with the mean, a Box-Cox-transformation of the data was conducted in order to meet the above mentioned requirements. If no reasonable transformation was found, then the non-parametric Kruskal-Wallis-Anova followed by multiple comparisons (post-hoc) was used. (This test was used for base saturation and concentrations of Ca, Mg and Mn in leaf litter type).

Pearson Correlations were conducted to analyze the relationship between litter nutrients (Ca, Mg and Mn) and soil properties. Linear multiple regression analyses were used to detect variables influencing soil chemical properties. Four theoretically possible variables were examined: (1) the proportion of beech, (2) ash or (3) lime leaf litter to total leaf litterfall and (4) the clay content.

3.4 Results

3.4.1 Clay content of the mineral soil of the clusters

The clay content was higher at TB 60 (22% to 31%) than at TB 100 (16% to 19%; Table 3.1). The clay content did not differ between 0-10 cm and 10-20 cm soil depths. We found no relationship between the clay content and the cluster variants. Only at TB 60 in 10-20 cm, the clay content in beech clusters tended to be lower than in ash clusters ($p<0.1$).

3.4.2 Production and composition of leaf litter

In 2008, 3.6 to 5.3 Mg ha^{-1} of litterfall was produced at our study site. The production of litterfall did not differ between the two subsites or between variants. Nevertheless, litterfall tended to be lower in pure beech clusters than in ash-lime clusters ($p<0.1$).

The nutrient content of the leaf litterfall (calculated means over all clusters) in 2008 differed significantly between the species (Table 3.2). The Ca and Mg contents were lowest in beech leaf litter and the Mg content was highest in ash leaf litter. Beech leaf litter showed lowest N concentrations and the highest C:N ratio and lignin:N ratio among the investigated species. Ash leaf litter showed the lowest Mn concentration among the investigated species. The composition of beech, ash and lime leaf litter was not influenced by the cluster species.

The variants differed in their composition of leaf litterfall in 2008. Beech leaf litter was present in all variants with 7.9% to 99.5% mass (dry matter). However, its contribution to total leaf litterfall decreased in the order pure beech clusters > mixed clusters with beech present > clusters without beech present. Ash and lime leaf litter did not exceed 63.9% (ash) and 44.4% (lime) in the respective single species clusters. In pure beech clusters almost no ash and lime leaf litter was present. The proportion of a leaf litter type to total leaf litterfall ($LL_{(tree\ species)}/LL_{total}$) did not correlate with the clay content, except for the proportion of ash leaf litter to total leaf litterfall (LL_{ash}/LL_{total}) at TB 60 in 10-20 cm depth ($R^2=0.23$, $p<0.05$).

The C:N ratio in mixed litterfall collected varied significantly between pure ash clusters (34.4) and clusters with beech present (43.7-45.4; Table 3.2). Further, it varied significantly between ash-lime clusters (36.9) and pure beech clusters (45.4). The N content in litterfall of ash clusters was higher than of all clusters containing beech. Furthermore, it was higher in ash-lime than in beech clusters.

Table 3.2 Nutrient contents, C:N ratio and lignin:N ratio of leaf litterfall in 2008 of different species (upper part; means from all clusters) and of mixed litterfall (leaves and fruits) of different cluster variants (lower part; mixed calculation of the contents in species litter with the proportion of the species litter to total litterfall). Mean with standard deviation in brackets.

Different lower case letters indicate significant differences between the variants at a significance level of $p < 0.05$ (Scheffe Test for C, N and C:N; Kruskal-Wallis-Anova for Ca, Mg and Mn). There were no differences between the two subsites (TB 60, TB 100) and the mean includes results from both subsites. Lignin:N ratios are results from mono species clusters at TB 60.

Variant	Ca	Mg	Mn	C	N	C:N	Lignin:N
			[mg g^{-1}]				
Leaf litter type							
Beech	16.2^{a} (1.4)	1.5^{a} (0.2)	0.6^{b} (0.2)	493.2^{c} (6.3)	9.9^{a} (0.9)	50.1^{b} (4.7)	7.5^{b} (0.8)
Ash	24.1^{b} (3.3)	2.7^{c} (0.6)	0.1^{a} (0.03)	471.8^{a} (8.3)	14.8^{b} (2.4)	32.6^{a} (4.8)	4.0^{a} (0.8)
Lime	22.5^{b} (2.9)	2.0^{b} (0.4)	0.5^{b} (0.1)	486.7^{b} (7.9)	15.3^{b} (1.6)	32.2^{a} (3.2)	4.4^{a} (0.6)
Mixed litterfall of cluster variants							
Beech	16.6^{a} (1.8)	1.50^{a} (0.19)	0.61^{b} (0.19)	493.3^{a} (6.6)	11.0^{a} (1.3)	45.4^{c} (6.0)	NA
Ash	20.0^{b} (1.0)	2.23^{c} (0.29)	0.28^{a} (0.08)	483.4^{a} (3.9)	14.2^{c} (1.6)	34.4^{a} (3.6)	NA
Lime	19.3^{ab} (1.6)	1.63^{ab} (0.06)	0.55^{ab} (0.11)	491.7^{a} (4.9)	13.6^{abc} (0.6)	36.3^{abc} (2.0)	NA
Beech-Ash	19.3^{ab} (1.8)	2.00^{bc} (0.40)	0.41^{ab} (0.23)	486.2^{a} (8.8)	11.2^{ab} (0.8)	43.7^{bc} (2.9)	NA
Beech-Lime	18.1^{ab} (1.7)	1.69^{ab} (0.29)	0.47^{ab} (0.10)	488.8^{a} (8.6)	11.2^{ab} (1.1)	43.9^{bc} (4.0)	NA
Ash-Lime	20.0^{b} (2.4)	2.00^{bc} (0.32)	0.44^{ab} (0.16)	484.5^{a} (5.0)	13.2^{bc} (1.2)	36.9^{ab} (3.3)	NA

The concentration of Ca and Mg in mixed litterfall was lowest in beech clusters (16.6 and 1.5 mg g^{-1}, respectively; Table 3.2) and highest in clusters containing ash (19.3-20.0 and 2.0-2.2 mg g^{-1}, respectively). The concentration of Mn in litterfall was lowest in ash clusters (0.3 mg g^{-1}) and highest in beech clusters (0.6 mg g^{-1}). It was negatively correlated with the pH of the topsoil (0-10 cm) at both subsites (Figure 3.2).

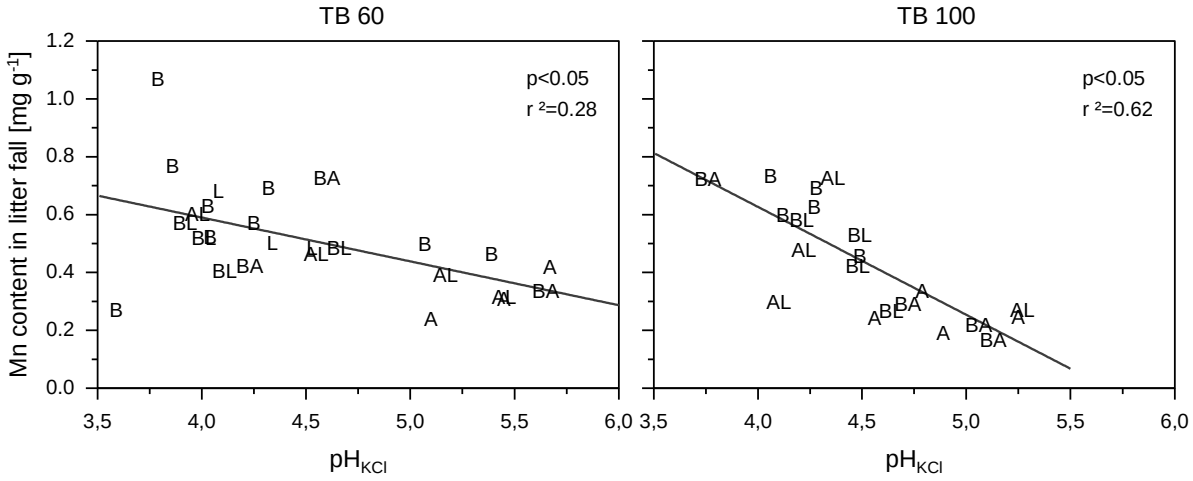

Figure 3.2 Relationship between topsoil pH (0-10 cm) and Mn content in litter fall. Pearson correlations are displayed by a linear slope. The cluster variants: A: Ash, B: Beech, L: Lime, BA: Beech-Ash, BL: Beech-Lime, AL: Ash-Lime

3.4.3 Characterization of the forest floor

The forest floor dry mass (median) in June varied between 0.5 Mg ha^{-1} under pure ash clusters and 2.2 Mg ha^{-1} under beech and beech-lime clusters (Table 3.3). There was a tendency towards lower forest floor masses in pure ash clusters than in beech and beech-lime clusters ($p<0.1$).

Table 3.3 Stock of C_{org} and N_t and C:N ratio of the forest floor and the mineral soil at the different cluster variants; mean with standard deviation in brackets; forest floor dry matter: median with minimum and maximum values in brackets. Different letters indicate significant differences between the variants at a significance level of $p < 0.05$ (Scheffe Test). If not separately displayed, then no differences between the two subsites (TB 60, TB 100) existed and the mean includes results from both subsites.

Variant	forest floor dry matter [Mg ha^{-1}]		C_{org} [Mg ha^{-1}]			N_t [Mg ha^{-1}]			C:N		
	TB 60	TB 100	forest floor	0-10 cm	10-20 cm	forest floor	0-10 cm	10-20 cm	forest floor	0-10 cm	10-20 cm
Beech	1.3^{a} (1.0-1.8)	2.2^{a} (2.0-3.3)	0.42^{c} (0.09)	30.7^{a} (3.2)	21.3^{a} (3.7)	0.016^{b} (0.004)	2.2^{a} (0.2)	1.7^{a} (0.3)	26.4^{a} (4.4)	13.9^{a} (0.7)	12.2^{a} (0.6)
Ash	0.5^{a} (0.4-0.6)	0.5^{a} (0.4-1.4)	0.16^{a} (0.06)	37.1^{b} (6.3)	23.6^{a} (4.0)	0.007^{a} (0.004)	2.7^{b} (0.4)	2.0^{a} (0.3)	24.1^{a} (4.4)	13.5^{a} (0.4)	11.8^{a} (0.4)
Lime	1.3^{a} (0.8-1.6)	NA	0.20^{ab} (0.05)	27.1^{a} (2.0)	18.9^{a} (3.5)	0.009^{ab} (0.001)	2.1^{a} (0.3)	1.6^{a} (0.3)	21.7^{a} (2.1)	13.1^{a} (1.1)	11.8^{a} (1.3)
Beech-Ash	0.6^{a} (0.4-0.7)	1.5^{a} (1.1-2.2)	0.24^{ab} (0.07)	32.5^{ab} (4.4)	21.3^{a} (3.8)	0.008^{a} (0.002)	2.4^{ab} (0.3)	1.7^{a} (0.3)	31.1^{a} (5.7)	13.5^{a} (0.6)	12.2^{a} (1.0)
Beech-Lime	1.4^{a} (1.1-1.5)	2.2^{a} (1.6-3.0)	0.39^{bc} (0.11)	32.3^{ab} (7.3)	21.7^{a} (3.4)	0.015^{b} (0.004)	2.3^{a} (0.4)	1.8^{a} (0.3)	26.0^{a} (4.1)	13.9^{a} (1.1)	12.0^{a} (0.4)
Ash-Lime	0.6^{a} (0.3-1.4)	0.8^{a} (0.6-1.4)	0.21^{a} (0.08)	32.7^{ab} (6.7)	19.9^{a} (3.9)	0.008^{a} (0.004)	2.4^{ab} (0.6)	1.7^{a} (0.4)	28.3^{a} (5.7)	13.8^{a} (0.6)	12.1^{a} (0.7)

Stocks of C_{org} were higher in pure beech clusters (0.42 Mg ha^{-1}) than in all other variants except beech-lime clusters (0.39 Mg ha^{-1}). The latter differed significantly from pure ash (0.16 Mg ha^{-1}) and ash-lime clusters (0.21 Mg ha^{-1}; Table 3.3). The stock of N_t in the forest floor was significantly lower in all clusters with ash present than in pure beech and beech-lime clusters and varied from 7.0 kg ha^{-1} in pure ash clusters to 16.2 kg ha^{-1} in pure beech clusters. The C:N ratio of the forest floor did not differ between the two subsites or between variants (Table 3.3).

3.4.4 Soil organic carbon and total nitrogen content in the mineral soil

In 0-10 cm soil depth, the stock of C_{org} was significantly higher under pure ash clusters (37.1 Mg ha^{-1}; Table 3.3) than under pure beech (30.7 Mg ha^{-1}) and pure lime clusters (27.1 Mg ha^{-1}). The stock of N_t in the topsoil was significantly higher under pure ash clusters (2.7 Mg ha^{-1}) than under all variants without ash (2.1-2.3 Mg ha^{-1}). The stocks of C_{org} and N_t did not differ between the variants in 10-20 cm soil depth. The C:N ratio in the mineral soil was similar in all variants (Table 3.3).

Multiple regression analyses revealed that at TB 60, the stocks of C_{org} and N_t were strongly correlated with the clay content in both depths. In addition, in 0-10 cm LL_{ash}/LL_{total} contributed to the variability of C_{org} (multiple R^2=0.59) and N_t stocks (multiple R^2=0.72). At TB 100, where the variability of the clay content was low, LL_{ash}/LL_{total} was the most important variable explaining the variability of N_t (multiple R^2=0.56) and C_{org} stocks (R^2=0.24) in 0-10 cm depth. At the depth of 10-20 cm, the clay content was the only variable which correlated with stocks of C_{org} (R^2=0.39) and N_t (R^2=0.40).

3.4.5 Soil acidity and exchangeable cations

The soil pH and base saturation were higher under ash than beech clusters in 0-10 cm depth. There were no differences in the pH and base saturation between variants in 10-20 cm depth (Table 3.3).

The dominant exchangeable cation was Ca^{2+} which contributed up to 91.5% in 0-10 cm and 93.5% in 10-20 cm to the CEC, respectively. Neither the proportion of Ca^{2+} to the CEC nor the stock of exchangeable Ca^{2+} differed between the variants (Table 3.4). However, at TB 60, the latter tended to be higher in pure ash clusters than in clusters without ash ($p<0.1$). The stock of exchangeable Mg^{2+} in 0-10 cm depth was higher in ash clusters than in beech clusters at both sites. Further, at TB 60, it was higher in ash-lime clusters than in beech clusters. There were no differences between variants in 10-20 cm depth (Table 3.4).

In 0-10 cm, the percentage of exchangeable Al^{3+} was lower in pure ash clusters than in beech and beech-lime clusters (the latter only at TB 60, Table 3.4). Further it also tended to be lower in pure ash clusters than pure lime clusters ($p<0.1$). The contribution of Mn^{2+} to the CEC as well as the stock of exchangeable Mn^{2+} did not differ between variants in both depths.

At both sites in 0-10 cm depth, LL_{ash}/LL_{total} explained a large proportion of the variation of the soil pH. The clay content was the second most important factor which contributed to differences in soil pH (TB 60: multiple $R^2=0.45$, TB 100: multiple $R^2=0.62$). The pH in 10-20 cm depth was mainly related to soil clay content ($R^2=0.36$ and $R^2=0.41$ at TB 60 and TB 100, respectively).

At TB 60 in 0-10 cm depth, the clay content explained more than 50% of the variation in the stocks of exchangeable Mg^{2+} and Ca^{2+}. In addition, the abundance of beech litterfall reduced the stock of exchangeable Mg^{2+} (multiple $R^2=0.73$) and ash litterfall increased the stock of exchangeable Ca^{2+} (multiple $R^2=0.64$). In contrast to these results, at TB 100 (the site with the more uniform distribution of clay), clay did not influence the stock of exchangeable Mg^{2+}. Here, a simple linear regression with LL_{ash}/LL_{total} showed the strongest correlation and explained 64% of the variation of exchangeable Mg^{2+}. Comparably, ash was also the most important variable in multiple regression analysis with the stock of exchangeable Ca^{2+}, which additionally was influenced by the clay content (multiple $R^2=0.52$). In general, the stocks of exchangeable Mg^{2+} and Ca^{2+} in the mineral soil (0-10 cm) correlated positively with the input of the respective cation with the litterfall (Figure 3.3).

Table 3.4: Overview of the soil chemical properties of the different cluster variants (depth: 0-10 cm and 10-20 cm); mean with standard deviation in brackets. Base saturation and exchangeable aluminum are displayed as median with their 25%-75% percentile in bracket brackets, because their residuals were not normally distributed. If not listed separately, then no differences between the two subsites (TB 60, TB 100) existed and the mean includes results from both subsites.
Different lower case letters indicate significant differences between the variants, A and B show significant differences between the two study sites at a significance level of $p < 0.05$ (Scheffe Test; Kruskal-Wallis-Anova for Base saturation).

Soil depth (cm)	Variant	pH_{KCl}	Base saturation [%]	Contribution to CEC [%]								stock of exchangeable cations [kg ha^{-1}]				
				Ca^{2+}		Mg^{2+}		Mn^{2+}		Al^{3+}		Ca^{2+}		Mg^{2+}		Mn^{2+}
				TB 60	TB 100	TB 60^{A}	TB 100^{B}	TB 60	TB 100	TB 60	TB 100	TB 60^{B}	TB 100^{A}	TB 60	TB 100	
0-10	Beech	4.3^{a} (0.5)	87.7^{a} (76.8-93.9)	75.0^{a} (16.2)	81.2^{a} (8.5)	4.7^{a} (0.5)	6.2^{a} (1.0)	3.9^{a} (1.7)	3.7^{a} (1.4)	7.8^{b} (2.2-19.7)	3.7^{b} (2.8-7.1)	1895^{a} (868)	1677^{a} (428)	69.7^{a} (19.3)	75.5^{a} (14.4)	113.3^{a} (33.4)
	Ash	5.1^{b} (0.4)	97.9^{b} (96.6-98.4)	91.5^{a} (1.1)	86.1^{a} (2.7)	5.0^{ab} (0.8)	8.6^{a} (1.6)	1.6^{a} (0.4)	2.7^{a} (0.7)	0.0^{a}	0.3^{a} (0.1-0.6)	4114^{a} (724)	2345^{a} (695)	134.7^{b} (7.8)	137.0^{b} (12.8)	94.7^{a} (8.1)
	Lime	4.3^{ab} (0.2)	91.8^{ab} (85.4-93.8)	79.5^{a} (6.3)	NA	7.9^{c} (1.9)	NA	3.6^{a} (0.6)	NA	3.6^{ab} (2.5-9.5)	NA	1762^{a} (166)	NA	106.1^{ab} (26.9)	NA	109.8^{a} (25.6)
	Beech-Ash	4.7^{ab} (0.6)	97.5^{ab} (93.5-97.8)	89.0^{a} (3.1)	82.4^{a} (13.7)	5.3^{ab} (0.8)	6.9^{a} (0.9)	2.6^{a} (1.1)	2.6^{a} (1.1)	0.9^{ab} (0.0-2.4)	0.3^{ab} (0.2-10.5)	2968^{a} (611)	2380^{a} (879)	105.8^{ab} (21.8)	115.9^{ab} (24.4)	100.5^{a} (25.2)
	Beech-Lime	4.3^{ab} (0.3)	93.3^{ab} (83.8-96.0)	77.2^{a} (8.4)	85.5^{a} (3.5)	6.2^{abc} (0.7)	6.9^{a} (0.2)	3.5^{a} (0.9)	2.8^{a} (0.5)	10.2^{b} (3.1-15.2)	0.9^{ab} (0.7-3.9)	2258^{a} (910)	2081^{a} (204)	108.2^{ab} (35.1)	101.2^{ab} (6.9)	112.6^{a} (32.9)
	Ash-Lime	4.6^{ab} (0.6)	95.1^{ab} (89.7-98.2)	82.1^{a} (13.3)	82.8^{a} (4.9)	6.7^{bc} (0.4)	7.9^{a} (1.1)	2.8^{a} (1.4)	2.8^{a} (1.2)	0.2^{ab} (0.0-10.6)	3.2^{ab} (1.6-5.6)	3114^{a} (1584)	2024^{a} (649)	145.1^{b} (57.9)	116.8^{ab} (33.9)	102.2^{a} (24.0)
10-20	Beech	4.5^{a} (0.7)	96.4^{a} (72.1-97.6)	77.0^{a} (19.0)	84.3^{a} (14.1)	3.6^{a} (0.9)	5.5^{ab} (0.8)	2.5^{a} (1.4)	2.0^{a} (1.1)	9.9^{a} (1.2-22.9)	0.8^{a} (0.3-1.1)	2123^{a} (1043)	2355^{a} (870)	59.5^{a} (27.1)	88.7^{a} (18.3)	74.9^{a} (32.0)
	Ash	5.0^{a} (0.5)	98.9^{a} (95.9-99.4)	93.5^{a} (0.8)	89.1^{a} (3.3)	3.9^{a} (0.5)	5.0^{a} (0.1)	0.6^{a} (0.2)	2.1^{a} (0.9)	0.0^{a}	1.0^{a} (0.1-3.0)	4340^{a} (628)	2319^{a} (683)	109.6^{a} (21.7)	77.7^{a} (18.3)	56.7^{a} (19.7)
	Lime	4.0^{a} (0.2)	81.8^{a} (66.7-88.4)	71.3^{a} (11.6)	NA	5.4^{a} (1.0)	NA	2.8^{a} (0.4)	NA	13.5^{a} (7.9-27.5)	NA	1545^{a} (256)	NA	71.0^{a} (13.4)	NA	85.8^{a} (23.0)
	Beech-Ash	4.8^{a} (0.8)	98.5^{a} (96.4-99.0)	91.8^{a} (2.1)	79.9^{a} (23.6)	4.3^{a} (0.7)	5.2^{a} (0.6)	1.4^{a} (0.7)	1.4^{a} (0.6)	0.6^{a} (0.0-1.6)	0.2^{a} (0.1-21.4)	3204^{a} (622)	2582^{a} (1152)	91.7^{a} (26.6)	98.5^{a} (33.5)	57.9^{a} (17.2)
	Beech-Lime	4.5^{a} (0.6)	97.2^{a} (81.1-98.4)	74.9^{a} (12.6)	90.4^{a} (1.4)	4.7^{a} (0.6)	5.7^{ab} (0.3)	2.1^{a} (0.7)	1.4^{a} (0.3)	14.2^{a} (5.9-22.3)	0.3^{a} (0.1-0.9)	2397^{a} (1245)	2706^{a} (262)	89.0^{a} (39.6)	102.9^{a} (7.1)	69.9^{a} (16.7)
	Ash-Lime	4.4^{a} (0.7)	92.0^{a} (81.5-98.8)	76.2^{a} (29.5)	78.3^{a} (9.6)	5.0^{a} (1.1)	6.6^{b} (0.6)	1.7^{a} (1.1)	2.6^{a} (1.3)	0.2^{a} (0.1-28.0)	8.6^{a} (3.9-13.4)	3006^{a} (1782)	1839^{a} (629)	112.3^{a} (54.3)	93.5^{a} (24.8)	73.5^{a} (26.0)

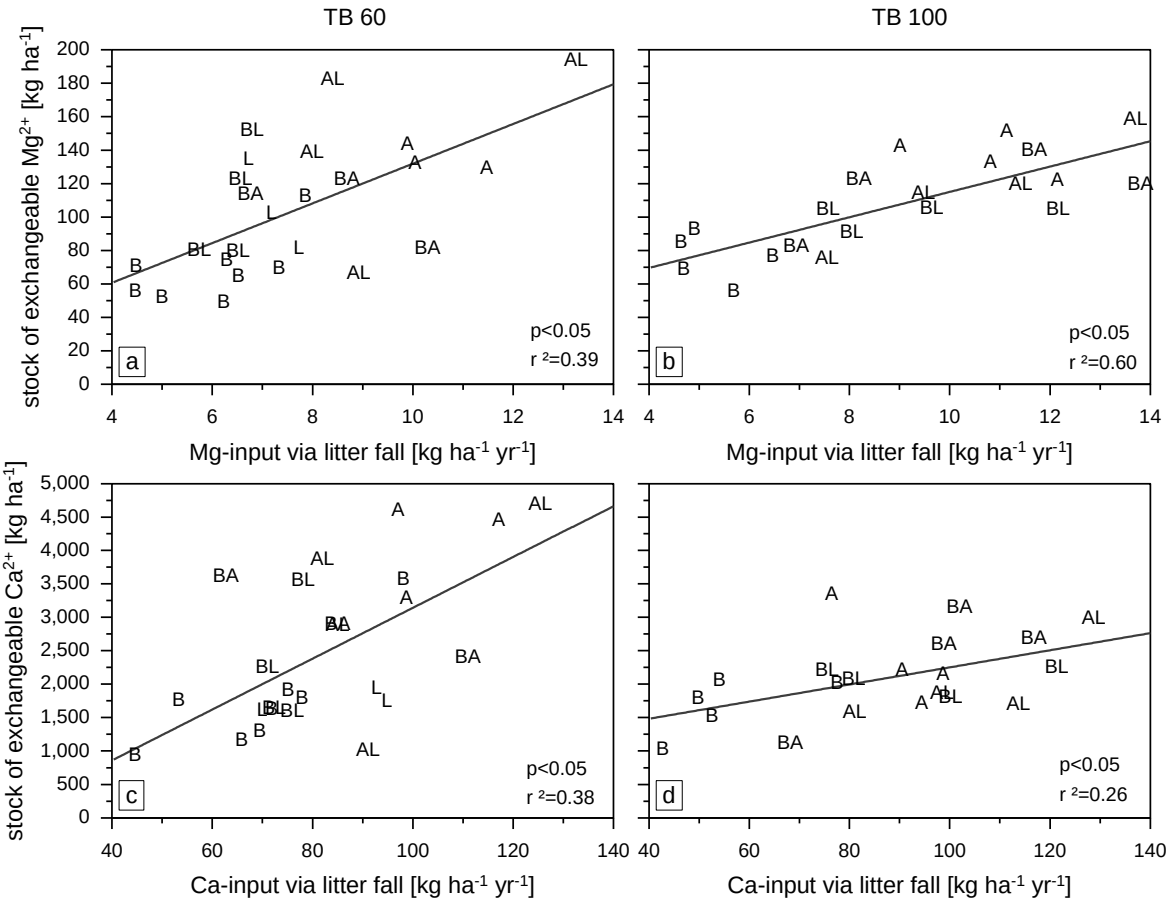

Figure 3.3 Influence of Mg input (a, b) and Ca input (c, d) via litterfall on the stock of the respective exchangeable cation in 0-10 cm depth at TB 60 and TB 100. Significant linear correlations (Pearson; p<0.05) are displayed by a linear slope. The cluster variants: A: Ash, B: Beech, L: Lime, BA: Beech-Ash, BL: Beech-Lime, AL: Ash-Lime

3.5 Discussion

3.5.1 Effects related to the clay content

The results show that, even though clusters were chosen in two small areas (TB 60 and TB 100), the clay content in the upper 20 cm of the mineral soil varied considerably, in particular at the site with the thinner loess cover (TB 60, Table 3.1). The clay content did not differ between the cluster variants. However, at TB 60, the clay content in 10-20 cm depth tended to be slightly lower in beech clusters than in ash clusters. The uniform distribution of the clay content over the cluster variants, in particular at TB 100, provided a reasonable basis for our analysis of species related effects on small scale differences in soil chemical properties. We have not determined the variability of subsoil clay content and thus we cannot fully exclude that the distribution of ash and lime was influenced by subsoil properties. However, we assume that subsoil effects on species distribution are unlikely at our experimental sites because ash and lime were growing side by side with beech and root systems were overlapping.

Multiple regression analyses revealed that in 10-20 cm depth, the clay content was the dominant variable causing variations in soil acidity and nutrient stocks at both subsites. This is in line with clay contents' general ability to affect CEC and exchangeable base cations (Guckland et al. 2009), soil acidity (Bredemeier et al. 1990) and organic matter storage (von Lützow et al. 2006).

In the upper 10 cm of the soil, the abundance of ash leaf litterfall and the clay content explained most of the variations in soil acidity and soil nutrient stocks at both subsites. The importance of ash leaf litterfall as an explaining variable increased with decreasing variation in the clay content, and it was the dominant control of small scale variability of soil acidity and nutrient stocks at our subsite TB 100. Covariance analyses revealed comparable results for both subsites concerning differences in soil properties between cluster variants (Table 3.4), indicating that we successfully disentangled the effect of the clay content and tree species on soil chemical properties.

3.5.2 Fuzziness of approach

Litterfall composition of mono-species clusters revealed that litterfall originated mainly but not exclusively from cluster trees. In particular, beech leaf litterfall from non-cluster trees was found in our litter traps.

The contribution of trees from outside the cluster to litterfall inside the clusters was expected, since it is well known that litter dispersal of different deciduous tree species in mature stands clearly exceeds the distance from our sampling point to the stems of the cluster trees. Results from studies on litter dispersal suggest that most of the leaf litter falls down within a distance of about 18 m from the stem (Ferrari and Sugita 1996; Rothe 1997; Staelens et al. 2004). However, this distance depends on several factors such as canopy structure, leaf size and wind velocity. Rothe et al. (2002) and Holzwarth et al. (2011) pointed out that soil samples of a given point in a mature deciduous forest are influenced by the tree species within a radius of about 10 m. Thus our results do not reflect conditions in mono-species stands, larger groups of single tree species or well defined mixtures of different species, but rather represent natural conditions in a highly diverse deciduous mixed forest. This implies the fuzziness of a heterogeneous mixture of different tree species, which reduces possible effects of tree species on soil properties.

3.5.3 Effects related to leaf litter composition

Our results show that the distribution of ash induced small scale variations in soil chemical properties, such as soil acidity or nutrient stocks, in a beech-dominated

temperate mixed broadleaved forest. The influence of ash was most pronounced in the forest floor and in the topsoil (0-10 cm) and could be related to leaf litter composition. Nordén (1994) discovered partly contrasting effects of tree species on base saturation in the topsoil compared to deeper horizons and reasoned that in the topsoil, leaf litterfall is the dominant control on soil acidity (higher pH value under lime trees than under beech or oak). Hansen et al. (2009) figured that the accumulation of C and nutrients in the forest floor are basically controlled by decomposition of litterfall. Our results agree with the general observations that effects of tree species on soil chemical properties appear mainly in the topsoil (Augusto et al. 2003; Guckland et al. 2009; Hagen-Thorn et al. 2004; Mareschal et al. 2010).

3.5.3.1 Nutrient contents in litterfall

We found clear species-specific differences in leaf litter composition of trees growing at the same site (Table 3.2). Such differences are considered to be intrinsic species-specific traits. In line with our results, several studies found lower quantities of base cations but a higher Mn content (in comparison to only ash litter) and a higher C:N ratio and lignin:N ratio in beech litterfall compared to ash and lime litterfall (Jacob et al. 2009, 2010; Reich et al. 2005; Vesterdal et al. 2008). Besides the tree species itself, other factors like tree age (Vesterdal et al. 2008), soil fertility status (Sariyildiz and Anderson 2005) or annual variations (Jacob et al. 2009) may cause variations in litterfall chemistry of a single species. Meier et al. (2005) who analyzed nutrient returns with litterfall in beech forests found intermediate variations in Ca and Mg return and large variations in Al and Mn return with litterfall across a soil fertility gradient. There was no evidence in our study that the observed small scale variation of the topsoil chemistry affected litter composition.

The horizontal and vertical expansion of nutrient uptake by the root system is generally much larger than the observed differences in soil chemistry which are restricted to the upper 10 cm of the soil (Leuschner et al. 2004). For the Hainich National Park, Meinen et al. (2009a) found that 63-77% of fine roots are concentrated in the upper 20 cm of mineral soil, but there were still fine roots in depths larger than 40 cm of mineral soil. However, root activity may differ from fine root biomass distribution and can be very variable and allows subsoil resource use (Lehmann 2003).

3.5.3.2 Organic carbon and total nitrogen in mineral soil and forest floor

We found higher stocks of C_{org} and N_t in the forest floor under beech than under ash, but stocks of C_{org} and N_t in the mineral soil (0-10 cm) were smaller under beech and lime than under ash (Table 3.3). The results point at a faster turnover rate of ash litterfall

compared to beech litterfall resulting in a faster, more efficient nutrient return to the soil (Jacob et al. 2009; Oostra et al. 2006; Vesterdal et al. 2008). Carbon accumulation in the forest floor depends on several interacting factors: The most important are litter quality (i.e. the lignin content and the lignin:N ratio, compare Berg 2000 and Inagaki et al. 2004), soil fertility and activity of soil biota. The higher lignin and lower N content resulting in a higher lignin:N ratio of beech leaf litterfall, compared with ash and lime, results in a generally higher recalcitrance and slower rate of decomposition (Finzi et al. 1998a; Jacob et al. 2010; Melillo et al. 1982). Our results are in line with the conclusion of Guckland et al. (2009), Vesterdal et al. (2008) and Kooijman and Cammeraat (2010) that C accumulation in the forest floor of deciduous tree stands is largely determined by the abundance of beech litterfall. Vesterdal et al. (2008) observed that forest floor C_{org} and N_t stocks were related to C:N ratio of litterfall, which agrees with the findings in our study. Leuschner et al. (2006) analyzed the soil nutrient status in 50 European beech stands. They found that the stock of N in the forest floor was closely related to the content of exchangeable Al^{3+} in the mineral soil, indicating that elevated Al^{3+} contents negatively influence the activity of soil organisms due to Al toxicity and reduce decomposition and incorporation of organic matter into the mineral soil by bioturbation. This might additionally explain differences in stocks of N between our cluster variants because we found highest contents of exchangeable Al^{3+} in the soils of pure beech clusters (Table 3.4). The C:N ratio of the organic layer did not reflect the higher N content of ash and lime leaf litterfall compared to beech (compare Table 3.2 and Table 3.3). We assume that this is a result of the sampling time in June, because ash and lime litterfall was already decomposed at this time and the sampled forest floor consisted mainly of beech litterfall.

3.5.3.3 Tree species effect on soil acidity and exchangeable cations

The mixture of different broadleaved tree species resulted in a spatial variability of topsoil chemistry (e.g. soil acidity, exchangeable base cations). Our results show that this variability was largely determined by the abundance of ash leaf litterfall, which had highest contents of Mg and Ca.

Our results agree with the conclusion of Neirynck et al. (2000) that the surface soil nutrient status is influenced by the ability of different tree species to improve or maintain soil productivity via nutrient uptake and redistribution. Augusto et al. (2002) summarized effects of tree species on soil fertility and concluded that the acidifying effect of beech and oak on soil pH was higher than for all other deciduous trees. The ability of tree species to reduce acidification and increase the nutrient availability in topsoils was mainly related to the Ca and Mg concentration in litterfall and the litter ash alkalinity (Dijkstra 2003; Noble and Randall 1999; Reich et al. 2005).

Our results confirm the assumption of Guckland et al. (2009) of having detected a beech gradient effect on soil acidity and nutrient contents in Hainich National Park. However, the abundance of ash showed an even more profound influence on soil acidity than the abundance of beech. Our results indicate that the addition of ash leaf litter in beech dominated stands on loess over limestone reduced soil acidification and led to higher stocks of exchangeable macro nutrients such as Mg^{2+} or Ca^{2+}. A positive effect of ash on topsoil fertility was also observed in other studies (Hagen-Thorn et al. 2004; Neirynck et al. 2000; Oostra et al. 2006). In some cases it was difficult to separate effects of tree species from effects induced by heterogeneity of the soil texture (Alriksson and Eriksson 1998; Guckland et al. 2009) or soil parent material (Augusto et al. 1998). We were able to separate these effects at least at TB 100 (i.e. effects of clay content and tree species) and the results show that the abundance of ash leaf litterfall significantly contributed to the variability in soil acidity and stocks of exchangeable base cations. This effect was restricted to the top 10 cm of mineral soil. The effect of tree species on the redistribution of Ca and Mg in the soil profile through nutrient uptake and litterfall and mineralization depend on soil properties such as the nutrient availability and buffer capacity at different soil depths (Augusto et al. 2002; Meier et al. 2005; Noble and Randall 1999). In our clusters, the biological pumping of base cations from the subsoil was of great importance, because loess has a rather low buffer capacity and thus tends to form strongly acid forest soils (Guckland et al. 2009). Guckland et al. (2009) supposed that the ameliorating effects through nutrient uptake from the deep soil layers, litterfall and mineralization differed between species and counteracted the accumulation of acid cations at the exchange complex. Thus, the replacement of exchangeable base cations was minimized (Guckland et al. 2009). The vertical extension of tree species effects on soil properties probably depends on soil texture and the related pH buffer capacity and CEC of soils e.g. Nordén (1994) found species related effects on soil acidity and exchangeable cations down to a depth of 70 cm on a sandy site with 2-3% clay.

We found no clear effect of lime on soil acidity and stocks of exchangeable nutrients (Table 3.4). In contrast, several studies detected higher pH values, base saturation and nutrient stocks in soils under lime than under beech (Nordén 1994; Neirynck et al. 2000; Hagen-Thorn et al. 2004). Neirynck et al. (2000) and Hagen-Thorn et al. (2004) compared soil properties of adjacent plots of monospecific stands and therefore did not have any litter mixture of different tree species. In our study (i.e. tree species standing in mixture in a mature forest), the mixture of different litter types led to blurs, which are usual in natural conditions. We assume, that the effect of lime on soil properties might have been more pronounced in larger groups of lime, where the admixture of beech litterfall is smaller. However, additional studies are required to be able to capture and quantify the influence of tree species distribution on the variability of soil properties in different locations and, in

a further step, to distinguish between general and site-specific species-induced influences on soil properties.

The Mn content in litterfall correlated negatively with the pH in the upper mineral soil at both subsites. This can be explained by the dissolution of Mn oxides with decreasing pH, which results in a greater bioavailability of Mn (Schachtschabel 1957). Our results suggest that the higher Mn content in beech leaf litter than ash leaf litter was influenced by the stronger soil acidification under beech.

Overall, our results indicate that in a diverse stand the abundance and distribution of individual tree species accounted for the variation in soil chemical properties and the sum of these species make up the soil chemical properties of the whole forest stand. Jacob et al. (2009) came to the same conclusion concerning litter decomposition rates. Guckland et al. (2009) also suggested that they rather detected a beech gradient than a biodiversity effect as a cause of decreasing soil acidification and an increase of base cations in more diverse stands (one-species stands were all of beech).

Besides the influence of leaf litter quality on soil properties, tree species can alter soil properties through various factors. Among the most important are the capacity of tree species to intercept atmospheric deposition (Augusto et al. 2002; Talkner et al. 2010), variations in the amount and distribution of throughfall (Augusto et al. 2002; Barbier et al. 2009), stemflow (Falkengren-Grerup 1989; Koch and Matzner 1993), root growth (Lehmann 2003; Meinen et al. 2009b) and spatial and temporal differences in water and nutrient uptake (Augusto et al. 2002; Bittner et al. 2010). Stemflow of different tree species in the Hainich National Park was analyzed by Krämer and Hölscher (2009) and Talkner et al. (2010). It was 2-6% of total precipitation (while throughfall was between 66 and 77% of total precipitation) and it was lower for ash and lime than for beech (Krämer and Hölscher 2009). The results support the observation that stemflow of beech increases soil acidification near the trunk (Falkengren-Grerup 1989; Koch and Matzner 1993). Since this acidifying effect is restricted to a small distance from the trunk (<1.5 to 2 m; Falkengren-Grerup 1989) it cannot explain the different soil acidity in the center of our tree clusters. Talkner et al. (2010) found that deposition of Ca and Mg via throughfall was lower and acid deposition was higher in pure beech stands than in mixed species stands in the Hainich National Park. This observation was explained by canopy processes which resulted in different canopy leaching rates of Ca and Mg. The results suggest that differences in canopy exchange processes and deposition between the studied tree species might additionally have influenced differences of soil chemical properties in our tree clusters.

Root distribution, composition of root litter and rhizosphere properties are further factors which may cause species specific effects on soil properties (Calvaruso et al. 2011;

Hinsinger et al. 2005). Meinen et al. (2009a, b) determined total root biomass and root distribution of different tree species close to our cluster sites. They found no evidence of spatial root system segregation or elevated root biomass in multi-species sites. Fine root biomass of a single tree within the distance of 2-5 m (cluster radius) from the stem was approximately 400-600 g m^{-2} (0-40 cm depths) and did not differ between species. Meinen et al. (2009b) found a high degree of root system overlap in mixed stands. The change of fine root biomass with increasing stem distance suggest that fine roots in our soil samples originated mainly from the three cluster trees. Lang (2008) found that N and Mg contents of tree fine roots in the Hainich National Park were higher for ash than beech. These differences of root composition might have contributed to the observed effects of ash on soil chemical properties.

3.6 Conclusion

Our results show that the presence of ash in a species-rich (although beech dominated) temperate forest on a luvisol of loess over limestone reduced soil acidification and enlarged the stocks of exchangeable base cations, organic carbon and total nitrogen in the topsoil (0-10 cm). The results on litterfall quality and distribution indicate that these changes of topsoil properties were caused, to a large extent, by differences in leaf litterfall chemistry. The distribution of ash resulted not only in aboveground diversity of stand structure but also caused distinct small scale belowground diversification of the soil habitat. The results from the different tree clusters show that small scale variability of soil chemical properties was not only driven by species mixture and identity but also by the spatial distribution of individual species (e. g. grouping of ash increases the range of variation of chemical soil properties). Thus, ash leaf litter not only reduced soil acidity and increased nutrient availability but also led to an increased diversity of the soil habitat in beech stands. The soil clay content was the primary factor which explained spatial variability of soil acidity, soil organic carbon content, and exchangeable base cations. The influence of ash on chemical topsoil properties was only dominant in stands with low variability of soil clay content. We found no influence of clay content or cluster species on the composition of beech, ash and lime leaf litter.

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4 PARTITIONING OF C AND N DURING DECOMPOSITION OF ^{13}C- AND ^{15}N-LABELED BEECH AND ASH LEAF LITTER[2]

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4.1 Abstract

The aim of this study was to determine the influence of leaf litter type (i.e. European beech – *Fagus sylvatica* L. and European ash – *Fraxinus excelsior* L.) and leaf litter mixture on the partitioning of leaf litter C and N during decomposition. In a mature beech stand of Hainich National Park, Thuringia, Germany, undisturbed soil cores (Ø 24 cm) were transferred to plastic cylinders and the original leaf litter was either replaced by $^{13}C^{15}N$ labeled beech or ash leaf litter, or leaf litter-mixture treatments in which only one of the two leaf litter types was labeled. Leaf litter-derived CO_2-C flux was measured biweekly over a period of one year. Partitioning of leaf litter C and N to the soil and microbial biomass was measured five and ten months after the start of the experiment. Ash leaf litter decomposed faster than beech leaf litter. The decomposition rate was related to initial leaf litter lignin and Ca concentrations. The mixture of both leaf litter types led to enhanced decomposition of ash leaf litter. However, it did not affect beech leaf litter decomposition. After five and ten months of in-situ incubation, recoveries of leaf litter-derived C and N in the O-horizon (7-20% and 9-35%, respectively) were higher than in the mineral soil (1-5% and 3-8%, respectively) showing no leaf litter type or leaf litter mixture effect. Partitioning of leaf litter-derived C and N to microbial biomass in the upper mineral soil (< 1% of total leaf litter C and 2-3% of total leaf litter N) did not differ between beech and ash. The results show that short term partitioning of leaf litter C and N to the soil was similar for ash and beech leaf litter under standardized field conditions even though mineralization was faster for ash leaf litter than for beech leaf litter.

4.2 Introduction

Tree species may affect chemical soil properties via leaf litter quality (Guckland et al. 2009; Langenbruch et al. 2012) and thus via different decomposition rates of the leaf litter (Hättenschwiler et al. 2005; Jacob et al. 2010). Leaf litter quality and decomposition rates are defined over the lignin content or lignin:N ratio, their C:N ratio, via different nutrient concentrations in the leaf litter, i.e. Ca (Melillo et al. 1982; Berg 2000; Vesterdal et al. 2008; Jacob et al. 2010) or via physical parameters such as leaf tensile strength (Pérez-Harguindeguy et al. 2000).

Leaf litter mixture of different species can further affect litter decomposition. Most studies considering leaf litter mixtures of broadleaved species found either additive or positive mixture effects on the decomposition of individual leaf litter types (Gartner and Cardon 2004 and references therein); the latter often occurred in N-rich substrates (Wardle et al. 1997). Contrasting results exist concerning nutrient release rates. While Wardle et al. (1997) found in their study on leaf litter mixtures of several functional groups

that nutrient release rates were lower in mixes than in corresponding monocultures, Jacob et al. (2009) found no evidence for a species richness effect on nutrient release rates. Whether leaf litter mixtures lead to additive or non-additive decomposition seems to depend on the abundant leaf litter species (Gartner and Cardon 2004), the site properties (Jacob et al. 2010) and the mixing ratio (Salamanca et al. 1998), and no general pattern could be observed (Hättenschwiler et al. 2005).

Several studies analyzed the effect of different tree species and species mixtures on C and N cycling (Finzi et al. 1998a; Lovett et al. 2004; Baum et al. 2009; Trum et al. 2011). Results indicate that mineralization of leaf litter (Baum et al. 2009) and partitioning of leaf litter C and N to the mineral topsoil (Vesterdal et al. 2008; Langenbruch et al. 2012) was higher under ash than under beech.

In this study, we aimed to determine (1) whether decomposition of leaf litter and partitioning of leaf litter C and N differ between tree species with considerably different leaf chemistry, i.e. European ash and European beech, and (2) whether a mixture of both leaf litter types influences these processes compared to leaf litter of individual species. We hypothesized that (1) the faster decomposition of ash leaf litter is associated with a greater partitioning of leaf litter C and N to mineral soil and soil microbial biomass in comparison to beech leaf litter, and (2) a mixture of beech and ash leaf litter leads to additive effects on the partitioning of leaf litter C and N. We used $^{13}C^{15}N$-labeled leaf litter and followed the decomposition and partitioning of leaf litter-derived C and N via isotopic measurements of the organic C (C_{org}), total N (N_t), microbial biomass (MB) and soil CO_2 flux.

4.3 Material and methods

4.3.1 Study site

The study was conducted in a beech forest in the North of Hainich National Park (Thuringia, Germany), near the village “Mülverstedt” (51°06’N, 10°27’E). The elevation of the site is 370 m a.s.l. The mean annual temperature is 7.5°C and the mean annual precipitation is 670 mm (Meteomedia, station Weberstedt/Hainich, 51°06’N, 10°27’E). The forest has existed for over 200 years and contains mature trees aged 100 to 200 years. In 1997, the Hainich became a National Park (Mölder et al. 2006). The beech forest grows on a Luvisol (IUSS Working Group WRB 2006) developed from loess underlain by Triassic Limestone. The topsoil (0-10 cm) contained 3% sand, 82% silt and 15% clay (Guckland et al. 2009). According to the morpho-functional classification of humus forms by Zanella et al. (2011), the forest floor was classified as a dysmull (OL+OF) to hemimoder (OL+OF+discontinuous OH). The topsoil (0-5 cm) of the study site was rather

acid with a pH_{KCl} of 3.3 and a base saturation of 26%. The mean CEC in 0-5 cm was 86 $mmol_c$ kg^{-1} and the C:N ratio was 20.

4.3.2 Leaf litter

For the experiment, leaf litter of European beech and European ash was chosen, because they significantly differ in their chemistry (Jacob et al. 2010) and in the influence on soil chemical properties (Guckland et al. 2009; Langenbruch et al. 2012). In order to detect possible differences between the leaf litter types in the partitioning of C and N during decomposition processes, we labeled leaf litter with ^{15}N and ^{13}C. Young ash and beech trees were grown in a greenhouse under $^{13}CO_2$-enriched atmosphere ($\delta^{13}C$ of ~300 ‰ V-PDB) for one vegetation period and supplied with a $^{15}NH_4{}^{15}NO_3$-containing nutrient solution (~44 atom% ^{15}N). For reference, ash and beech leaf litter with natural abundance of ^{13}C and ^{15}N were sampled in Hainich National Park. All leaf litter samples were air dried. A subsample of each leaf litter type was ground in a mixer mill (RETSCH MM2, Haan, Germany) and C_{org} and N_t content was measured with an automated C and N analyzer (Heraeus Elementar Vario EL, Hanau, Germany). The isotopic composition was measured with an IRMS Delta Plus (unlabeled samples) or an IRMS Delta C (labeled samples) (Finnigan MAT, Bremen, Germany). Lignin content was determined using the acetylbromide method (Brinkmann et al. 2002). Contents of cations were analyzed after pressure digestion with concentrated nitric acid (Chander et al. 2008) at the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Kleve, Germany).

4.3.3 Experimental setup

The experiment was installed in four blocks within 50x50 m from Dec. 9th to Dec. 12th 2008. Each block contained three replicates per treatment. Six treatments were established: (1) pure labeled beech leaf litter (PL-beech), (2) pure labeled ash leaf litter (PL-ash), (3) 1:1-mixture of labeled beech and unlabeled ash leaf litter (ML-beech), (4) 1:1-mixture of labeled ash and unlabeled beech leaf litter (ML-ash), (5) pure unlabeled beech leaf litter (PU-beech), (6) pure unlabeled ash leaf litter (PU-ash), with “P” indicating pure treatments, “M” mixed treatments, “L” labeled leaf litter and “U” unlabeled leaf litter.

Intact soil cores of 24 cm in diameter and a thickness of approximately 5 cm were taken and transferred into plastic cylinders (mesocosm) which were closed with a 50 µm gauze at the bottom to prevent roots from growing into the mesocosms. The mesocosms were placed back to their place of origin. They were installed at least 1 m apart from each other and 2 m apart from tree stems. While the older, partly humified fraction of the organic layer (OF+OH) remained (in the following text referred to as “O-horizon”), the

original freshly fallen aboveground litter (L) was removed and replaced with 14.4 g dry weight of the respective experimental leaf litter (in treatments with litter mixtures, 7.2 g dry weight of each species was placed onto the soil; in the following text referred to as "L-horizon"). Finally, the soil cores were closed with a fly gauze on top to keep aboveground litter from falling in. The mesocosms were kept free of plants throughout the experiment. The experimental site was fenced in to keep wild boars, red deer and roe deer out.

4.3.4 CO_2 emission

The CO_2 emission was measured biweekly at one replicate per block of each treatment (n = 4) over a period of one year via the closed-chamber technique (Blackmer et al. 1980; Hutchinson and Mosier 1981; Högberg and Ekblad 1996), using a plastic lid that was placed on top of the mesocosm. The chamber remained closed for 60 minutes and gas samples of 15 ml were taken 0, 20, 40 and 60 minutes after chamber closure using a gastight syringe and transferring the gas into evacuated LABCO EXETAINER of 12 ml volume (Labco Limited, Buckinghamshire, United Kingdom). The CO_2 concentration and its $\delta^{13}C$:^{12}C ratio was measured with an IRMS Delta Plus with GP interface and GC-Box (Thermofisher, Bremen, Germany). Flux rates were calculated from the increase in CO_2 concentration within single soil chambers by fitting concentrations linearly (Eq. 4.1) and quadratically (Eq. 4.2; Wagner et al. 1997) against the minutes of chamber closure:

Equation 4.1: CO_2 [ppm] = a+b*time [min]

Equation 4.2: CO_2 [ppm] = a+b*time [min]+c*time^2 [min].

For the quadratic model, the slope at chamber closure (time= 0 min) described the increase of CO_2 per minute in the chamber (Wagner et al. 1997) and could be calculated by differentiating Equation 2 with respect to time yields (Eq. 4.3).

Equation 4.3: $\Delta(CO_2)/\Delta$ (time) = b+2c*time [min]

For both models the letter b described the increase of CO_2 per minute in the chamber.

Daily fluxes were then calculated using equation 4.4:

Equation 4.4: $$CO_2 - C\left[\frac{mg}{m^2 d}\right] = 24 \times 60 \times \frac{b\left[\frac{ppm}{min}\right] \times 12\frac{g}{mol} \times P[Pa] \times V[m^3]}{1000 \times 8.314\frac{J}{mol \times K} \times (273.2 + T[°C]) \times A[m^2]}$$

In equation 4.4, b stands for the parameter from the model with the lower AIC (Eq. 4.1 or 4.2), P for the air pressure, V for the chamber volume, A for the chamber area and T for the temperature measured 10 cm above the soil surface.

The isotopic composition of the respired CO_2 was obtained from the Y-axis intersection of the Keeling plot (Keeling 1958; Yakir and Sternberg 2000) by plotting the $\delta^{13}C/^{12}C$ ratio of the individual measurement points linearly against 1/CO_2 [ppm].

4.3.5 Partitioning of litter C and N to the O-horizon, mineral soil and microbial biomass

Five (May 5th 2009) and ten (October 20th 2009) months after leaf litter application, one replicate per block of each treatment (n = 4) was sampled. Two cores with a diameter of 5 cm were taken from each mesocosm. These cores were divided into L-horizon, O-horizon and mineral soil. The latter was further divided into soil increments of 1 cm thickness. All divisions were sieved (< 2 mm) and each a subsample was dried, ground and analyzed by an automated C and N analyzer (Heraeus Elementar Vario EL, Hanau, Germany) for their C and N content. The abundance of ^{13}C and ^{15}N was determined by isotope ratio mass spectrometry (IRMS Delta plus and IRMS Delta C, Finnigan MAT, Bremen, Germany). Soil microbial biomass was determined by the chloroform fumigation extraction (CFE) method (Brookes et al. 1985 and Vance et al. 1987). However, we used 0.05 *M* K_2SO_4 solution instead of 0.5 *M* K_2SO_4 solution for the extraction, because large quantities of salt hamper the determination of isotopes using EA-IRMS (Potthoff et al. 2003). Extracted C and N were measured using a DIMATOC 100 TOC/TNb analyzer (Dimatec, Essen, Germany). The microbial biomass (MB) C and N was calculated via equation 4.5 (Wu et al. 1990) and equation 4.6 (Brookes et al. 1985; Joergensen and Mueller 1996).

Equation 4.5: $C_{MB}\,[\mu g\ g^{-1}] = (C_{fum}\,[\mu g\ g^{-1}] - C_{non}\,[\mu g\ g^{-1}])/0.45$

Equation 4.6: $N_{MB}\,[\mu g\ g^{-1}] = (N_{fum}\,[\mu g\ g^{-1}] - N_{non}\,[\mu g\ g^{-1}])/0.54$

C_{fum} stands for the C_{org} extracted from fumigated soil and C_{non} stands for C_{org} extracted from non-fumigated soil (N analog). The extracts from the CFE-analysis were further freeze-dried (CHRIST DELTA II, Osterode Harz, Germany) and subsequently measured for their $\delta^{13}C/^{12}C$ ratio and $\delta^{15}N/^{14}N$ ratio using the IRMS Delta plus (Finnigan MAT, Bremen, Germany). Microbial biomass ^{13}C was calculated by equation 4.7 (Dijkstra et al. 2006). $^{15}N_{MB}$ was calculated analog to $^{13}C_{MB}$ by substituting the C components in equation 4.7 with the respective N components.

Equation 4.7: $$atom\%^{13}C_{MB} = \frac{atom\%^{13}C_{fum} \times C_{fum}\left[\frac{\mu g}{g}\right] - atom\%^{13}C_{non} \times C_{non}\left[\frac{\mu g}{g}\right]}{C_{fum}\left[\frac{\mu g}{g}\right] - C_{non}\left[\frac{\mu g}{g}\right]}$$

4.3.6 Calculation of leaf litter recoveries

The proportions of leaf litter-derived C (for N analog) in the analyzed samples (mineral soil, L- and O-horizon, CO_2-flux) were calculated via Equation 4.8 (Balesdent and Mariotti 1996).

Equation 4.8:

$$Proportion\ of\ litter\ derived\ C\ [\%] = \frac{atom\%^{13}C_{treatment} - atom\%^{13}C_{ref}}{atom\%^{13}C_{labelled\ litter} - atom\%^{13}C_{ref}} \times 100\%$$

Here, atom%$^{13}C_{treatment}$ stands for the atom%^{13}C of the analyzed fraction in the labeled treatments, atom%$^{13}C_{ref}$ for the atom%^{13}C of the analyzed fraction in PU-beech and atom%$^{13}C_{labeled\ litter}$ for the atom%^{13}C of the initial beech or ash leaf litter. The results were converted into recovery of leaf litter-derived C (%) according to eq. 4.9 (N analog).

Equation 4.9: $Recovery\ of\ litter\ derived\ C\ [\%] = \frac{C_{total}[mg] \times \frac{proportion\ of\ litter\ derived\ C\ [\%]}{100\%}}{C_{litter}\ [mg]}$

C_{total} stands for the amount of C in the soil increment, the O-horizon or the MB in the mesocosm and C_{litter} for the amount of C that was introduced to the mesocosm with the labeled litter.

Cumulated litter derived CO_2-C was calculated by assuming that the recovery of litter C in the daily CO_2 flux was identical to the first measurement in the first half and to the second measurement in the second half of the period between two measurements. The calculated cumulative CO_2 fluxes cannot be considered absolute values or compared with values of other studies, because CO_2 fluxes are highly variable with time and biweekly measurements are not enough to determine total CO_2-C losses from the added leaf litter. However, the results can be used for a comparison between the treatments of our study.

4.3.7 Statistical analyses

Anovas followed by Tukey`s HSD Tests were conducted to detect possible differences in the chemical composition between different leaf litter types at the beginning of the experiment, and in the L-horizon after five and ten months of decomposition. Paired t-tests were used to detect possible differences in the partitioning of leaf litter-derived C and N between PL-beech and PL-ash, the partitioning of ash litter derived C and N between PL-ash and ML-ash and the partitioning of beech litter derived C and N between PL-beech and ML-beech. In total, three t-tests needed to be done for each variable. Therefore, a correction of the p-value was carried out using the method of Hajek and Sidak (Eq. 4.10, see Equation 2.15 in Bortz et al. 1990).

Equation 4.10: $p = 1 - (1 - p')^{\frac{1}{n(n-1)/2}}$

In equation 4.10, p' is the significance level of 0.05, n is the number of tested groups (here 3) and p is the corrected p-value. In case that the residuals were not normally distributed, a Mann-Whitney-U-Test was conducted instead of a t-test (May 2009: litter-derived C and N in the forest floor (ML-ash), litter-derived C and N in the mineral soil (ML-beech), October 2009: litter-derived C in the mineral soil (ML-beech and PL-ash)). Anovas with repeated measures followed by Tukey`s HSD Tests were used to detect possible interdependencies between treatments and time or depths concerning the partitioning of litter C and N. Simple linear and forward stepwise multiple regression analyses were conducted to determine the initial litter chemistry parameters that best explained losses of litter mass, C and N.

4.4 Results

4.4.1 Litter composition

Carbon and nitrogen in labeled leaf litter were significantly enriched in ^{13}C and ^{15}N compared to the unlabeled reference leaf litter (Table 4.1). Beech leaf litter had higher C and lignin concentrations than ash leaf litter. Further, unlabeled beech leaf litter had higher C:N and lignin:N ratios than unlabeled ash leaf litter, while these ratios did not differ between the two labeled leaf litter types. The N concentration was higher in labeled leaf litter than in unlabeled leaf litter. This was probably related to the N fertilization of the trees in the greenhouse with ^{15}N-containing nutrient solution. The Ca concentration was higher in ash leaf litter than in beech leaf litter.

Table 4.1 Chemical composition of the leaf litter types (means and standard deviation, n=12 (labeled litter) and n=4 (non-labeled litter), for lignin: n=4). The concentrations of C, lignin, N and Ca were tested for significant differences (ANOVA, Tukey`s HSD Test, $p<0.05$). Different letters indicate significant differences between litter types.

Leaf litter	C [mg g^{-1}]	$\delta^{13}C$	Lignin [mg g^{-1}]	N [mg g^{-1}]	atom% ^{15}N	C:N	Lignin:N	Ca [mg g^{-1}]
beech	507^{d} (1)	-29 (0)	301^{d} (3)	8.7^{a} (1.1)	0.365 (0.001)	58	35	9.9^{a} (0.1)
labelled beech	492^{c} (1)	118 (2)	241^{c} (4)	21.3^{d} (0.4)	1.500 (0.078)	23	11	12.5^{b} (0.4)
ash	487^{b} (1)	-28 (1)	201^{b} (2)	11.4^{b} (0.3)	0.367 (0.001)	43	18	24.6^{c} (0.2)
labelled ash	456^{a} (2)	155 (5)	178^{a} (2)	19.9^{c} (0.9)	9.307 (0.527)	23	9	25.8^{d} (0.8)

4.4.2 CO_2 emission

Soil respiration showed a clear seasonal pattern (Fig. 4.1A) and was positively related to soil temperature. It did not differ between treatments (data not shown). Leaf litter-derived CO_2 emission contributed 2-37% (mean 9%) to the soil respiration measured at a sampling date. It basically followed the seasonal pattern until the early summer, after which it slowly declined (Fig. 4.1B).

Leaf litter derived CO_2 emissions where higher in PL-ash than PL-beech until May 7th 2009. Further, ash leaf litter was mineralized faster in ML-ash than PL-ash until May 7th 2009 (Fig. 4.1B). After this date, no more differences between the treatments were observed. Cumulated fluxes gave a similar picture (Fig. 4.1C): After one year, 23-25% of beech leaf litter C, 33% of ash leaf litter C in PL-ash and 40% in ML-ash were mineralized.

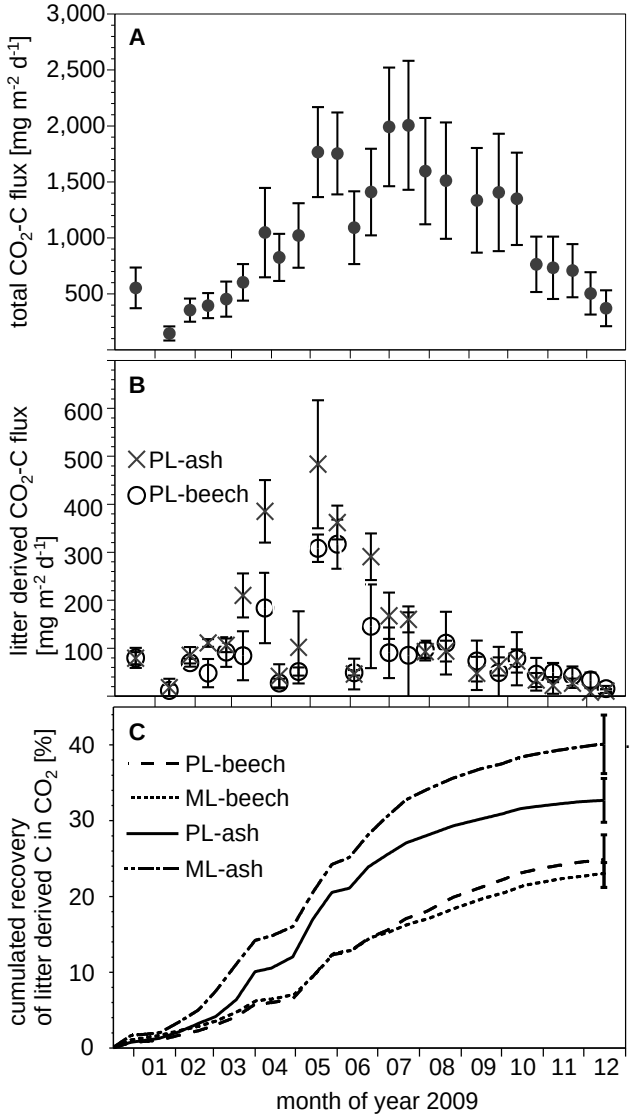

Figure 4.1 Total (A) and litter-derived (B) fluxes of CO_2 over a period of one year and (C) cumulated recovery of litter derived C in CO_2 flux. Total CO_2 flux is a mean over all variants. Displayed are means with standard deviation (n=4; n=24 for total fluxes).

4.4.3 Changes of the leaf litter layer

Mass loss proceeded faster for ash leaf litter than for beech leaf litter (Table 4.2). Five months after the start of the experiment (May), 90% of PU-beech leaf litter and 48% of PU-ash leaf litter remained in the L-horizon. After ten months (October), the major part of the leaf litter applied had disappeared from the L-horizon and only 24-36% of beech leaf litter and 3-7% of ash leaf litter remained. Leaf litter mass loss was positively related to the initial leaf litter Ca concentration (R^2=0.31 after 5 months and R^2=0.62 after 10 months) and negatively to the initial leaf litter lignin concentration (R^2=0.59 after 10 months).

There also occurred a net N loss (total N: ^{15}N and ^{14}N) during leaf litter decomposition (Table 4.2). After ten months, only 3-13% of the total N added as ash leaf litter remained in the L-horizon. In PU-beech leaf litter, the percentage of net N loss was lower than in any other leaf litter type. Further, it was only half of the percentage of C loss from the leaf

litter. Net leaf litter N loss after ten months was best explained by the initial leaf litter lignin concentration (R^2=0.69, negative relationship).

The leaf litter C:N ratio strongly decreased during the first 5 months of decomposition (Table 4.1 and 4.2). It remained lower in PL-ash and PL-beech leaf litter compared to the unlabeled leaf litter. Changes of the C:N ratio from May to October were not significant, except for a relatively small increase from May to October in PU-ash leaf litter.

Table 4.2 Proportion of leaf litter mass (%) remaining as well as the proportion of N remaining in the leaf litter (% of initially added) and its C:N ratio after 5 and 10 months of decomposition. Displayed are means with their standard deviation in brackets (n=4). Different letters indicate significant differences between treatments (p<0.05).

Treatment	May 5th 2009			Oct. 20th 2009		
	Litter mass [%]	N [%]	C:N	Litter mass [%]	N [%]	C:N
PU-beech	90^{b} (2)	127^{a} (6)	35^{c} (5)	36^{b} (2)	60^{b} (12)	31^{b} (5)
PL-beech	74^{b} (8)	78^{a} (20)	19^{a} (3)	24^{ab} (18)	24^{a} (16)	17^{a} (1)
PU-ash	48^{a} (13)	72^{a} (21)	27^{b} (2)	3^{a} (2)	3^{a} (3)	35^{b} (5)
PL-ash	73^{b} (9)	102^{a} (24)	15^{a} (1)	7^{a} (12)	13^{a} (21)	18^{a} (4)

4.4.4 Leaf litter C and N in the O-horizon and mineral soil

Mean total recoveries of leaf litter-derived C and N in the O-horizon and mineral soil (0-4 cm) after ten months were 13-23% (C) and 22-32% (N), respectively (Fig. 4.2 and 4.3). This made up approximately 20-30% (mean) of the total leaf litter C loss (calculated from the remaining carbon in the L-horizon). Recovery of leaf litter-derived C and N in the mineral soil (0-4 cm) was at most 5 and 8%, respectively, for both sampling dates (Fig. 4.2 and 4.3). The measured values in the O-horizon and mineral soil varied up to ~100% around the mean and no differences between treatments were observed in the recovery of leaf litter derived C and N. The recovery of leaf litter-derived C in C_{org} increased from May (1-2%) to October (up to 5%) while there was no significant difference in the recovery of leaf litter-derived N in N_t between May and October. The mean recovery of leaf litter-derived C (7-20%) and N (9-35%) in the O-horizon was much higher than in the mineral soil after five and ten months (Fig. 4.2 and 4.3).

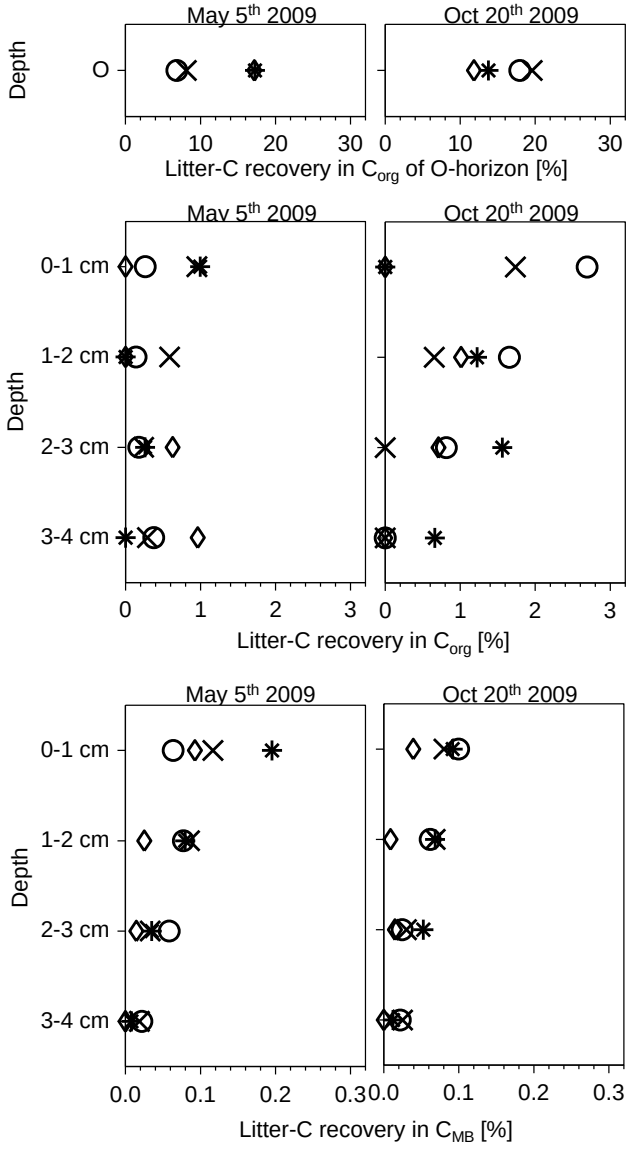

Figure 4.2 Litter-derived C in C_{org} and C_{MB} on May 5th 2009 and October 20th 2009. Displayed are means (n=4). ×: PL-ash derived C; ✳: ML-ash derived C; ○: PL-beech derived C; ◊: ML-beech derived C. More litter C was recovered in the O-horizon than in the mineral soil ($p<0.05$). There were no significant differences between treatments and interdependencies between treatments and mineral soil depths.

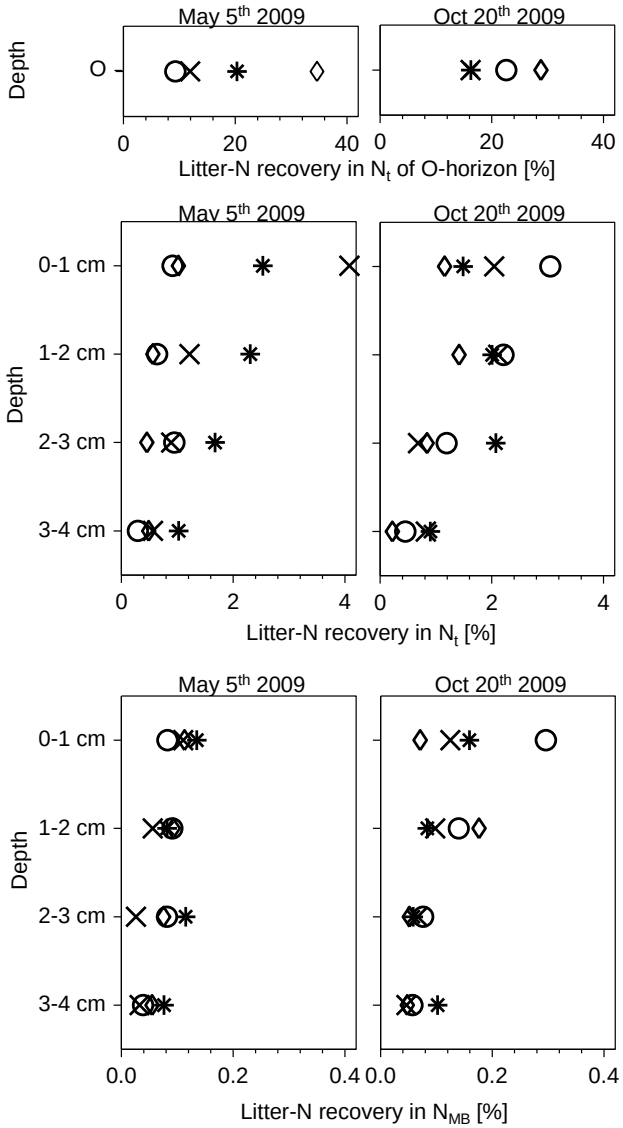

Figure 4.3 Litter-derived N in N_t and N_{MB} on May 5th 2009 and October 20th 2009. Displayed are means (n=4). ×: PL-ash derived N; ✳: ML-ash derived N; ○: PL-beech derived N; ◊: ML-beech derived N. More litter N was recovered in the O-horizon than in the mineral soil ($p<0.05$). There were no significant differences between treatments and interdependencies between treatments and mineral soil depths.

4.4.5 Leaf litter C and N in microbial biomass

In the upper mineral soil (0-4 cm), MB contributed approximately 2-3% to N_t (Table 4.3) and 1% to C_{org}. The recoveries of leaf litter derived C and N in MB did not differ between treatments and were <1% of the initial leaf litter C and N. The measured values varied up to ~100% around the mean. Five months after leaf litter application (May), leaf litter-derived N in microbial biomass accounted for 6-7% of total ash leaf litter derived N in N_t and the proportion was 12-13% for beech leaf litter (Table 4.3). Ten months after litter application, no differences were observed anymore. The recoveries of leaf litter-derived C and N (the latter only in October) in MB declined with increasing depth (Fig. 4.2 and 4.3)

and they did not change from May to October (one exception: in ML-beech less beech leaf litter-derived N was incorporated into the MB in October).

Table 4.3 Proportion (%) of N_{MB} to N_t in the top mineral soil (0-4 cm) calculated for total N and for the litter-derived N at two sampling dates. Displayed are means from the depth increments with the standard deviation in brackets (n=4). Different lower case letters (a,b) indicate significant differences between the treatments ($p<0.05$).

Treatment	Total N N_{MB}:N_t (%)		Litter-derived N N_{MB}:N_t (%)*	
	May 5th	Oct 20th	May 5th	Oct 20th
PL-beech	2.6 (0.4)^{b}	2.5 (0.8)^{a}	11.5 (5.6)^{ab}	9.0 (5.2)^{a}
ML-beech	2.7 (0.7)^{b}	3.2 (0.8)^{b}	13.2 (7.8)^{b}	9.2 (12.2)^{a}
PL-ash	1.8 (1.0)^{a}	2.8 (0.7)^{ab}	6.6 (4.7)^{a}	7.5 (4.2)^{a}
ML-ash	2.9 (0.9)^{b}	2.4 (0.8)^{a}	6.3 (5.2)^{a}	5.1 (3.8)^{a}

* Since for a lot of samples the recovery of litter-derived C was close to zero or zero, calculation of C_{MB}:C_{org} ratios for litter derived C and a comparison of these values were not reasonable.

4.5 Discussion

4.5.1 Leaf litter mass loss and CO_2 emission

4.5.1.1 Effects of leaf litter type

We determined two proxies for leaf litter decomposition that can be used to compare decay of leaf litter from different species (Berg 2000; Ngao et al. 2005; Jacob et al. 2010; Kammer and Hagedorn 2011; Vesterdal et al. 2012): leaf litter mass loss and emission of leaf litter-derived CO_2-C.

During the first five months of the experiment, ash leaf litter was mineralized faster than beech leaf litter (proxy: leaf litter-derived CO_2 emission). Similarly, Melillo et al. (1982) found higher mass losses in leaf litter low in lignin than in leaf litter high in lignin. In agreement with our results, Vesterdal et al. (2012) found lowest C turnover rates in beech and highest C turnover rates in ash leaf litter in a common garden experiment design in Denmark. After May 7th, leaf litter-derived CO_2-C flux did not differ anymore between treatments and it slowly declined, suggesting decomposition being in the second phase, the breakdown of lignin and other more recalcitrant compounds (Berg 2000). The results show that the different composition of beech and ash leaf leaf litter affected decomposition and mineralization particularly in the initial phase of leaf litter decay.

Both proxies of leaf litter decomposition (i.e. leaf litter-derived CO_2 emission and leaf litter mass loss) showed that ash leaf litter was decomposed more rapidly than beech leaf litter even though these proxies covered different processes (i.e. mineralization of leaf litter carbon and all processes which contributed to loss of leaf litter dry weight). The

difference between these proxies could be partly explained by the recovery of leaf litter-C in the O-horizon and in the upper mineral soil. However, there was still a considerable gap in the mean total recovery of leaf litter-derived ^{13}C of 11-40% for ash leaf litter and approximately 30% for beech leaf litter. This gap most likely resulted from the uncertainty in the cumulated leaf litter-derived CO_2 fluxes, which were based on biweekly measurements. Leaching of leaf litter-derived dissolved organic carbon to deeper soil layers was of minor importance (Scheibe, personal communication). Nonetheless, the results provide reasonable information on differences of the mineralization dynamics of beech and ash leaf litter. In addition, they show that leaf litter mass loss and faster decomposition of ash leaf litter than of beech leaf litter was mainly driven by leaf litter mineralization.

Different leaf litter quality parameters were found to either have a rate-enhancing (N and Ca content) or rate-retarding (lignin content, C:N, lignin:N) effect on the decomposition process (Melillo et al. 1982; Berg 2000; Jacob et al. 2010). Even though differences between labeled ash and labeled beech leaf litter were either not existing (C:N ratio) or considerably smaller (lignin:N ratio, N content) than usually observed between these two species (e. g. Jacob et al. 2010), we still found that labeled ash leaf litter was decomposed more rapidly than labeled beech leaf litter and could relate this to the initial litter Ca and lignin concentration. This is in accordance with findings from Melillo et al. (1982) who suggested that in substrates where N was not limiting possibly the lignin content is a better predictor for decomposition rate than the lignin:N ratio.

4.5.1.2 Effects of leaf litter mixture

Mixture of ash and beech leaf litter had a positive effect on the mineralization rate of ash leaf litter but did not affect beech leaf litter mineralization. In contrast to our results, Jacob et al. (2009, 2010) reported that in mixture with leaf litter from other deciduous species, decomposition of beech leaf litter can be accelerated but also retarded depending on the site characteristics such as the moisture regime and the time period in which the decomposition was investigated. Several studies found synergistic mixture effects on the decomposition of leaf litter from various tree species (Hättenschwiler et al. 2005 and references therein), but there also exist studies that found purely additive effects on leaf litter decomposition (Ball et al. 2008; Lummer et al. 2012). Hättenschwiler et al. (2005) named four possible reasons for non-additive mixture effects: (1) nutrient transfer from one leaf litter to another, (2) influences related to specific leaf litter compounds, (3) improved microclimatic conditions or habitat diversity in leaf litter mixtures and (4) interactions across trophic levels. However, they further pointed out that the processes controlling additive and non-additive effects on leaf litter decomposition in mixtures are still

not fully understood. In the present study, beech leaf litter probably functioned as a microhabitat for decomposers and thus enhanced decomposition of ash leaf litter. Since both of our labeled leaf litter types had a clearly higher N content than both non-labeled leaf litter types, a possible synergistic effect related to N transfer on the decomposition of labeled leaf litter can be excluded in our study.

4.5.2 Release and immobilization of nitrogen

Nitrogen release and immobilization of exogenous N can occur simultaneously during leaf litter decomposition (Cotrufo et al. 2000). By May, the absolute amount of N in the unlabeled beech leaf litter increased and indicated a net N immobilization (Zeller et al. 2000). External N may be provided by the canopy throughfall (Downs et al. 1996), diffusion from the O-horizon (Berg 1988) or via import through soil fauna, fungi or bacteria (Setälä et al. 1996; Lummer et al. 2012). The ^{15}N-labeled litter enabled more detailed insights in the release and immobilization of N during decomposition (Ventura et al. 2009):

Equation 4.11: $gross\ N - loss\ [\%] = (1 - \left(\frac{^{15}N_{excess}\ [mg] in\ the\ sampled\ litter}{^{15}N_{excess}\ [mg] in\ the\ initial\ litter}\right) \times 100)$

Mean gross loss of the initial labeled beech leaf litter N was 60% after five months and 87% after 10 months of decomposition. Mean gross loss of the initial labeled ash leaf litter N was 40% after five months and 91% after 10 months of decomposition. Gross and net releases of leaf litter N are influenced by initial leaf litter N content (Cotrufo et al. 2000). The high rates observed in our study for beech leaf litter are non-typical and probably a result of the exceptionally high N content of the labeled beech leaf litter (Cotrufo et al. 2000).

4.5.3 Partitioning of leaf litter C to the O-horizon and the mineral soil

Mean total recovery of C from leaf litter in the O-horizon and top mineral soil (0-4 cm) explained approximately 20-30% of the total C mass loss from the L-horizon. Similarly, Ngao et al. (2005) recovered 80% of the annual beech leaf litter C loss in the CO_2 efflux and assumed that the other 20% were partitioned to the mineral soil, and Kammer and Hagedorn (2011) found approximately 8% of initial beech leaf litter C in the top 2 cm of mineral soil, while 31% were mineralized after 12 months of decomposition. The limited transport of leaf litter C to the mineral soil (<5% after 10 months of decomposition) has been also documented previously for conifers (e.g. Bird and Torn 2006).

Our results indicate that the partitioning of ash and beech leaf litter C was similar under the same environmental conditions, which contradicts the repeatedly reported higher topsoil C stocks under ash than under beech (Vesterdal et al. 2008; Guckland et al. 2009;

Langenbruch et al. 2012). This discrepancy may have several reasons: First, the composition of beech and ash leaf litter typically differs a lot more than we observed for our labeled leaf litter types (e.g. Jacob et al. 2009, 2010; Langenbruch et al. 2012). Second, in our experiment, the ash leaf litter was introduced to a beech forest soil and therefore was exposed to exactly the same soil conditions as the beech leaf litter. Usually, soil conditions are more favorable for leaf litter decomposition (e.g. higher pH and nutrients) under ash than under beech (Guckland et al. 2009; Jacob et al. 2009; Langenbruch et al. 2012). Third, the positive effect of ash leaf litter on soil properties is considered a long-term tree species effect and is hardly apparent after a few months.

There were no differences between mixed and pure treatments for leaf litter-derived C in the O-horizon and mineral soil. On the one hand, this resulted from the similar partitioning of ash and beech leaf litter C to the O-horizon and mineral soil. On the other hand, we expected additive effects, because the abundance and proportion of beech and ash leaf litter was found to be more important for C stocks in the O-horizon and topsoil than the species richness (Guckland et al. 2009; Langenbruch et al. 2012).

4.5.4 Partitioning of leaf litter N to the O-horizon and the mineral soil

Recovery of leaf litter-derived N in the O-horizon (9-35%) and mineral topsoil (<8%) followed the same pattern as the distribution of litter C for both species. Similarly, Zeller et al. (2001) found that after two years, >50% of the N derived from the beech leaf litter remained in the forest floor and the upper two centimeter of mineral soil. The low total recovery of N from the leaf litter in our results points to effective N uptake by trees since mycorrhiza and root tips underneath the mesocosms were enriched with ^{15}N compared to the control (Seven, personal communication). There was no evidence of a significant amount of N leaching to deeper soil layers (Talkner, personal communication) or emission from the soil (Guckland et al. 2010). Our results show short term partitioning of N from the leaf litter. The small total N stock of the O-horizon ($\approx$ 30 g N m^{-2}) indicates fast N cycling and little long-term N-accumulation in the organic layer.

Results indicate that under similar decay conditions, e.g. precipitation, soil conditions and microbial biomass, no difference occurred in the short-term partitioning of beech and ash leaf litter N to the mineral soil. Usually, ash leaf litter contains a lot more N than beech leaf litter (e.g. Langenbruch et al. 2012; Vesterdal et al. 2012). The unusually high N content in labeled beech leaf litter and the resulting small difference between labeled beech and labeled ash leaf litter probably contributed to our results. Further, the more favorable soil conditions (e.g. lower soil acidity) under ash than beech (Guckland et al. 2009; Jacob et al. 2009; Langenbruch et al. 2012) may favor N partitioning from the leaf litter to the mineral soil under natural conditions. This assumption is supported by findings

of Langenbruch et al. (2012) and Vesterdal et al. (2008), that the concentration of N_t is higher in the topsoil under ash than beech, and that this elevated N_t concentration is related to the proportion of ash leaf litter to total leaf litter input (Langenbruch et al. 2012).

4.5.5 Partitioning of leaf litter C and N to soil microbial biomass

The low recovery of leaf litter-derived C and N in soil MB (<1% for both leaf litter types) is in line with findings by Zeller et al. (2001), who detected 0.5-1.5% of the N derived from beech leaf litter as part of the soil microbial biomass after six months (0-10 cm depth). We observed no species effect on the incorporation of leaf litter C and N into MB, which could be due to the similar N content of our labeled leaf litter.

Our results show a preferential incorporation of N from leaf litter into MB, which is in line with the observation of Bird and Torn (2006) who found that 4-28% of the vertically moved ^{15}N from Ponderosa pine needles was recovered as microbial ^{15}N. This indicates that MB prefers N from fresh leaf litter for their metabolic growth. Similar to our results, Lummer et al. (2012) found no effect of leaf litter type or mixture on the soil MB. However, they discovered a higher proportion of saprophytic fungi in beech leaf litter than in ash leaf litter. Further, in their decomposition study of ^{13}C-labeled straw and root residues of crimson clover (*Trifolium incarnatum* L.) and ryegrass (*Lolium multiflorum* Lam.), Williams et al. (2006) discovered that MB feeds substrate specifically. This indicates that other microbial communities might have been involved in the decomposition of ash leaf litter than of beech leaf litter. This hypothesis could be checked by ^{13}C analyses of the PLFAs and should be included in future studies.

4.6 Conclusion

As hypothesized, mass loss was higher and mineralization was faster for ash leaf litter than beech leaf litter. Mineralization of ash leaf litter was enhanced when mixed with beech leaf litter indicating non-additive effects of the leaf litter mixture. In contrast to our hypothesis, no leaf litter type effect on the partitioning of leaf litter C and N to the O-horizon, the mineral soil or the MB was observed, possibly due to the similar lignin:N ratios and the very high N concentrations in both labeled leaf litter types. Our results describe short-term partitioning of leaf litter C and N during decomposition under standardized field conditions. They do not display long-term effects of leaf litter decomposition.

4.7 Acknowledgements

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5 PARTITIONING OF LITTER C DURING DECOMPOSITION OF ^{13}C-LABELED BEECH AND ASH LEAF AND ROOT LITTER - A LABORATORY INCUBATION EXPERIMENT

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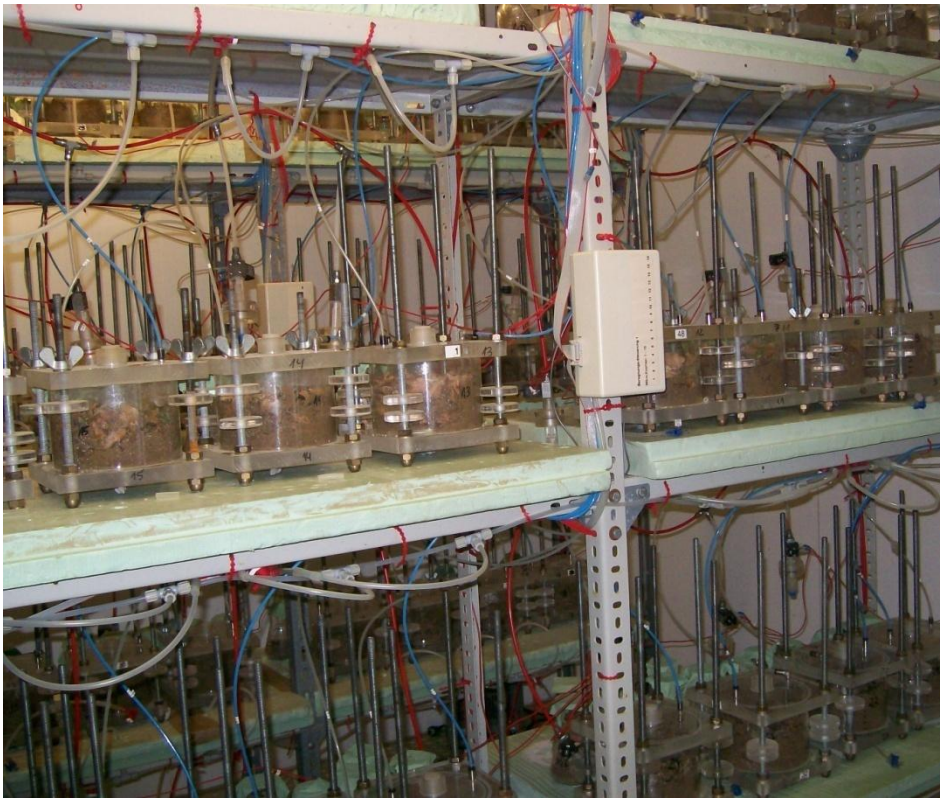

5.1 Abstract

The aim of this study was to detect the influence of litter type and litter mixture on the partitioning of litter-derived C during decomposition. In a laboratory incubation experiment (litter-soil mixture), the partitioning of litter C during decomposition to CO_2, dissolved organic C (DOC), microbial biomass C (C_{MB}) and light (particulate organic C) and heavy density fractions (mineral-associated C) was examined. Mesofauna and macrofauna were excluded from the soil. Decomposition of ^{13}C-labeled fine root and leaf litter of European ash (*Fraxinus excelsior* L.) and European beech (*Fagus sylvatica* L.) was compared during 206 days of incubation at 17°C air temperature and 21% (by mass) soil moisture. More litter-derived C was emitted as CO_2 from ash leaves (34%) and roots (29%) than from beech leaves (24%) or roots (23%). Further, incorporation of litter derived C into C_{MB} was higher for ash (0.7-1.0%) than beech (0.2-0.4%). Litter derived DOC was negligible after 206 days. Four to twelve percent of litter derived C was found in the heavy fraction. We observed no differences between the litter types concerning DOC and density fractions. Mixture of ash and beech leaf litter enhanced the decomposition of ash leaf litter (positive mixture effect) while the decomposition of all other litter types (beech leaf litter as well as ash and beech root litter) showed purely additive effects. The present study shows that (1) the litter decomposition may show deviant behavior in litter mixtures compared to pure variants and therefore transfer of results from the decomposition of litter species in pure to mixed treatments is limited, and (2) root litter decomposes more slowly than leaf litter, indicating a longer residence time of partly decomposed root litter C in the soil. Consequently, root litter shall be included in future studies on soil organic matter formation.

5.2 Introduction

Temperate forests provide an important sink for atmospheric CO_2. They sequester 0.6-0.7 Pg C per year (Goodale et al. 2002) which is approximately half of the annual uptake of the global land biosphere (1.4 Pg C per year) (Battle et al. 2000), even though forests and woodlands make up only 36% of the temperate land area (Goodale et al. 2002). Besides the incorporation of atmospheric CO_2-C into the plant biomass, this sink function is ascribed to large C stocks in forest soils. In Europe the relation of plant biomass to soil organic C (SOC) is almost 1:2 (Goodale et al. 2002).

Soil organic C storage was found to be affected by tree species (Finzi et al. 1998a; Langenbruch et al. 2012; Oostra et al. 2006; Vesterdal et al. 2008). For example, SOC stocks are higher under ash than under beech (Langenbruch et al. 2012; Oostra et al.

2006), while in the forest floor it is vice versa (Langenbruch et al. 2012; Vesterdal et al. 2008). Thus, it is of outstanding interest to understand how the C storage in forest soils and forest floor may be increased by an appropriate choice of tree species. However, the underlying processes are not sufficiently understood (von Lützow et al. 2006).

Among other reasons, differences in C stocks result from different decomposition rates depending on tree species, which in turn could entail differences in C partitioning. Jacob et al. (2009) and Vesterdal et al. (2012) found slower decomposition of European beech (*Fagus sylvatica* L.) leaf litter than European ash (*Fraxinus excelsior* L.) leaf litter. Similarly, Scheu and Schauermann (1994) found that ash root litter decomposed faster than beech root litter. A slower decomposition of leaf litter leads to higher C stocks in the forest floor, while faster decomposition leads to a higher partitioning of litter C to the mineral soil (Vesterdal et al. 2008). Not only litter of different species shows different decomposition behavior, but also leaf and root litter of the same species. Most studies report slower decomposition of root litter than of the respective leaf litter (Hansson et al. 2010; Heim and Frey 2004; Uselman et al. 2007). The different decomposition rates have often been explained by differences in the chemical composition between leaves and roots. Litter decay rate was positively related to the concentration of different base cations (Hobbie et al. 2007, 2010; Silver and Miya 2001; Vesterdal et al. 2012) and negatively to the lignin content or lignin:N ratio (Chen H. et al. 2002; Hobbie et al. 2007, 2010; Mao et al. 2011; Melillo et al. 1982; Silver and Miya 2001; Vesterdal et al. 2012). Due to its longer mean residence time, root litter contributes to a significant portion to the C fixation in the soil (Rasse et al. 2005; Tefs and Gleixner 2012) indicating that root litter decomposition is of higher importance than leaf litter decomposition to the C budget in the soil (Bird et al. 2008; Bird and Torn 2006; Hansson et al. 2010; Mambelli et al. 2011).

Few studies so far have examined the partitioning of leaf or root litter C in the course of decomposition (Bird et al. 2008; Bird and Torn 2006; Fahey et al. 2011; Fröberg et al. 2007a; Kammer and Hagedorn 2011). During litter decomposition, litter derived C may be mineralized or enter the soil, e.g. as dissolved organic carbon (DOC) or dissolved inorganic C, via microbial biomass (MB) or the soil fauna. The main pathway during litter decomposition is mineralization. In two studies on beech litter decomposition, about 30-37% of beech litter derived C was mineralized after one year (Kammer and Hagedorn 2011; Ngao et al. 2005), while only 4% was leached as DOC (Kammer and Hagedorn 2011). Incorporation into MB seems to be of the same order of magnitude as leaching via DOC: For example, Fahey et al. (2011) found 2-3% of sugar maple (*Acer saccharum* Marshall) leaf litter C in MB down to 10 cm depth. At least in the short-term, stabilization of litter C by organo-mineral association is also of minor importance. Bird et al. (2008) found approximately 3.4% of needle litter C and 1.8% of root litter C of Ponderosa pine (*Pinus*

ponderosa Laws.) associated to minerals in the top 20 cm of the soil after six months of decomposition.

Under natural conditions, often a mixture of litter from different species exists at one site. Many studies found non-additive effects of leaf litter mixing on decomposition (e.g. Wardle et al. 1997), most of them positive (Gartner and Cardon 2004; Salamanca et al. 1998). Jacob et al. (2009) found in a 22 months' litterbag study in Hainich National Park that most species, including beech, were decomposed faster in species rich stands than in mono-species stands. In contrast, some studies found purely additive decomposition rates in litter mixtures (Ball et al. 2008; Blair et al. 1990; Klemmedson 1992). Whether litter mixtures lead to additive or non-additive decomposition rates seems to depend on the abundant litter species (Gartner and Cardon 2004), the site properties (Jacob et al. 2009, 2010) and the mixing ratio (Salamanca et al. 1998) and no general pattern could be observed so far (Hättenschwiler et al. 2005). Research on decomposition of root litter in mixture is, to the best of our knowledge, lacking for forest ecosystems. We found two studies that compared root litter decomposition of herbs and grasses or arctic species in mixed and pure variants, which reported both positive and negative mixture effects on the decomposition rates (de Graaff et al. 2011; Robinson et al. 1999). The mechanisms controlling these effects are unknown so far. This emphasizes great need for further research on this topic.

In the present study, we investigated the decomposition of ^{13}C-labeled leaf and root litter of European beech and European ash through microorganisms during a 206-day incubation experiment. We compared the decomposition of litter species in pure and mixed variants and followed the partitioning of the litter-C into the emitted CO_2, the light (LF) and heavy fraction (HF), the DOC and the MB. We tested the following hypotheses:

(1) The decomposition of ash leaf litter is faster than of beech leaf litter and faster decomposition is associated with a greater partitioning of litter C to the HF and the soil MB.
(2) Decomposition of root litter is slower than of leaf litter because of a higher content of lignin in roots than in leaves.
(3) The mixture of beech and ash litter affects the partitioning of C from the respective litter type, but the partitioning of litter C of the litter mixture is additive.

5.3 Materials and methods

5.3.1 Mineral soil

The mineral soil was collected from a beech stand in Hainich National Park, Thuringia, Germany and consisted of about 50% each of Ah and Al horizon (Finnern et al. 2005). The topsoil (0-30 cm) contained 2-3% sand, 82-83% silt and 15% clay (Guckland et al. 2009). The pH measured in 1 *M* KCl-solution was 3.6 and the base saturation amounted 44.3%. The cation exchange capacity was 54.5 $mmol_c\ kg^{-1}$ of which Al^{3+} and Ca^{2+} accounted for 43% and 37%, respectively. The C:N ratio was 13.

The soil was frozen for 7 days at -20°C in order to kill the mesofauna and macrofauna before incubation, but to ensure the survival of the microbial biomass (Martens 1995). Thereafter the soil was air dried and sieved to 4 mm.

5.3.2 Study species

For the experiment, leaf and fine root litter of European beech and European ash was chosen, because these litter types differ in their chemistry (Langenbruch et al. 2012; Scheu and Schauermann 1994), such as lignin contents, the lignin:N ratio or C:N ratio, which might lead to different decomposition rates (Jacob et al. 2010; Melillo et al. 1982; Scheu and Schauermann 1994). In order to detect possible differences between the litter types in the partitioning of C during decomposition, we used ^{13}C labeled leaf and fine root litter, which derived from young ash and beech trees that were grown in a greenhouse under $^{13}CO_2$-enriched atmosphere (~300δ‰ V-PDB) for one vegetation period. For reference, ash and beech leaves and fine roots with natural abundance of ^{13}C were sampled in the Hainich. The roots were cut from young live trees and carefully washed to free them from minerals. All litter types were air dried prior to the experimental setup.

5.3.3 Experimental setup

The incubation experiment was carried out using an automated microcosm system (Hantschel et al. 1994) and run for 206 days (April – November 2010) with a soil moisture of 21% (mass) and at a constant air temperature of 17°C. This equals a year in 17°C-days (defined as the sum of temperatures (negative temperatures were treated as zero) in 2008 in Hainich National Park divided by 17°C).

The following variants were installed with three replicates each: (1) bare soil (control), (2) soil with incorporation of pure labelled beech leaf litter (PL-$beech_{leaf}$), (3) soil with

incorporation of 1:1 mixture of labelled beech and non-labelled ash leaf litter (ML-beech$_{leaf}$), (4) soil with incorporation of pure labelled ash leaf litter (PL-ash$_{leaf}$), (5) soil with incorporation of 1:1 mixture of labelled ash and non-labelled beech leaf litter (ML-ash$_{leaf}$), (6) soil with incorporation of pure labelled beech root litter (PL-beech$_{root}$), (7) soil with incorporation of 1:1 mixture of labelled beech and non-labelled ash root litter (ML-beech$_{root}$), (8) soil with incorporation of pure labelled ash root litter (PL-ash$_{root}$) and (9) soil with incorporation of 1:1 mixture of labelled ash and non-labelled beech root litter (ML-ash$_{root}$), whereas "P" indicates pure variants, "M" mixed variants and "L" labelled litter. Five gramm (dry matter (DM)) of the litter (in mixed variants 2.5 g of each litter type) were mixed with 600 g dry soil from Hainich National Park and incorporated in plastic cylinders (microcosms) with an inner diameter of 14.2 cm and a height of 9.6 cm. The microcosms were sealed with a lid that had an air inlet and an air outlet port. A continuous flow of 10-15 ml min^{-1} of fresh air through the microcosm headspace allowed gas exchange.

5.3.4 Laboratory analyses

5.3.4.1 Chemical composition of initial litter

A subsample of each litter type was ground finely, to pass through a 1 mm sieve in an Ultra Centrifugal Mill ZM 1000 (RETSCH, Haan, Germany). Part of the ground material was then weighed into tin capsules and measured for the C and N content by an automated C and N analyzer (Heraeus Elementar Vario EL, Hanau, Germany) and the isotopic composition by an IRMS Delta Plus (non-labeled samples) or an IRMS Delta C (labeled samples) (Finnigan MAT, Bremen, Germany). For the analysis of the plant components, 100 mg of the ground material was extracted with a methanol:chloroform:water (MCW; 2:2:1) mix and prepared for isotopic analyses as described in Pollierer et al. (2009). After the extraction, a pellet remained, from which lignin and holocellulose contents were extracted as described in Allen (1974) and then weighed into tin capsules. The isotopic composition of the plant components was measured using an IRMS Delta XP (Thermo Electron Cooperation, Bremen, Germany) at the Center for Stable Isotope Research and Analyses, University of Goettingen (KOSI).

5.3.4.2 CO_2-flux measurements

Carbon dioxide concentration of the air input ("in") and exhaust air ("out") of each microcosm (3 times a day) and of calibration gases (9 times a day) was measured continuously using an automated gas chromatographic system as described by Loftfield et

al. (1997) and Flessa and Beese (1995, 2000). The CO_2 production of the soil-litter mixture was calculated using equation 5.1.

Equation 5.1: $V_0[ml\ min^{-1}] = \frac{273}{273+17°C} \times \frac{flow\ [ml\ min^{-1}]}{1000000} \times (C_{out}[ppm] - C_{in}[ppm])$

Here, "flow" is the continuous flow of air through the microcosm, "C_{out}" stands for the CO_2-concentration of the out flowing air from the microcosm and "C_{in}" for the CO_2-concentration of the inflowing air.

Measurements were counted as valid, if the calibration quality (calculated from 100 minus the average percentage deviation of the measured points from the resulting calibration curve) was at least 95%. For each day, averages were calculated from the valid measurements and extrapolated to daily production rates using equation 5.2.

Equation 5.2: $CO_2 - C[mg\ d^{-1}] = \frac{V_0}{Vm} \times 12.0107[g\ mol^{-1}] \times 60\ \mathrm{min\ h^{-1}} \times 24\ h\ d^{-1}$

Here, Vm stands for the volume of the gas at a specific temperature and may be calculated via the general gas equation (Equation 5.3):

Equation 5.3: $Vm = \frac{1mol \times 8.314472J\ mol^{-1} \times \frac{1}{K} \times (273K+17°C)}{101.325hPa}$

The daily fluxes were then cumulated over the 206 days of incubation.

For the detection of labeled litter mineralization rates, output air from each microcosm was sampled in LABCO EXETAINER of 12 ml volume (Labco Limited, Buckinghamshire, United Kingdom) by connecting the EXETAINER with a needle to the exhaust tube for 30 minutes. A second needle allowed the flow and complete gas exchange in the EXETAINER. Additionally, samples of the input air were collected for analysis. Sampling was conducted every three days during the first six weeks of the experimental period and once a week thereafter. The CO_2-concentrations and the $\delta^{13}C/^{12}C$ ratio in the gas samples were measured using an IRMS Delta plus with GP interface and GC-Box (Thermo Fisher, Bremen, Germany) at the KOSI. Samples were introduced with an Autosampler (CTC-Analytics AG, Zwingen, Switzerland). The atom%^{13}C of the CO_2 that respired from the soil litter mixture was calculated via Equation 5.4.

Equation 5.4:

$$atom\%^{13}CO_2{-}C_{treatment} = \frac{atom\%^{13}C_{out} \times CO_2 - C_{out}\ [ppm] - atom\%^{13}C_{in} \times CO_2 - C_{in}\ [ppm]}{CO_2 - C_{out}[ppm] - CO_2 - C_{in}[ppm]}$$

The litter-derived CO_2-C emission was used to determine dynamics of litter mineralization. The amount of litter-derived dissolved CO_2 is insignificant in our through-flow incubation approach.

5.3.4.3 Dissolved organic C

For the measurement of DOC at different times during the experiment, 300 ml Erlenmeyer flasks were filled with a mixture of 60 g soil and 0.5 g litter (DM), i.e. the mixing ratio was identical with that of the microcosms. On days 9 and 29, three replicates of each variant were destructively harvested. The soil-litter mixture was transferred into 200 ml polyethylene bottles. The bottles were then filled with 120 ml of water. The bottles were shaken for 30 min at 120 rpm. The extract was first filtered through paper filters (Whatman 595 ½, 4-7 µm and Whatman 589/1 ashfree, 12-25 µm, Springfield Mill, UK) and then through mixed cellulose ester membranes (Whatman ME 25; 0.45 µm, Springfield Mill, UK). The filtrate was frozen at -18°C until measurement. Dissolved organic C in the filtrate was measured using a TOC Analyser 5050 (Shimadzu, Duisburg, Germany). The $\delta^{13}C/^{12}C$ ratio in the DOC was measured in bulk mode at a Delta V Advantage (Thermo Fisher, Bremen, Germany) coupled to an LC Isolink (Thermo Fisher, Bremen, Germany) at the KOSI.

5.3.4.4 Density fractionation

In order to separate the heavy fraction (HF; organic matter associated with mineral surfaces) from the light fraction (LF; partly decomposed free and occluded particulate organic matter), a modification of the density fractionation method after Christensen (1992) was conducted using sodium polytungstate $Na_6(H_2W_{12}O_{40})*H_2O$ (SOMETU, Berlin, Germany, "SPT") with a density of 1.8 g cm^{-3}.

Briefly, the soil-litter-mixture was dried at 40°C until of constant weight. Subsequently, 10 g samples were weighed into centrifuge tubes and filled with 40 ml of the SPT-solution. Further, 10 glass beads (∅ 5 mm) were added. The tubes were sealed and shaken for 16 h at 80 rpm. Then, the solution was centrifuged at 3,800 g for one hour. The supernatant (LF) was filtered (Whatman ME 25; 0.45 µm, Springfield Mill, UK) and washed with 2 l of H_2O_{dem} in order to remove the salt residues. The filter residue was transferred into glass dishes and dried at 40°C to constant weight. The centrifugation residue (HF) was washed three times with H_2O_{dem} (centrifuge, 5,100 g, each 10 min). The residue was transferred into glass dishes and dried at 40°C until of constant weight. The dry fractions were reweighed and then ground in a planetary ball mill (RETSCH PM 4000, Haan, Germany). The ground material was weighed into tin capsules and measured at the automated C and N analyzer (Heraeus Elementar Vario EL, Hanau, Germany) for the C content. The $\delta^{13}C/^{12}C$ ratio was measured with the IRMS Delta plus (Finnigan MAT, Bremen, Germany) at the KOSI. Labeled LF samples were measured at the IRMS Delta C (Finnigan MAT, Bremen, Germany).

5.3.4.5 Microbial biomass

Microbial biomass C (C_{MB}) was measured at the end of the incubation time (day 206). Litter pieces were removed from the fresh soil by hand. Ten grams of the fresh material was weighed into glass bottles of 100 ml and then closed with a lid. The samples were then used for chloroform-fumigation-extraction (CFE), as described in Brookes et al. (1985) and Vance et al. (1987), however using 0.05 *M* K_2SO_4-solution instead of 0.5 *M* K_2SO_4-solution for the extraction, because large quantities of salt hamper the determination of isotopes using EA-IRMS (Potthoff et al. 2003). Joergensen (1995) extracted $CHCl_3$-labile MB with 0.01 *M* $CaCl_2$ solution and found that the amount is comparable to 0.5 *M* K_2SO_4 extractable MB. We therefore assume that this applies also for 0.05 *M* K_2SO_4 solution. Since we cannot say this with absolute certainty, we are referring here to the 0.05 *M* K_2SO_4 extractable MB as C_{MB} instead of C_{mic}.

Because of a high background of dead organic C, the samples were pre-extracted with 0.05 *M* K_2SO_4-solution by shaking for 30 min at 200 rpm followed by centrifugation (Mueller et al. 1992). The supernatant was discarded. A subsample of the residue was dried at 105°C for 24 h to determine the water content. From the remainder, two 5 g-samples were weighed into 150 ml glass bottles. One set of samples was fumigated with chloroform for 24 hours at 25°C under low pressure prior to the extraction with 20 ml of 0.05 *M* K_2SO_4-solution by shaking for 30 min at 200 rpm. The other set of samples was directly extracted with 20 ml of 0.05 *M* K_2SO_4-solution. The extracted samples were filtered through folded and black ribbon filters (Whatman 595 ½ and Whatman 589/1, Springfield Mill, UK) and then measured using a DIMATOC 100 (Dimatec, Essen, Germany) for their C content. The C_{MB} was then calculated via equation 5.5 (Joergensen 1996; Jörgensen 1995; Wu et al. 1990).

Equation 5.5: $C_{MB} = (C_{fum} - C_{non})/0.45$

C_{fum} stands for the C_{org} extracted from fumigated soil and C_{non} stands for C_{org} extracted from non-fumigated soil in µg g^{-1}. The values in the denominator describe the extractable part of the C_{MB}.

*q*MB is defined as the percentage of C_{MB} to organic carbon. As a proxy for organic carbon, we take the sum of LF-C and HF-C.

Equation 5.6: $qMB = \frac{C_{MB}\,(\frac{mg}{kg})}{(LF\ C + HF\ C)\,(\frac{mg}{kg})} \times 100\%$

The microbial activity can be defined by the metabolic quotient *q*CO_2 (Anderson and Domsch 1990,1993):

Equation 5.7: $qCO_2 = \frac{CO_2\text{-}C\ (\frac{\mu g}{kg*h})}{C_{MB}\ (\frac{mg}{kg})}$

For the determination of the isotopic composition of the C_{MB}, the extracts from the CFE-analysis were freeze-dried (CHRIST DELTA II, Osterode Harz, Germany). The salt was then weighed into tin capsules and measured for their $\delta^{13}C/^{12}C$ ratio using the Isotope Ratio Mass Spectrometry (IRMS Delta plus, Finnigan MAT, Bremen, Germany) at the KOSI. The isotopic composition of the C_{MB} was then calculated by Equation 5.8, as described in Dijkstra et al. (2006).

Equation 5.8: $atom\%^{13}C_{MB} = \frac{atom\%^{13}C_{fum} \times C_{fum}\left(\frac{\mu g}{g}\right) - atom\%^{13}C_{non} \times C_{non}(\frac{\mu g}{g})}{C_{fum}\left(\frac{\mu g}{g}\right) - C_{non}(\frac{\mu g}{g})}$

5.3.5 Calculations of litter recoveries

The proportion of litter derived C in the analyzed components was calculated via Equation 5.9 (Balesdent and Mariotti 1996).

Equation 5.9: $proportion\ of\ litter\ derived\ C = \frac{atom\%^{13}C_{treatment} - atom\%^{13}C_{control}}{atom\%^{13}C_{labelled\ litter} - atom\%^{13}C_{control}}$

Here, $atom\%^{13}C_{treatment}$ stands for the $atom\%^{13}C$ of the analyzed component in the labeled variants, $atom\%^{13}C_{control}$ for the $atom\%^{13}C$ of the analyzed component in the control and $atom\%^{13}C_{labeled\ litter}$ for the $atom\%^{13}C$ of the initial labeled beech or ash litter.

These were then converted into recovered litter derived C (%) (Eq. 5.10).

Equation 5.10: $Recovery\ of\ litter\ derived\ C\ (\%) = \frac{C_{total}(mg) \times proportion\ of\ litter\ derived\ C}{C_{litter}\ (mg)}$

C_{total} stands for the amount of C of the analyzed component in the microcosm and C_{litter} for the amount of C that was introduced to the microcosm with the labeled litter.

5.3.6 Statistical analyses

In order to detect possible differences between variants, an ANOVA or an ANOVA with repeated measures followed by a Tuckey-HSD Test ($p<0.05$) was conducted using Statistica 10.0 (StatSoft, Inc., 2010). In cases when the residuals were not normally distributed and/or the standard deviation was not homogenous and correlated positively with the mean, a Box-Cox transformation of the data was conducted. If no reasonable transformation was possible, a Kruskal-Wallis-Anova was conducted instead (recovery of litter C in HF, proportion of litter derived C to total DOC at day 29, proportion of cumulated litter derived C to total cumulated CO_2 at day 29). Simple and stepwise forward multiple

linear regression analyses ($p<0.05$) were conducted to find the best explaining litter chemistry parameters on the mineralization of litter-C.

5.4 Results

5.4.1 Litter chemistry

The litter types differed in their chemical composition (Table 5.1). Beech contained more lignin and a higher lignin:N ratio than ash for both leaf and root litter. Lignin content and the lignin:N ratio were higher in root litter than in the respective leaf litter for both beech and ash, while the C:N ratio between root and leaf litter only differed for ash. Beech leaf litter contained a higher C:N ratio than ash leaf litter. The same was true for non-labeled root litter. Considering the groups of non-labeled litter and labeled litter separately, beech litter contained a higher concentration of holocellulose than ash litter.

Lignin was depleted in ^{13}C while holocellulose was enriched in ^{13}C compared to the overall $\delta^{13}C$ value in the respective litter. Only in the case of labeled ash leaf litter were both lignin and holocellulose depleted in ^{13}C (compared to the overall $\delta^{13}C$ value of labeled ash leaf litter). Here, the highest label (133.1 $\delta‰^{13}C$, data not shown) was found in lipids and pigments. However, the relative deviations of the ^{13}C atom% of lignin and holocellulose to the ^{13}C atom% of the total C were within ±1%.

Table 5.1 Chemical composition of the initial litter. L refers to labeled litter, NL refers to non-labeled litter, $\delta^{13}C$ (‰) refers to the standard V-PDB.

Litter type		C		Lignin		Holocellulose		C:N	Lignin:N
		mg g^{-1}	$\delta^{13}C$ (‰)	mg g^{-1}	$\delta^{13}C$ (‰)	mg g^{-1}	$\delta^{13}C$ (‰)		
Leaf litter	L-beech	464	86,8	118	79,2	135	96,6	34	9
	NL-beech	492	-28,7	158	-30,5	211	-27,0	47	15
	L-ash	441	113,4	51	104,8	95	103,2	30	3
	NL-ash	460	-26,2	110	-28,0	165	-24,4	26	6
Root litter	L-beech	493	92,9	161	91,7	150	106,4	34	11
	NL-beech	514	-30,2	211	-31,0	233	-29,5	42	17
	L-ash	465	152,3	81	149,1	113	164,1	39	7
	NL-ash	484	-30,5	127	-31,4	155	-29,5	22	6

5.4.2 CO_2-efflux

The total and litter derived CO_2-efflux was highest during the first 40 days of incubation with the maximum occurring around day 10 (Figure 5.1). Mean cumulated total CO_2-emissions after 206 days of incubation was lower in the control (408 mg CO_2-C kg^{-1} DM) than in all variants with litter addition. Mean cumulated total CO_2-C efflux in litter variants after 206 days was lowest in PL-beech$_{root}$ (1343 mg CO_2-C kg^{-1} DM) and highest in ML-beech$_{leaf}$ (1692 mg CO_2-C kg^{-1} DM), possibly due to a fast mineralization of the ash leaf litter in this variant.

After 206 days, more ash leaf litter than beech leaf litter derived C was emitted (Table 5.1). This was related to a higher CO_2-efflux of ash litter derived C at the beginning of the experiment as can be seen from higher CO_2-fluxes during the first 20 days (Figure 5.1). After 206 days by trend ($p<0.1$) more ash root litter than beech root litter C was recovered in CO_2 (Table 5.2). Further, the recovery of ash leaf litter was higher than the recovery of ash root litter. No such effect was observed for beech root and leaf litter. Mixture of litter types seemed to have an enhancing effect for the decomposition of ash leaf litter, because the recovery of litter C was higher in ML-ash$_{leaf}$ than in PL-ash$_{leaf}$ (Table 5.2). However, no such effect was observed for ash root litter. Cumulated CO_2-fluxes of beech litter were not affected by litter mixture (Table 5.2).

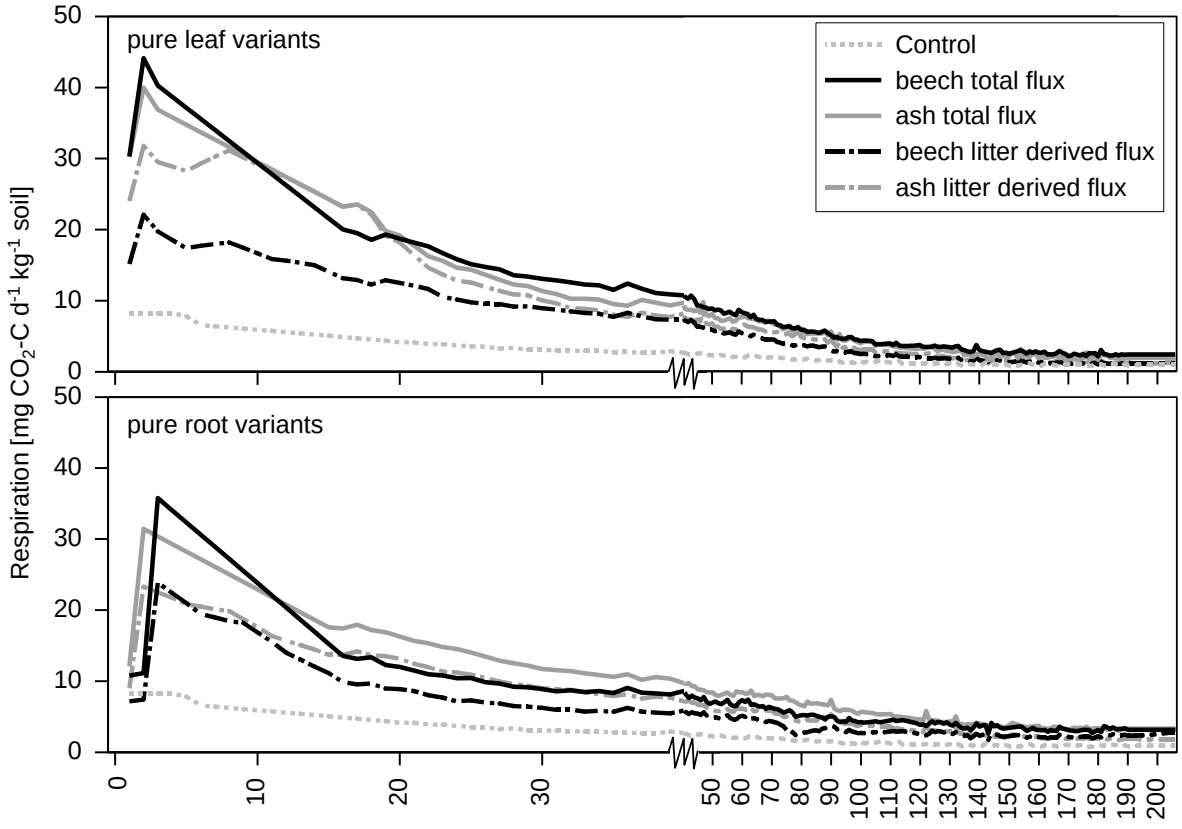

Figure 5.1 Total and litter derived CO_2-respiration of pure leaf (upper graphs) and pure root (lower graphs) treatments. Further, the daily CO_2-respiration of the control is presented. Displayed are daily means of the three repeats per variant.

Regression analyses revealed that litter chemistry could explain differences in litter derived CO_2-effluxes (data not shown). The concentration of N was not among the explaining variables, which was possibly related to very similar concentrations of N in all litter types (12.0-14.8 mg g^{-1}). Considering cumulated litter derived CO_2-effluxes over 206 days, among the best explaining variables were the lignin:N ratio (R^2=0.82) and the lignin concentration (R^2=0.79), both having a rate retarding effect on litter derived CO_2-effluxes.

Table 5.2 Recoveries of litter derived C at the end of the experiment (day 206) in cumulated CO_2 (CO_2-C), the light fraction (LF-C) and heavy fraction (HF-C), the microbial biomass (C_{MB}) and dissolved organic C (DOC). Displayed are means with standard deviation in brackets. Different lower case letters indicate significant differences between variants (p<0.05).

Variant	Recoveries of litter derived C (%)				
	CO_2-C	LF-C	HF-C	C_{MB}	DOC
PL-beech$_{leaf}$	24.2^{a} (1.1)	49.2 (7.9)	7.2^{ab} (2.3)	0.36^{ab} (0.09)	0.058^{ab} (0.022)
ML-beech$_{leaf}$	26.1^{ab} (1.5)	52.0 (18.2)	6.6^{ab} (0.7)	0.33^{ab} (0.04)	0.042^{ab} (0.008)
PL-ash$_{leaf}$	34.0^{c} (1.9)	40.3 (28.2)	7.5^{ab} (0.6)	0.65^{bc} (0.11)	0.064^{b} (0.007)
ML-ash$_{leaf}$	38.8^{d} (1.8)	51.5 (9.7)	12.2^{b} (5.3)	0.64^{bc} (0.17)	0.035^{ab} (0.006)
PL-beech$_{root}$	23.0^{a} (2.5)	33.0 (10.4)	3.7^{a} (1.0)	0.22^{a} (0.02)	0.028^{a} (0.008)
ML-beech$_{root}$	24.4^{a} (0.8)	67.8 (21.5)	4.9^{ab} (0.8)	0.26^{a} (0.06)	0.049^{ab} (0.004)
PL-ash$_{root}$	29.2^{b} (0.2)	73.1 (27.5)	7.8^{ab} (0.6)	0.97^{c} (0.23)	0.059^{ab} (0.003)
ML-ash$_{root}$	30.4^{bc} (0.9)	48.6 (13.9)	8.0^{ab} (0.8)	0.49^{bc} (0.06)	0.045^{ab} (0.010)

5.4.3 Dissolved organic C

Total water extractable DOC declined for all variants from day 9 over day 29 to day 206 (Figure 5.2). Litter derived DOC did not differ between day 9 and day 29, but declined until day 206. Total DOC of all variants with root litter did not differ from the control at days 9 and 29, while variants with leaf litter were higher compared to the control (except for PL-beech$_{leaf}$ at day 29). At day 206, total DOC of variants with leaf litter did not differ from the control any longer, while ML-beech$_{root}$ and PL-ash$_{root}$ were higher than the control at this day (Figure 5.2).

At the beginning of the experiment (days 9 and 29), the variants showed significant differences in the recovery of litter derived C in DOC. It was higher for PL-ash$_{root}$ (0.53% (day 9) and 0.28% (day 29)) than PL-beech$_{root}$ (0.05% (day 9) and 0.03% (day 29)) and it was higher for PL-ash$_{leaf}$ (0.39% (day 29)) than PL-beech$_{leaf}$ (0.31% (day 9) and 0.19% (day 29)). Further, the recovery of litter derived C in DOC was lower for PL-beech$_{root}$ than PL-beech$_{leaf}$. However, differences between variants had vanished by the end of the experiment (day 206; Table 5.2).

We found no mixture effects in the recovery of litter derived DOC at any time of the experiment. Similarly, no mixture effects occurred considering proportions of labeled litter derived C to total DOC because pure variants always showed twice the proportion of labeled litter derived C than the respective mixed variant. However, the proportion of labeled litter derived C to total DOC differed significantly between ash and beech as well as between leaf and root litter of one species at the beginning of the experiment (day 9). These differences were found for both, pure and mixed variants, e.g. ML-ash$_{leaf}$ (19%) > ML-ash$_{root}$ (13%) > ML-beech$_{leaf}$ (7%) > ML-beech$_{root}$ (2%).

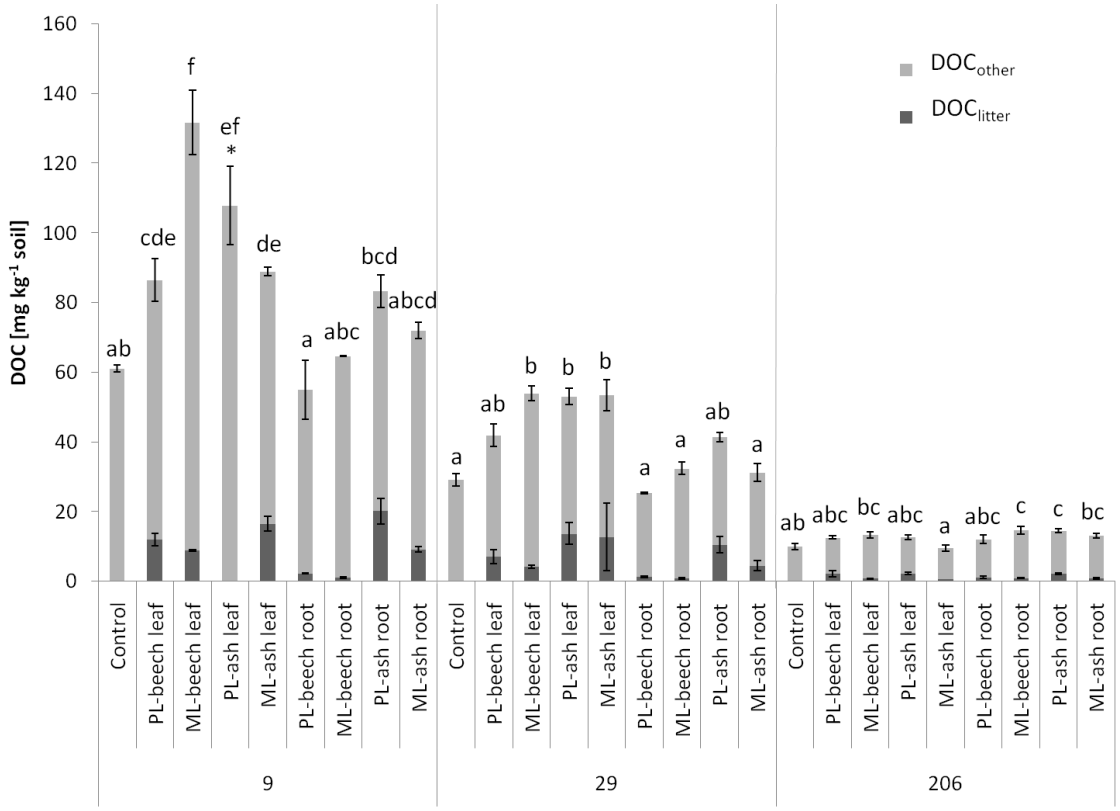

Figure 5.2 Total DOC at days 9, 29 and 206. The bars of the total DOC are divided into labeled litter derived DOC (DOC$_{litter}$) and DOC derived from older soil organic matter or unlabeled litter (DOC$_{other}$). Displayed are means with standard deviations. Different letters indicate significant differences between variants in total DOC concentration within one sampling day.

*: DOC$_{litter}$ could not be calculated because the measurement failed for this variant. Here, DOC$_{other}$ implies the entire DOC (derived from litter plus older soil organic matter).

5.4.4 Density fractions

In the control, 18% of the SOC was found in the LF and 82% in the HF. It was different from all variants with added litter where on average 37% of SOC was found in the LF and 63% in the HF. The litter variants did not differ significantly from each other (data not shown).

The recovery of litter derived C in HF and LF did not differ between variants, probably due to a high variation within one variant. Most litter derived C was still left in LF (average 55%) and 4-12% were found in the HF (Table 5.2). The proportion of litter derived C to total C in HF varied from 0.9-4.3% and was lower for PL-beech$_{root}$ (1.7%) than PL-ash$_{root}$ (3.8%). No such difference was observed between the two leaf litter types and no mixture effects were observed. In the LF, the proportion of litter derived C to total C ranged from 5-57% and did not differ significantly between variants.

5.4.5 Microbial biomass

Microbial biomass C averaged 83 mg kg^{-1} soil and was neither higher in variants with litter than in the control nor did it differ between the litter variants (data not shown). The metabolic quotient qCO_2 at day 206 differed between variants. It was lowest in the control (0.39±0.04). All variants including roots and ML-beech$_{leaf}$ had a significantly higher qCO_2 than the control with values ranging from 1.27±0.12 in ML-beech$_{leaf}$ to 2.53±0.83 in PL-beech$_{root}$. It was lower in PL-beech$_{leaf}$ (1.17±0.54) than in PL-beech$_{root}$. The *q*MB was lowest in PL-beech$_{root}$ (0.40±0.14%) and highest in the control (0.89±0.11%). It was negatively linearly correlated with qCO_2 (R^2=0.55).

Recovery of litter derived C in C_{MB} was higher for ash root litter (0.97%) than for beech root litter (0.22%), and by trend higher for ash leaf litter (0.65%) than for beech leaf litter (0.36%, Table 5.2). The recovery of litter derived C in C_{MB} did not differ between leaf and root litter within tree species. Mixture effects were observed neither for the recovery of litter derived C in C_{MB} (Table 5.2) nor concerning the proportion of litter derived C to total C_{MB} (data not shown). In pure variants, the proportion of litter derived C to total C_{MB} was significantly different between PL-beech$_{leaf}$ and PL-ash$_{root}$ and increased in the order: PL-beech$_{leaf}$ (15±3%) < PL-beech$_{root}$ (17±6%) < PL-ash$_{leaf}$ (26±6%) < PL-ash$_{root}$ (36±8%).

5.5 Discussion

The decomposition experiment was conducted in a laboratory under constant air temperature and soil moisture conditions. No plants were included. This enabled us to analyze purely litter type related effects and to avoid seasonal effects and variables related to weather extremes, like droughts, heavy rainfall, freezing and melting events. Further, the soil was free of soil mesofauna and macrofauna. Thus, decomposition was exclusively mediated by microbial biomass. We are aware of the artificial conditions and the associated restrictions, i.e. that we cannot transfer the absolute values obtained in this study to field conditions in the forest. However, by the controlled laboratory conditions natural fluctuations were minimized and thus differences between the variants were more clearly revealed as they would have been in a field experiment.

5.5.1 CO_2-efflux

Our findings that 24% of the beech leaf litter C was lost via the CO_2-efflux agree with those of Kammer and Hagedorn (2011) and Ngao et al. (2005), who found that mineralization was the main pathway of C loss from decomposing litter over one year (31-37% of beech leaf litter C).

Mineralization differed between litter types, which was, among others, related to the lignin:N ratio as often found in the literature for decomposition rates (Heim and Frey 2004; Jacob et al. 2010; Melillo et al. 1982; Silver and Miya 2001; Taylor et al. 1989). Similar to the results of our study, Jacob et al. (2009, 2010) and Vesterdal et al. (2012) found faster decomposition of ash leaves compared with beech leaves. Further, Baum et al. (2009) found more pronounced increases in CO_2-emissions during litter fall in mixed stands of beech, ash and maple species and the least pronounced increase in a pure beech stand in Hainich National Park. Also ash roots (29% of initial litter C) were mineralized faster than beech roots (23% of initial litter C) in our study. This is in accordance with results from Scheu and Schauermann (1994) who found higher C losses from ash fine roots (~40%) compared to beech fine roots (~15%) after 12 months.

Root litter mineralization proceeded more slowly than leaf litter mineralization in the case of ash litter. This is in agreement with findings in several studies on various tree species (Bird and Torn 2006; Hansson et al. 2010; Uselman et al. 2007; Wang et al. 2010). However, no different mineralization rates were observed for beech roots and leaves. Yet, this agrees with results from Heim and Frey (2004) who also observed no differences in the mineralization of beech leaves and roots. One possible explanation

could be that the lignin:N ratio differed only slightly between the leaf and root litter of labeled beech while it was twice as high in labeled ash root litter than leaf litter.

We found synergistic mixture effects on the CO_2-emission of ash leaf litter derived C, which is in line with the majority of findings on the decomposition of litter mixtures (Gartner and Cardon 2004; Hättenschwiler et al. 2005; Salamanca et al. 1998). In contrast, Jacob et al. (2010) found no mixture effect on ash litter mass loss. One possible explanation for synergistic effects on decomposition is the enriched microhabitat structure in mixtures (Chapman et al. 1988; Hättenschwiler et al. 2005).

Mineralization of beech litter was not affected by litter mixture. Similar to our results, Jacob et al. (2009, 2010) found that at a given site, the decomposition rate of beech litter did not differ between mono- and mixed-litterbags (Hainich National Park). However, decomposition of beech litter after 22 months increased with increasing species richness (Jacob et al. 2009). This means that a litter mixture itself does not enhance decomposition of beech litter but the interaction of environmental conditions determines the decomposition. Further, one theory is that a transport of N from N-richer to N-poorer litter enhances the decomposition of the latter (Chapman et al. 1988). However, this is not necessarily the case (Hättenschwiler et al. 2005) and seems to depend less on the height of difference between the litter types in mixture (Hoorens et al. 2003) rather than on the total N concentration in the litter mixture (Wardle et al. 1997). Supportively, Lummer et al. (2012) found that the N-transport is mainly conducted by saprophytic fungi, while bacteria rather hamper the transport. While bacteria occur more in N-rich substrates, saprophytic fungi dominate in N-poor substrates. It is possible that ash could have a positive effect on the decomposition of beech litter in a substrate that is limited in N, again indicating mixture effects being site specific.

Decomposition studies on root mixtures are very scarce and we are not aware of any that were conducted on root mixtures of temperate tree species. Nonetheless, for grassland species, de Graaff et al. (2011) found higher soil CO_2-efflux rates of root litter in mixture compared to pure variants. In their study on decomposition of root litter from arctic species, Robinson et al. (1999) found both positive and negative non-additive effects on the decomposition of root mixtures. In contrast to these two studies, we found no differences in the mineralization of beech and ash root litter between pure and mixed variants pronouncing the great need for decomposition studies of root mixtures.

5.5.2 Dissolved organic C

Most of the DOC in this study was derived from older SOM and not from the fresh litter. This goes in line with findings by Flessa et al. (2000) that from a soil which had been under maize cultivation for 37 years, only one third of the DOC was maize derived, while the other two third derived from older SOM. Similarly, Fröberg et al. (2007a) found that only 9% of the DOC that was leached from the organic layer in a spruce (*Picea abies* L.) forest derived from fresh needles.

Dissolved organic C was highest in the beginning and lowest in the end of our experiment, indicating that DOC was either mineralized (Don and Kalbitz 2005; Hansson et al. 2010; De Troyer et al. 2011), incorporated by microbial biomass (Uselman et al. 2007), precipitated (Kalbitz et al. 2000; Scheel et al. 2007) or had undergone organo-mineral association (Fröberg et al. 2007b; Kalbitz et al. 2005; Kalbitz et al. 2000; Kammer and Hagedorn 2011). Rapid loss of DOC from litter was repeatedly mentioned in the literature (Berg 2000; Don and Kalbitz 2005; Fröberg et al. 2007a; Hansson et al. 2010). Hansson et al. (2010) found that most mineralization of DOC (7-45%) takes place within the first three days of decomposition. Similarly, De Troyer et al. (2011) found that maize litter-C in DOC peaked during the first three days (maximum 3% of the added C). This indicates that DOC in our litter could have been somewhat higher than we observed, and explains why leaching of litter-C was slightly lower than in most literature (less than 1% after 9 days compared to 1-4% in other studies (Don and Kalbitz 2005; Kalbitz et al. 2006; Kammer and Hagedorn 2011).

Although leaching of litter-C was of minor importance in our experiment, litter type strongly affected the partitioning of litter C to DOC, which is in accordance to findings of other studies (Bird and Torn 2006; Hansson et al. 2010; Kalbitz et al. 2006; Uselman et al. 2007). In general, ash litter contained higher amounts of DOC than beech litter. Recovery of litter derived C in DOC was lower in PL-beech$_{root}$ than in PL-beech$_{leaf}$ in the beginning of the experiment, which is in line with findings in the literature (Bird and Torn 2006; Hansson et al. 2010; Uselman et al. 2007) for various broadleaved and coniferous tree species. However, recovery of litter derived C in DOC did not differ between PL-ash$_{leaf}$ and PL-ash$_{root}$. We are not able to explain this finding. Nevertheless, differences between variants were restricted to the first 29 days of our experiment and the DOC deriving from litter was very low after 206 days of incubation, which is in line with Kalbitz et al. (2006), who found this pattern during the first phase of litter decomposition, before the start of lignin degradation (Berg 2000).

No mixture effects were observed on the litter-derived DOC. To the best of our knowledge, no studies exist that compared the leaching of litter C between pure and

mixed variants, but this result corresponds to our expectations, since the leaching is in the first hand related to the amount of soluble C in the litter and the hydrological conditions in the soil (Kalbitz et al. 2000).

5.5.3 Density fractions

We found no differences between variants in the recovery of litter derived C in the LF as a result of the high variation in the LF within one variant. The reason for this high variation probably finds its origin in the sampling design, as a subsample of 10 g was taken from the whole soil-litter mixture which was 605 g in total. Although we were very careful to sample representatively by homogenizing the soil-litter mixture as good as possible, even a small inhomogeneity may lead to large errors in the recovery of litter-C in the LF. Also variations in the total recovery of the litter-C (as sum of the recovery in CO_2, LF and HF) most likely find their origin in the strong fluctuation in the LF, which is why the latter data will be considered with care.

In contrast to our results (no litter type or litter mixture effect), Bird et al. (2008) found that after 10 months more needle (~4.5%) than root litter C (~3.2%) was mineral associated and recoveries in the HF in their study were in the same magnitude as the values in our study (4-12%). The slight differences are probably related to species specific effects, however due to the few studies on this topic, no general conclusion can be drawn.

In general, our findings contradict the repeatedly reported higher topsoil C stocks under ash than under beech (Guckland et al. 2009; Langenbruch et al. 2012; Oostra et al. 2006; Vesterdal et al. 2008). This discrepancy may have several causes. In this experiment, the ash litter was introduced to the exact same soil as the beech litter. However, usually, the soil preconditions under ash are much better (higher pH and nutrients) than under beech (Guckland et al. 2009; Jacob et al. 2009; Langenbruch et al. 2012). Further, the positive effect of ash leaf litter on the soil properties might be very lengthy and therefore detectable only after a very long time. Thus, short term decomposition experiments (<1 year) cannot fully picture the decomposition process and might lead to miss-conclusions. It is therefore highly recommended to run decomposition experiments over longer time periods, i.e. 5-10 years to be able to prove this assumption. In addition, it is possible that the positive influence of ash leaf litter on the soil C storage was not mediated by soil microorganisms. Thus, soil fauna, such as earthworms possibly could be responsible for a higher C storage under ash, as the abundance of earthworms lead to an increase of C in the soil (Scheu 1997).

5.5.4 Microbial biomass

After 206 days of incubation, an effect of litter addition, litter type or mixture on the concentration of C_{MB} could not be observed, which is in line with findings from a laboratory study by Lummer et al. (2012). In contrast to our results, Thoms et al. (2010) found an increase of the total amount of phospholipid fatty acids (PLFA) in 0-20 cm soil depth with increasing tree species diversity (and thus decreasing beech abundance) in Hainich National Park. However, this difference may be related either to the two different methods used (CFE versus PLFA) or to more favorable soil conditions in the species rich stands, as microbial biomass of nearly all groups was correlated with various soil chemical (such as pH, soil C, N, P stock) and physical (clay content) parameters. As we only determined C_{MB} after 206 days of incubation, however, we cannot exclude, that it was enhanced shortly after the litter addition.

Although C_{MB} did not differ between the variants at day 206, the microbial activity was significantly increased in variants with litter compared to the control. The mean qCO_2 on day 206 in the control was 0.4 which is rather low for acidic soils. In the variants containing litter the average qCO_2 ranged from 0.8 in PL-ash_{leaf} to 2.5 in PL-$beech_{root}$, which falls in the range of variation of previously published values (0.5-2.4) for acidic forest soils (Anderson and Domsch 1993; Blagodatskaya and Anderson 1998; Malchair and Carnol 2009). The qCO_2 was negatively correlated with the *q*MB, indicating that the microbial activity per C_{MB} increased with increasing availability of C. This is in agreement with the results of Malchair and Carnol (2009).

Overall, the amount of microbial biomass C in our study was rather low compared to results from other beech forest soils (Joergensen et al. 1995; Malchair and Carnol 2009). This most probably finds its cause in the experimental design, since we had rather low concentrations of organic C (1.1-1.6%, data not shown) in our microcosms compared to other studies, where the organic C in acidic soils ranged from 5% to more than 20% (Blagodatskaya and Anderson 1998; Joergensen et al. 1995; Malchair and Carnol 2009). However, *q*MB of the investigated treatments fell within the range of previously published values of 0.5-2.3% for acidic forest soils (Anderson and Domsch 1993; Blagodatskaya and Anderson 1998; Joergensen et al. 1995; Malchair and Carnol 2009).

The recovery of litter derived C in C_{MB} was below 1% for all variants which is lower than the observed 3% of maize derived C in the incubation study in a greenhouse by Rottmann et al. (2010). Still, these values are in the same order of magnitude and the differences probably are related to the species from different plant groups. Supportively, the recovery of ash leaf and root litter derived C in C_{MB} was higher than of beech leaf and root litter derived C, respectively. No differences were observed in the recovery of root compared to

leaf litter derived C. Contrary to this, Moore-Kucera and Dick (2008) found a higher incorporation of Douglas fir (*Pseudotsuga menziesii* Mirb.) needle litter derived C into microbial biomass compared to root litter derived C, indicating possible species related differences. No mixture effects were observed on the partitioning of ash and beech root litter C to C_{MB}. We are not aware of any study that analyzed mixture effects on the partitioning of litter C to the C_{MB} so far.

5.6 Conclusion

Litter type affected the partitioning of litter C, which was especially pronounced in the efflux of litter derived CO_2-C, the main decomposition pathway, but also in the incorporation of litter C into MB or the leaching of DOC from decomposing litter during the first 29 days. The results of our study showed that (1) ash litter was decomposed more rapidly than beech litter, (2) initial decomposition was slower for root litter than leaf litter due to a higher lignin content and lignin:N ratio, and (3) the litter decomposition in mixtures may, but does not necessarily, behave differently than in pure variants and therefore it cannot necessarily be calculated from the pure variants. Even though clear species specific differences were found in the decomposition, no differences were observed in the partitioning of litter C to the HF, i.e. the association to the mineral surfaces. This indicates that in the short term, litter type or litter mixture does not affect C sequestration in the soil under identical soil conditions and the exclusion of mesofauna and macrofauna. We like to point out that our results prohibit drawing general conclusions concerning long term effects that are related to decomposition processes in the late stages.

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6 SYNTHESIS

Recently, one aim of forestry has become to replace monocultures of mainly conifers through introducing various broadleaved tree species in order to reduce the danger of hazards, such as soil deterioration or susceptibility to pests. The discussion about biodiversity vs. species identity effects on biochemical interactions in broadleaved forests is therefore of great interest. A crucial factor for the vitality of a forest is the nutrient availability and base saturation in the soil. To understand to which extent tree species or tree species mixtures may influence these soil properties was the central topic of this study.

Investigating the influence of tree species on soil properties is associated with many difficulties. For example, separation of the influence of land use history, parent material or soil physical parameters, such as the clay content, from those arising from the vegetation, such as the tree species, is difficult in field studies with highly diverse vegetation and variable soil preconditions. The aim of the graduate school 1086 “The role of biodiversity on biogeochemical cycles and biotic interactions in temperate deciduous forests” was to reduce side effects, such as the clay content and the land use history and to maintain as homogeneous soil conditions as possible, while at the same time researching in an old grown natural species-rich forest. This aim was achieved with careful choice of study site (Hainich National Park) and experimental design. In the present work,

(1) the investigation of soil properties under different tree species took place in a spatially limited study area within the forest site, the so-called “tree clusters”.
(2) the C and N partitioning during decomposition of $^{13}C^{15}N$-labelled litter of different tree species was investigated in a field mesocosm study that was carried out on a small spatial area in a beech forest.
(3) the third experiment, also a litter decomposition study, was conducted under even more controlled conditions as a laboratory study with constant temperature and soil moisture, absence of meso-and macrofauna, and exclusion of plants.

In the course of this synthesis, the results from the three studies will be evaluated collectively in order to draw reliable conclusions concerning the influence of leaf and root litter of different tree species on the soil organic matter (SOM), the nutrient stocks and the soil acidity.

6.1 Soil acidification and nutrients

Small-scale differences in the chemical properties of the humus layer and the topsoil (0-10 cm) were related to the abundant tree species (beech - *Fagus sylvatica* L., ash - *Fraxinus excelsior* L. and lime - *Tilia* spec.). An important control variable was found to be the composition of the leaf litter, as the stocks of base cations, such as Mg^{2+} and Ca^{2+},

were linearly related to the respective nutrient return with the leaf litterfall. Thus, nutrient return with leaf litter ameliorated the soil nutrient availability. Nutrient uptake from the subsoil via tree roots and transport to the leaves may function as an important biological pumping and higher stocks of base cations in the topsoil slow soil acidification. The ameliorating effect was species dependent. Ash litter, which contained highest stocks of base cations, slowed the acidification and increased stocks of exchangeable Mg^{2+} and Ca^{2+} in the topsoil.

6.2 Soil organic matter, C and N cycling

Tree species affected the SOM storage and the stocks of N_t in the forest floor and mineral soil. The stocks of C and N in the forest floor were primarily related to the abundance of beech leaf litter, because of its slower decomposition compared to ash and lime leaf litter. In contrast, the stocks of C and N in the topsoil were positively influenced by the abundance of ash litter. The two decomposition studies revealed that mineralization was the main pathway of decomposition and that ash litter was mineralized faster than beech litter (both roots and leaves). Further, mineralization of ash leaf litter was enhanced when mixed with beech leaf litter. However, recoveries of C and N in the mineral soil and association of litter C to minerals did not differ between the litter types. This might at first sight contradict the results found in the cluster study. However, the lacking difference in the partitioning of N to the mineral soil was related to two factors:

(1) The N content in the labeled beech leaf litter was considerably higher than it was typical for this litter due to the necessary N fertilization in the course of the labeling process and thus differences between beech and ash leaf litter in the N content were minor.

(2) The absolute amount of litter N found in the soil showed a positive linear relationship to the N content in the litter, i.e. the proportion of litter N recovered in the soil did not differ between litter types. Thus, the results from the mecocosm study are actually in line with those from the cluster study, because ash leaf litter usually contains more N than beech leaf litter. Hence, assuming a comparable litter production, more N is returned to the soil by ash leaf litter than by beech leaf litter and the stock of N increases over time, as long as no leaching or enhanced nutrient uptake from the topsoil occurs.

Regarding the SOM storage in the soil, the relationships are more complex. The differences between the studies allow the following four conclusions that in combination probably reveal the true relationships:

(1) The effect of litter becomes apparent only after very long periods of time and a year is too short to be able to show differences.
(2) Whether and how tree species affect the SOM storage depends on site conditions. The soil of the cluster-study was only slightly acidified and had a high base saturation, while the soil of the two litter decomposition experiments was relatively strong acidified, the proportion of aluminum in the soil was high and base saturation was rather low. This probably also affected the soil microbial and faunal community. In addition, in the microcosm study decay was limited to microbial breakdown. Thus, it is likely that the microbial biomass is not responsible for the differences in SOM in the soil under ash and under beech.
(3) Under natural conditions, the soil properties under ash are different than under beech, which is related to a higher nutrient return with ash litter and the ability of ash to slow soil acidification. However, in our two decomposition studies, all litter types were introduced to the exact same soil, thus prohibiting interactions that are related to soil preconditions.
(4) In the mesocosm experiment, the composition of the labeled beech litter was significantly different from the chemical composition usually observed for beech leaf litter. Further, the chemical composition of the labeled beech and labeled ash litter was rather similar, while in nature it usually differs considerably. Thus, decomposition of the labeled beech litter possibly behaved differently than a beech litter with typical chemical composition would have.

6.3 Ecological-silvicultural importance of the present findings

When considering the ecological and silvicultural importance of the present findings, the results should be viewed from different angles and the statements are limited to forests that grow on loess over limestone in a humid temperate climate. If the goal is

(1) to increase the persistence of a beech forest, it would be advisable to add ash trees in order to reduce the danger of long-term soil acidification and degradation.
(2) to increase biodiversity, it is recommended that ash should be mixed with beech trees in varying proportions, since the effect of tree species on soil chemical properties was already abundant on a very small scale and thus, the soil heterogeneity and with it the habitat diversity for soil organisms and herbaceous species could be increased.
(3) to increase the C sink function of the topsoil, stands with the highest possible proportion of ash should be striven for, because ash trees lead to higher C stocks

in the topsoil. The higher forest floor C storage under beech than under ash could not even compensate for the higher topsoil C storage under ash than under beech.

However, the influence of the clay content should not be underestimated. The cluster study showed that the clay content, even though varying less than the proportion of ash leaf litter to total leaf litter fall, already played a relevant role concerning topsoil C- and nutrient storages as well as pH. As soon as the clay content reached a certain variance (TB 60: 22-31%) it was even more important than the influence of the ash leaf litter, although the latter varied a lot more. This indicates that at sites where the clay content varies widely on a small spatial scale, the selection of tree species on the investigated parameters most likely only plays a minor role, while at sites were the clay content varies only very slightly on a large area, the selection of certain tree species could be relevant in order to enhance nutrient and C-stocks and to slow soil acidification.

6.4 General conclusion

The addition of ash to beech dominated stands has a positive effect on the nutrient storage in the soil and reduces soil acidification. Whether ash litter positively affects SOM storage seems to depend on several factors, among these the soil chemical preconditions. Although the mineralization of ash litter was enhanced in mixture with beech litter, there was no indication of a mixture effect on the stocks of C and N in the soil. All results indicate that in diverse stands, the species identity is more important than the diversity per se with respect to the formation of soil properties, as all species effects on the soil properties were linearly related to the initial chemical composition of the leaf and root litter. The effects of a litter type become visible only after a very long time period and a single application of ash litter probably has no significant effect on soil properties in beech forests. The results also show that soil properties in mixed stands vary on a small spatial scale, which leads to an enriched habitat diversity for soil organisms.

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Declaration of originality and certificate of authorship

I, Christina Langenbruch, hereby declare that I am the sole author of this dissertation entitled "Effects of nutrient cycling through litter of different broadleaved deciduous tree species on soil biochemical properties and the dynamics of carbon and nitrogen in soil". All references and data sources that were used in the dissertation have been appropriately acknowledged. I furthermore declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure. I certify that the manuscripts presented in chapters 3, 4 and 5 have been written by me as first author.

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E.A. Vockenhuber, C. Scherber, **C. Langenbruch**, M. Meißner, D. Seidel & T. Tscharntke (**2011**): Tree diversity and environmental context predict herb species richness and cover in Germany’s largest connected deciduous forest. Perspectives in Plant Ecology, Evolution and Systematics. 13: 111-119